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Transgenerational Epigenetic Inheritance in Mammals

Transgenerační epigenetická dědičnost u savců

MSc. THESIS

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I declare that I wrote my thesis independently and self-reliantly, under the supervision of Mgr. Jana Švorcová, PhD. and all the possible literature that has been used for completion of the thesis has been cited. This thesis nor a part of it was used for acquisition of any other degree of same or different value.

Prohlášení:
Prohlašuji, že jsem závěrečnou práci zpracovala samostatně, pod vedením Mgr. Jany Švorcové, PhD. a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

Prague, Deceber 16, 2016 Veronika Dostálová

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Contents

Abbreviation:

5-hydroxymethylcytosine	5hmC
5-methylcytosine	5mC
Alcohol Dehydrogenase	ADH
Acetaldehyde Dehydrogenase	ALDH
Alcohol-Use Disorder	AUD
Brain-Derived Neurotrophic Factor	BDNF
Competitive Endogenous RNAs	ceRNAs
Copy Number Variation	CNV
CpG Islands	CGIs
Differentially (hypo/hyper) Methylated Regions	DMRs
DNA (cytosine-5)-methyltransferase 1	Dnmt1
DNA (cytosine-5)-methyltransferase 3a	Dnmt3a
DNA (cytosine-5)-methyltransferase 3b	Dnmt3b
Encyclopedia of DNA Elements	ENCODE
Exocrine Gland-Secreting Peptide 1	ESP1
Exocrine Gland-Secreting Peptide 34	ESP34
Endogenous Retro Viruses	ERVs
Embryonic Stem Cell	ESC
Quantitative Trait Loci	QTL
Histone 3	H3
Histone 4	H4
Histone Acetyltransferase	HAT
Histone Deacetylase	HDAC
Human Genome Project	HGP
Inner-Cell Mass	ICM
Incomplete Lineage Sorting	ILS
In Vitro Fertilization	IVF
Large Intergenic Non-Coding RNAs	lincRNAs
Long Non-Coding RNAs	lncRNAs
Long Term Repeat Transposons	LTRs
Major Histocompatibility Complex	MHC
PIWI-interacting RNAs	piRNAs
Post-Translational Modifications	PTMs

RNA-Induced Silencing Complex	RISC
RNA Interference	RNAi
Short Interfering RNAs	siRNAs
Single-Strand RNA	ssRNA
Small Non-Coding RNAs	sncRNAs
Small Nucleolar RNAs	snoRNAs
Small Nuclear Ribonucleoprotein Polypeptide N	snRPN
Small Nuclear Ribonucleoprotein Polypeptide N gene	*SNRPN*
Transcription Start Sites	TSS
Ten-Eleven Translocation Oxidases	TEToxidases
TransgenerationalEpigenetic Inheritance	TEI

Abstrakt:

Transgenerational epigenetic inheritance in mamals is a widely discussed topic in today´s biology. Epigenetic modifications are molecules that play a crucial role in regulation of gene transcription. Epigenetic modifications regulate another epigenetic modification´s establishment. The extrinsic and the intrinsic cellular or organismal environment is involved within the establishment of epigenetic state. The molecules involved in epigenetic processes are able to regulate gene transcription in reaction to the environment and therefore these molecules partly shape the phenotype. Most importantly, epigenetic processes are affected by cellular or organismal history. A question emerges: Are these molecules able to transfer information through germline to subsequent generations? Does transgenerational epigenetic inheritance in mammals exist? Experimental data show it is so. What consequences this can mean in our understanding of evolution?

Key words: epigenetic modifications, transgenerational epigenetic inheritance, mammals, evolution, Lamarck, behavior

Abstract:

Transgenerační epigenetická dědičnost u savců je diskutované téma v současné biologii. Epigenetické modifikace jsou molekuly, které hrají zásadní roli v regulaci genové transkripce. Epigenetické modifikace regulují ustavování jiných epigenetických modifikací a na celkovém procesu epigenetického stavu se podílí vnitřní i vnější buněčné nebo organismální prostředí. Jelikož jsou tyto molekuly schopny regulovat genovou transkripci v odpovědi na prostředí, podílí se tak na fenotypových projevech organismu. Je důležité zmínit, že epigenetické procesy jsou ovlivňovány také buněčnou či organismální historií. Vyvstává otázka, zda mohou tyto molekuly předávat informaci skrze savčí pohlavní linii do dalších generací. Existuje transgenerační dědičnost u savců? Experimentální data ukazují, že je tomu tak. Jaký to může mít dopad na naše vnímání evoluce?

Klíčová slova: epigenetické modifikace, transgenerační epigenetická dědičnost, savci, evoluce, Lamarck, chování

1. Introduction

Epigenetic processes are generally responsible for the variety of different cell types in a body. Nowadays we arrive at the question of how much these processes could matter in transgenerational transfer and how these processes could modify the shape of evolution. The concept of transgenerational epigenetic inheritance is accepted in plants, for their somatic and germinal lines are not strictly divided, as in the case of animals. Continuity of germplasm is the generally accepted idea that germline and somatic line are completely separated during the life of an individual. Thus the events that happen in the somatic line, e.g. the cells that constitute our body, are supposed to have no possible effect on our germinal cells. Plants lack the Weismann barrier: using their somatic cells, plants can produce a body that contains the epigenetic changes present in the motherly somatic cell. In the case of animals, the situation is different, because the germ line is strictly divided from the somatic line. The division of the somatic and germinal line emerges with the specification of germ cells during gastrulation in every mammalian generation. A new organismal generation can rise from the germinal line only – a new mammalian organism comes only from germ cells of its parents. There are specific processes that keep the germ line free of acquired epigenetic changes, like deletions of methylations in preimplatation of the embryo and in primordial germ cells, or the replacement of histones by protamines. However, there are good reasons to pose further questions. How robust are these processes? Does epigenetic transmission through the germ line exist? How persistent can it be? If it does, what bearing can it have on our understanding of evolution? Among the arguments supporting the appropriateness of these questions are studies that show that a certain transgenerational transfer of acquired characteristics through the germ line exists. Even though we are not yet able to describe all the individual processes, a new field of evolutionary thinking has been opened.

The question whether the organic form is predetermined, or whether it acquires its shape during development, was as far as we know first posed by Aristotle. This question is an essential one running through various modes of biological thinking. However, answering this question appears to be intricate, reflecting the complexity of development itself. Aristotle used the term genesis (the Greek for ‘formation’ or ‘coming to being’). The term ´epigenesis´ was never actually used by Aristotle himself, though the process of epigenesis through which an organism goes during its life, gradually changing to acquire its form, was described in his treatise “On the Generation of Animals”. This term was later used in biological discussion in the 19th century, where the opposing theory was one of preformism, claiming that the form of an organism is predetermined, and that the final form of the body lies concealed in the germ. (Maienschein 2012).

Phenotypic plasticity has been described within organisms, because they are able to respond and adapt to environmental changes such as temperature, nutrition, presence of predators etc. Such changes are usually recorded in the form of epigenetic modifications of specific genetic loci. Whether a new adaptation can emerge through epigenetic changes, and whether it can be transmitted to the next generation, is a matter of dispute to which I will try to respond.

In this thesis, I aim to adjust the definitions of some terms concerned with epigenetics and epigenetic inheritance, to review the means of epigenetic modifications, to summarise the latest singular studies addressing the issue of transgenerational epigenetic inheritance (TEI) in mammals and, last but not least, I will contemplate the possible implications of these new findings for evolutionary thinking. I will ask if it is possible for TEI to be involved in evolution, and I will consider the possible ways it could take. I will question whether there is any evidence for inheritance of acquired characters. I will ask whether there could be heredity of acquired traits through TEI and whether there are any means by which we could rethink Lamarckian theory. I will pose the question if it is possible for evolutionary novelties to emerge through epigenetic variation, or whether epigenetic change is rather an evolutionary established switch between different underlying genes for a particular trait.

2 Defining of objectives

I will adjust terms concerning epigenetics and epigenetic inheritance and show the manifestation of the phenomena on empirical data of today'smolecular biology. I will ask if there is evidence in experimental data for transgenerational epigenetic inheritance in mammals and I will relate the findings in the context of today's evolutionary biology. I will ask wheather the findings imply changes of our understanding of evolution and if yes, I will reason the particular possible pathways of evolutionary nature.

3 Terms and Definitions

3.1. Epigenetics

Epigenetics is a domain of study that concerns cellular and physiological variations that are not caused by direct changes in DNA sequence. The field of study includes cellular processes that affect the transcription or translation of genes, alterations of the genome that do not represent changes in nucleotide sequence. It includes the description of processes leading from genotype to phenotype. The organism's or cell's fate is not predetermined simply by their genomic sequence,

but it is further influenced by external factors or by set conditions of the cell or organism itself (Szyf 2015).

The term epigenetics was first used by C. H. Waddington with a different meaning than we assign to it today. Waddington used the term "epigenetics" for a series of changes in gene expression that cells undergo during ontogenesis and that define the nature of the final appearance of an organ or (delete the a) specific cell type (Waddington, 1959). 'Epigenetics' in this original sense refers to the study of the way genes and their products bring the phenotype into being (Jablonka and Lamb, 2002).

"Some years ago, I introduced the word 'epigenetics,' derived from the Aristotelian word 'epigenesis,'which had more or less passed into disuse, as a suitable name for the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being." (Waddington 1969)

Waddington created the term "epigenetics" referring particularly to Aristotelian epigenesis, emphasizing development as a gradual and qualitative process, as well as building upon modern biology of that time – genetics (Jablonka and Lamb, 2002). 'Epi-' means 'upon or beyond' in Greek, Waddington thus pointed to the need for studies that would go further than genetics, for it is necessary to understand the processes that relate to the genome. C. H. Waddington illustrated the phenomenon of a cell's gradual specialization, creating a model (or metaphor) he called 'The Epigenetic Landscape', in which different possible fates of a specific cell are depicted as valleys on a wrinkled surface, with the cell represented by a ball moving upon it, gradually choosing between the possible paths to take (Jablonka and Lamb, 2002).

The definition of 'epigenetics' is problematic, hence it is based on what it is not. As its object of study lies outside the field of genetics, the primary and guiding branch of 20th century biology, it is not surprising that it has been generally disregarded in the sense that it has been incorporated into accepted evolutionary theories that are mainly based on genetics. This widely accepted 'modern synthesis', building upon natural selection, central dogma and population genetics, is elegant itself, nevertheless the issue becomes more complex when the novel findings are respected. The new empirical data leads us to explore the emerging field, and we are led to examine if, by some chance, our concepts of understanding evolution can be changed due to those novel empirical findings.

Since C. H. Waddington introduced the term epigenetics, the field of study has changed, broadened, and so has our understanding of the term. Eva Jablonka distinguishes between several ways of understandin the term. There is epigenetics in the Waddingtonian sense which is different

from the one of today. Further, epigenetics is often used as a synonym for epigenetic inheritance (further discussed in 3.2). Jablonka and Raz (2009) claim that it is crucial to distinguish between these terms, for epigenetic inheritance is a part of epigenetics, but not all of the epigenetic processes are hereditary.

"*Epigenetics is the study of the processes that underlie developmental plasticity and canalization and that bring about persistent developmental effects in both prokaryotes and eukaryotes. At the cellular level, these are the processes involved in cell determination and differentiation. At higher levels of biological organization, epigenetic mechanisms generate the context-dependent, selfsustaining interactions between groups of cells that lead to physiological and morphological persistence.*" (Jablonka and Raz, 2009)

3.2. Epigenetic inheritance

Epigenetic inheritance is a component of epigenetics. It occurs when phenotypic variations that do not stem from variations in DNA base sequences are transmitted to subsequent generations of cells or organisms (Jablonka and Raz, 2009). Epigenetic inheritance is either mitotic (somatic) or meiotic (gametic) and it concerns changes in gene expression that are adjusted without primary genetic sequence being compromised. Epigenetic inheritance is also called 'nonstable' (soft) inheritance, which refers to the fact that epigenetic changes vary in penetration. Several types of processes have been described by which cells preserve and pass on the change in epigenetic information. The ways in which epigenetic information is established are DNA methylation, histone modifications and non-coding RNAs (ncRNAs).

3.3. Somatic epigenetic inheritance

Epigenetic change and its inheritance seem to be one of the important aspects of mammalian development and cell differentiations, according to the latest studies (Reik et al., 2001; Broad et al., 2016; Lowdon et al., 2016; Perez et al., 2016). As embryonic pluripotent cells divide, while changing into various more and more specialized cell types, the informational change is truly of epigenetic nature, for we know one organism to have the same DNA in every single cell (excluding little variations caused by random mutations, transpositions or viral infections). This form of epigenetic inheritance is called 'somatic inheritance', for it is transmitted through the somatic line (Morgan et al., 1999). The developmental stimuli that led to the different cell phenotypes can be long gone, yet the cellular epigenetic memory preserves the required state.

3. 4. Gametic epigenetic inheritance

Epigenetic change and its inheritance seem to be one of the important aspects of mammalian development and cell differentiations, according to the latest studies (Reik et al., 2001; Broad et al., 2016; Lowdon et al., 2016; Perez et al., 2016). As embryonic pluripotent cells divide, while changing into various more and more specialized cell types, the informational change is truly of epigenetic nature, for we know one organism to have the same DNA in every single cell (excluding little variations caused by random mutations, transpositions or viral infections). This form of epigenetic inheritance is called 'somatic inheritance', for it is transmitted through the somatic line (Morgan et al., 1999). The developmental stimuli that led to the different cell phenotypes can be long gone, yet the cellular epigenetic memory preserves the required state.

Eva Jablonka distinguishes between two understandings of epigenetic inheritance: epigenetic inheritance in the broad sense, and cellular epigenetic inheritance which is a narrower aspect of it (Jablonka and Raz, 2009). Epigenetic inheritance in the broad sense is understood as inheritance of developmental variations that do not stem from DNA sequence or respond to the present environment. It can be represented either by cell-to-cell transmission of epigenetic variations, or by the transmission of information through social learning as well as by symbolic communication. The narrower understanding of epigenetic inheritance is cellular epigenetic inheritance, referring to epigenetic transmission in the somatic or germinal line, where the cell is considered the basic unit of the process. It is this narrower understanding of epigenetic inheritance that I will deal henceforth in my thesis. If the need arises to use the broader sense of the term, I will specify it.

Concerning mammals, the transmission from mother cell to daughter cell can be through chromatin marks or through RNAs. Within ciliates there are self-reconstructing 3D structures described, this is a form of structural inheritance. Within bacteria there are self-sustaining metabolic loops described, these are usually two variations of metabolic circuits, which can be switched from one to another and such a state can be heritable (Jablonka and Raz, 2009).

In the last two centuries, the Weismann barrier concept made gametic epigenetic inheritance impossible to include into the general biological paradigm. Gametic epigenetic inheritance was historically regarded as impossible due to the Weismann barrier paradigm. The concept of the paradigm relies on the presupposition that there is no communication between the somatic and the germinal line. Even though germ cells are maintained in a relatively stable milieu, not to get damaged, we cannot properly exclude the possible effects of the motherly environment.

The conditions in utero are known to have an effect on development through epigenetic processes. The developing organism is particularly sensitive to environmental changes, which may

lead to alteration of the epigenetic setting and affect the phenotype. In some cases, these changes may result even in TEI. Further, I will explain what factors need to be considered when testing for genuine TEI (5.), but first I will make a review of epigenetic modifications and molecules which are known so far.

4. Review on epigenetic modifications

4.1. Epigenetic modifications

Mechanisms responsible for epigenetic inheritance regulate potential transcriptional activation or inactivation of specific genes, sets of genes, genomic domains or chromosomes. One of the guiding principles of development is the dynamic change in gene activation/expression, regulated by specific epigenetic processes. There exist two main principles of epigenetic setting, according to the type of the final molecule that is involved. It can be the nucleic acids: various types of ncRNA or modifications to DNA. Looking at nuclear proteins, we can distinguish between post-translational modifications (PTMs) of the core histones, and the action of ATP-dependent chromatin remodelling enzymes (Swygert and Peterson, 2014).

4.2. DNA methylation

One of the most extensively studied epigenetic modifications is **DNA methylation**. It is widely accepted that DNA methylation is one of the crucial mechanisms involved in the inactivation of the X chromosome. Furthermore, DNA methylation can enhance or silence exon recognition during alternative splicing through specific epigenetic marking of exons (Yearim et al., 2015). In vertebrates, cytosine bases in the DNA sequence can be covalently modified by DNA methyltransferase enzymes to 5-methylcytosine (5mC). Also, a methylation of adenine has been found in mouse embryonal stem cells (ESCs) (Wu et al., 2016). Methyltransferases catalyze the transfer of methyl moiety from S-adenosylmethionine to the 5´ position on the cytosine ring (Drahovský and Morris, 1971). Methylation is widely used within the mammalian genome to prevent transcriptional initiation. DNA methylation promotes gene silencing on an inactive X chromosome or within imprinted genes as well as within retroviral sequences. Within development and cell differentiation, DNA methylation is known to change dynamically in dependency to the ongoing developmental processes (Lister et al., 2009). In addition, DNA methylation (or hydroxymethylation; see further Kriaucionis and Heintz, 2009) differs among specific cell types, and is essential for proper cellular differentiation (Ko et al., 2010).

There are several mechanisms by which DNA methylation alters gene expression. It can be

either a direct interference with the binding of transcription factors that recognize elements containing particular CG dinucleotide (Comb and Goodman, 1990) or it can trigger methylated DNA binding factors (Lewis et al., 1992) that recruit chromatin-inactivating complexes such as histone deacetylases (Jones et al., 1998; Nan et al., 1998) or histone methyltransferases (Fuks et al., 2003). Yet, there exist proteins that bind methylated as well as unmethylated DNA (Baubec et al., 2013); the nature of these processes and their function remains unclear. It appears that methylation in the body of a gene generally enhances gene activity – however, the specific mechanism is unknown (Hellman and Chess, 2007; Aran et al., 2011; Yang et al., 2014); whereas in the immediate proximity of transcriptional start site (TSS) (Jones, 2012), methylation causes gene silencing. Frequently there are methylated CpG-rich regions which are known as CpG islands (CGIs). These sites are separated by equivalent domains of unmethylated DNA (Antequera et al., 1990). The methylation state is heritable within a cellular lineage. Enzymatic activity of DNA (cytosine-5)-methyltransferase 1 (Dnmt1) forwards these epigenetic marks to the next cellular generation. When DNA is replicated, Dnmt1, having a high specificity for hemimethylated DNA, modifies the nascent strand according to the parental one (Gruenbaum et al., 1982).

DNA methylation is often referred to as 'symmetric' methylation, for it is present on both strands, and as such it is easily transferred to next cellular generations, representing the core process of somatic epigenetic inheritance. Mammalian germ cells and early embryos undergo global deletion of DNA methylation (Reik et al., 2001; Seisenberger et al., 2012). If the deletion of methylation were complete, it would be impossible to carry any information across generations. However, there are studies that describe the deletion as incomplete (Guibert et al., 2012). There are certain regions that escape general deletion: best known for escaping it are certain imprinted genes, transposable sequences and even certain single copy sequences are known to escape general deletion as well.

Dnmt1 adds methyl moiety on the nascent strand in the opposite position to the parental strand. Outside CGIs, DNA methylation also occurs: in these cases, it is referred to as non-CpG or 'asymmetric'. Even though it is rare within mammalian genomes, non-CpG methylation has been found in embryonic stem cells, where de novo methyl-transferases Dnmt3a and Dnmt3b appear to be highly active (Lister et al., 2009; for further reading see Tomizawa et al., 2012). In addition DNA methylation is influenced by histone-modifications state and linked enzymes (Fuks, 2005).

DNA hydroxymethylation: Ten-eleven translocation (TET) oxidases can further hydroxylate the methyl moiety, resulting in another epigenetic mark, 5-hydroxymethylcytosine (5hmC) (Ito et al., 2010; Tahiliani et al., 2009). DNA hydroxymethylation is considered to play a role functionally distinct from DNA methylation. DNA methylation is generally regarded to as having a silencing

function, whereas hydroxymethylation appears to enhance gene activity when present in the gene body. Hydroxymethylation is known to trigger transcription factors different from those DNA methylation recruits. Furthermore, hydroxymethylation is considered essential for the stage of pluriotency in germ cells. 5hmC DNA methylation in mammals is typically found within CpG dinucleotides.

4.3 Other means of DNA methylation

Non-CpG methylation has a specific function in plants, it is targeted to transposable elements by short interfering RNA (siRNA), it is considered to play a crucial role in gene silencing that is directed to the site (Mette et al., 2000). Non-CpG methylation has also been observed in early mouse embryos, appearing sometime between ovulation and the formation of a 2-cell embryo (Haines et al., 2001), which increases the suspicion of it having a specific function. Unlike somatic tissues, there has been non-CpG methylation found in mammalian embryonic cells, making 15-20% of total cytosine methylation (Ramsahoye et al., 2000). The experiments also indicate non-CpG methylation to be caused by de novo methyltransferases, as are DNA (cytosine-5)-methyltransferase 3a (Dnmt3a) and DNA (cytosine-5)-methyltransferase 3b (Dnmt3b) (Okano et al., 1999). Dnmt3a and Dnmt3b were found to be present in high levels in embryonic cells and in lower levels in somatic cells. Dnmt3a and Dnmt3b concentrations show distinct expression patterns during development, Dnmt3b appears to play an important role during early embryogenesis whereas Dnmt3a appears to be crucial for later embryonic and postnatal development. Dnmt3b was observed to have the ability to specifically methylate the centromeric minor satellite repeats in ESCs (Okano et al., 1999). Lower expression of the gene coding Dnmt3 showed in aging animals appears to correlate with decreased cognitive abilities of these animals (Oliveira et al., 2012), which would indicate a connection between de-novo methylation and cognition. Lower levels of Dnmt3a were also identified in cancer cells. Dnmt3a and Dnmt3b appear to play a role in the deactivation of the X chromosome and within gene silencing (Okano et al., 1999). Dnmt3 has the ability to methylate CpGs as well as non-CpGs (Ramsahoye et al., 2000).

In embryonic stem cells, almost one quarter of total methylation has been identified not to be in CpG context, thus it is possible to suspect embryonic stem cells of using different methylation mechanisms to control gene regulation (Lister et al., 2009). Cytosine hydroxymethylation is highly abundant in mouse brain, suggesting a role in the epigenetic control of neuronal function (Kriaucionis and Heintz, 2009). Hydroxymethylation also varies among different cellular populations, being highest within most specified tissues such as brain or kidney (Szwagierczak et al., 2010), and probably also playing a part in the development of an embryo (Ito et al., 2010) and

cellular differentiation (Ko et al., 2010). Hydroxymethylation was found to be frequent in promoter regions of coding genes in embryonic cells, in correlation with certain histone modifications present in the promoter sites. This indicates a possible function of hydroxymethylation as a regulator of activation of genes in developmental dependence (Pastor et al., 2011). 5-formylcytosine is a rare base found in mammals and probably also has functional roles (Bachman et al., 2015).

Recently discovered methylation of adenine in mice appears to correlate with gene silencing in certain transposomal regions, leading to their inactivation during embryonic stem cell differentiation (Wu et al., 2016).

DNA methylation also appears to play an important role in inter-species variation through hypermethylation of CpG sites and the hypomethylation of endogenous retro-viral elements (Hernando-Herraez et al., 2015). This has been observed in a unique global methylome study on primates by Hernando-Herraez et al. (2015), leading to a suggestion that DNA methylation could play the crucial role in the variation between species. Whether DNA methylation is the cause of species divergence, or rather its consequence, remains a matter for discussion. Focusing on the hypomethylation of endogenous retro viruses (ERVs), it can be argued that ERVs are species specific and are required for embryonic stem cell (ESC) maintenance (Lu et al., 2014). The state of methylation of ERVs can be considered crucial for further ontogenesis.

The divergence in methylation between species can be caused by differences in gene sequences or by sequence-independent mechanisms such as environmental factors or stochastic events. Different environmental factors are always present in the studies on non-model organisms, therefore the methylation can be altered by them. To bypass this problem, Hernando-Herraez et al. (2015) studied the differences in methylation on regions of incomplete lineage sorting (ILS). These regions happen to have higher similarity between species that are not the most closely related species generally. The results of these studies show the correlation of changes in gene sequence of ILS areas and changes in methylation. The methylation patterns appear to show the ILS as well. Further hypermethylated DNA showed concrete patterns of histone PTMs profiles that were different from those identified within hypomethylated DNA. DMRs were located distally to TSS with high abundance. It can be suggested that species specific DNA methylation patterns and the change in underlying DNA sequence are closely related phenomena. Human DMRs appear to be tissue specific, therefore it can be deduced that these are established during development. Interestingly DMRs associated with neuronal genes were found in human blood. Hernando-Herraez (2015) suggest changes in methylation to follow specific DNA sequences. Also, they found that hypermethylation at CpG sites is frequently coupled with rapid change in DNA sequence inthe

neighborhood of these CpG sites, it could be suggested that a loss of function of these regions is followed by accumulation of mutations. However, Hernando-Herraez et al. (2015) claim that establishment of specific histone modifications contradicts this 'loss-of-function-followed-by-accumulated-mutations' concept. They suggest these are rather complex regulatory epigenetic processes of co-opting DNA methylation, histone PTMs and underlying DNA sequence (Hernando-Herraez et al., 2015).

4.4. Histone modifications and the histone code

DNA in eukaryotes is organized together with core proteins into a complex structure called chromatin. The structure has a DNA protective function, but more importantly regulates the accessibility of genomic sequences, affecting replication, transcription or DNA repair. A recognizable unit of chromatin is a nucleosome consisting of 146 DNA base pairs organized into a superhelix around a histone octamer (Luger et al., 1997). An octamer contains two copies of histones H2A, H2B, H3 and H4. Nucleosomes follow one each other linked by 10-70 bp long segments of DNA that is called 'DNA-linker'. H1 histone is not a part of nucleosome but keeps linker DNA in a position that stabilizes the structure.

Nucleosome assembly creates a significant barrier for enzymes requiring access to DNA. Folding or unfolding of nucleosomal arrays takes a meaningful part in regulation of nuclear processes (Woodcock and Ghosh, 2010). The regulation of nucleosome structure is possible by two known means. One is through histone PTMs and the other is with ATP-dependent chromatin remodeling enzymes (Cosma et al., 1999; Clapier and Cairns, 2009). Histone PTMs remodulate chromatin folding (Shogren-Knaak et al., 2006). The other point is that certain histone modifications can mediate binding of regulatory proteins (Patel and Wang, 2013).

Chromatin is a highly responsive structure; it can react due to diverse external stimuli in the sense of regulating gene expression. One of the principles responsible for regulation of chromatin is through histone modifications (Bannister and Kouzarides, 2011). The histone parts, that are usually post-translationally modified, are N-terminal tails which can be altered by methylation, acetylation, phosphorylation, sumoylation or ubiquitination and others (Allfrey et al., 1964). Histone PTMs moderate chromatin state and also recruit ATP-dependent chromatin remodeling enzymes. Regulation of chromatin structure within histone modifications is highly complex, the way how they affect the chromatin setting differs according to the type of specific modification, its placement and the combination of neighboring modifications.

Acetylation of lysine residues on N-terminal tails was a first type of PTMs that has been identified in 1960´ (Allfrey et al., 1964). This is one of the most widespread modifications of

histones. Acetylation of lysine residues is believed to have activating potential in general. Acetyl moiety, being slightly negatively charged, decreases interaction of N-terminal tails with negatively charged phosphate groups of nucleic acid, loosening the structure of chromatin (Perry and Chalkley, 1982). Chromatin transformed into more relaxed structure decreases access to DNA and enables transcription. Acetylation is catalysed by the enzyme histone acetyltransferase (HAT) (Roth et al., 2001) and removed by histone deacetylase (HDAC) (Holbert and Marmorstein, 2005). Acetylation of lysine residues is mostly located to histone 3 (H3) or histone 4 (H4).

Methylation of histones appears to have multiple roles in epigenetic memory of activation and gene silencing (Peters and Schübeler, 2005). Methylation of arginine and lysine residues is catalyzed by lysine histone methyltransferases (HKMT) and histone demethylases (Shi et al., 2004; Smith and Denu, 2009; Rudolph et al., 2013).

Histone methylation acts as enhancing transcription or as suppressing it, depending on which effector protein is being recruited. Most usually, methylated residues are on N-terminal tails of H3 and H4. Types of histone methylation that usually lead to gene silencing are methylation of Lys9 on H3 (H3K9), H3K27 and H4K20. There are three types of histone methylation that typically lead to gene activation: methylation of Lys4 on H3 (H3K4), H3K36 and H3K79 (more examples are in The Mammals Histone Modification Table; Attachments). Unmethylated lysine 4 at H3 interacts with non-enzymatic regulatory factor Dnmt3L, which induces *de novo* methylation by the triggering or activation of Dnmt3A2. When the lysine 4 of H3 is methylated, this methylation-activating interaction is canceled (Ooi et al., 2007). Trimethylation of lysine 4 on histone 3 (H3me3K4) is found to be associated with promoter regions of active genes, and monomethylation of H3me1K4 is associated with enhancers.

Histone phosphorylation takes place on serines, threonines and tyrosines and is controlled by enzymes kinases and phosphatases that regulate the distribution of this modification. The proper mechanism by which histone phosphorylation affects the state of chromatin remains unknown, even though it is believed that histone phosphorylation plays a direct role in mitosis, cell death, repair, replication and recombination (Oki et al., 2007).

Ubiquitination, an addition of the protein ubiquitin to lysine, plays a role in regulating a variety of fundamental cellular processes, as protein degradation, gene transcription, DNA repair and replication, intracellular trafficking and virus particle budding are (Chen et al., 2015).

Sumoylation is a PTM that modifies lysine by attachment of small ubiquitin-like modifier (SUMO protein), this PTM acts within various nuclear and cytoplasmic processes and is known to act as repressing transcription (Nathan et al., 2006). Interestingly, sumoylation of Argonaute2 regulates process of RNA interference (RNAi) (Josa-Prado et al., 2015).

There are four classes of **ADP-ribosylation**: mono-ADP-ribosylation, poly-ADP-ribosylation, ADP-ribose cyclization, and formation of *O*-acetyl-ADP-ribose. ADP-rybosilation plays a role in intracellular signaling, transcriptional regulation, DNA repair pathways and maintenance of genomic stability, telomere dynamics, cell differentiation and proliferation, necrosis and apoptosis (Hassa et al., 2006; Hottiger, 2015).

Other **acylations** were found recently – crotonylation (Tan et al., 2011), 2-hydroxyisobutyrilation (Dai et al., 2014), propionylation, butyrylation (Chen et al., 2007), succinylation and malonylation (Xie et al., 2012). Those acylations appear to be mostly linked with TSS and gene activation. And differ from acetylation considering their properties related with their affinity to specific enzymes and persistence. However, proper function and regulation of these (as well as others) PTMs remains unclear. It has been proposed that crotonylation plays a role during spermatogenesis (Montellier et al., 2012), a highly specialized cell differentiation. For further information on acylations see Rousseaux and Khochbin (2015).

Histone PTMs appear to have a crucial functional role in chromatin remodeling and regulation of gene transcription. The effect of PTMs seems to have combinatorial basis as seen for example on the methylation above. The place, whether promotor or gene region; the amount of marks, whether mono-, di-, or trimethylation or the combination of marks; all these factors can be decisive in chromatin remodeling. In contrast to hyperacetylation, which operates basically by changing the charge of the specific molecular segment and hence changing the chemical properties of the area, other histone PTMs appear to have a code-like nature. A hypothesis of ´histone code´ has been designed (Jenuwein and Allis, 2001). It refers to genetic code and the informational potential of the DNA molecule that is extended thanks to the informational potential of combinatory histone PTMs patterns. This hypothesis proposes a histone epigenetic marking system to be a fundamental regulatory mechanism that has an impact on most chromatin-templated processes, referring to consequences for cell fate decisions.

There are plenty of histone PTMs that have been found recently (Li and Li, 2015), yet to recognize the enzymes that read them, erase them and write them, is still a matter of time. The meaning of all described histone PTMs is still left undiscovered; however, their presence appears to have a substantial impact on cellular fate. In the late haploid stage of spermatogenesis, histones are replaced by proteins called protamines: these proteins are considered crucial for sperm head condensation and stabilization of DNA (further described in chapter 5.1.).

Compared with DNA methylation with relatively low cellular level of DNA methyltransferases, histone PTMs tend to be more reversible, reacting on the actual cell state. Histone PTMs therefore seem to operate in reaction to the immediate change of conditions, such

as the dietary change (Vahid et al., 2015), whereas DNA methylation is more likely to guide the cell's differentiation and act within cellular heritability.

Chromatin structure is packaged into domains that are differently accessible for transcription. The chromatin domains are induced or formed due to the state of epigenetic modifications and it is considered that the whole chromatin structure is inherited within cellular lineage even though not all epigenetic modifications are transmitted to the next cellular generation. Are heterochromatin domains steadily conserved even after the original inducing stimulus faded away? Carone and Rando (2012) argue that this depends on the length of time that the chromatin has spent in a particular state. They make this assumption on the basis of experimental studies of chromatin structure performed by Hathaway et al. (2012), in which they present a model technique of induction of chromatin state through DNA binding domains without any environmental stimuli. The inducing domain leads to a concrete histone modification in the chromatin area and it has been observed that the histone PTMs were spreading over the surrounding regions, resulting in a transcriptional silent state. However, as mentioned before, the conservation of chromatin state appears to be dependent on time the inducing stimulus was present. When the recruiting stimulus was washed out after seven days, the cells were able to restore the transcriptionally active state they had maintained before. The different results appeared when the inducing stimulus was present for 4.5 weeks: the cells were not able to restore transcriptionally active state of chromatin. Furthermore, establishment of DNA methylation was observed within those cells. These findings point to an assumption that when the time of chromatin affecting stimuli persists for so long that DNA methylation is established, the chromatin state persists even after disappearance of the inducing stimulus, keeping the cell in the induced chromatin state, not able to restore the previous setting (Carone and Rando, 2012).

Histone PTMs also appear to play a role in the regulation of anti-sense transcription (Lavender et al., 2016). The function of anti-sense transcription is not yet fully understood; however, it often results in short-lived non-coding RNA transcripts – another form of epigenetic regulatory molecules.

4.5. Regulatory RNAs

Diverse classes of RNA, ranging from small non-coding RNAs (sncRNAs) to long non-coding RNAs (lncRNAs), have been identified to control genome-related processes. Non-coding RNAs (ncRNAs; Bakel and Hughes, 2009) are basically those that are not translated into protein, e. g. it is all RNAs except mRNA. There is tRNA and rRNA with a very well described and understood function. The ncRNAs appear to have a regulatory cellular function. When nucleolar RNA

production is impaired, disorganized chromatin regions are observed (Nickerson et al., 1989).

RNA history goes back to 1975, when it has been discovered that purified chromatin contained twice as much RNA as DNA, which led to supposition that RNA may constitute a structural chromatin component (Paul and Duerksen, 1975). Particular functional RNAs were discovered later on. Further Ribonuclease P (Rnase P) was discovered in 1978 (Stark et al., 1978), which is a ribozyme with rnase activity. Rnase P is known to have a function in tRNA processing 5 ´leader of precursor tRNA (Altman, 2000). A novel function of Rnase P has been discovered in 2000: Rnase P being essential for transcription of other small noncoding RNA genes and also acting as a transcription factor for polymerase III (Jarrous and Reiner, 2007). In 1982 signal recognition particle RNA (7sL) – the RNA component of signal recognition particle (a ribonucleoprotein that is known to target proteins to endoplasmatic reticulum in eukaryotes) was found (Walter and Blobel, 1982). The ncRNAs appear to have a regulatory cellular function. When nucleolar RNA production is impaired, disorganized chromatin regions are observed (Nickerson et al., 1989). In 1992 a mouse *Xist* gene product was identified as an X chromosome specific untranslated transcript, it has its chromatin regulatory function in X chromosome silencing of placental mammals (Brockdorff et al., 1992). The *Xist* gene is considered to be a pseudogene that evolved from previously active protein-coding gene (Duret et al., 2006). There followed other ncRNAs discoveries, pointing to chromatin regulatory functions (Quinodoz and Guttman, 2014).

4.5.1. lncRNAs

lncRNAs are more than 200 nucleotide long ncRNAs that are not translated into protein (Quinn and Chang, 2016). **lncRNAs** have been divided into several classes based on anatomical properties of their gene loci. Such classes are: antisense RNA (aRNA/asRNA) are complementary to mRNA, they overlap protein coding genes and may inhibit translation; intronic RNAs that are encoded within intronic sequences of protein-coding genes; overlapping transcripts – these are lncRNAs that overlap protein-coding genes and large intergenic RNAs (lincRNAs) that are encoded within intergenic sequences. These classes of lncRNAs may share some of their functional roles (Rinn and Chang, 2012).

Xist (Engreitz et al., 2013) or Neat1 (Mao et al., 2011) RNAs have a function in chromatin structure control. Neat1 is considered to have a function in paraspeckle (a dynamic chromatin structure nuclear domain probably funstioning within cell cycle regulation) assembly and maintenance (Mao et al., 2011). Even though lncRNAs expression is less abundant than expression of protein-coding genes, it appears to be more tissue-specific (Derrien et al., 2012; Ghosal et al., 2013). lncRNA have been identified to have key roles in the control of pluripotency in embryonic

cells and cell differentiation (Guttman et al. 2011; Ghosal et al. 2013). lncRNAs have activating or repressing effect on gene expression and its regulation by divers mechanisms, including their role of competitive endogenous RNAs (ceRNAs) (Ghosal et al. 2013). Some lncRNAs function as precursors for miRNAs and piRNAs (Derrien et al., 2012). lncRNAs also mediate activation or repression of genes through histone methylation. Some lncRNAs are related to the regulation of cellular cycle (Hung et al., 2011). Large intergenic non-coding (lincRNAs) are lncRNAs that do not overlap with coding transcripts (Ghosal et al., 2013), these are commonly regarded as byproducts of background transcription. Low abundance and poor evolutionary conservation of lincRNAs, in comparison to sncRNA classes, underlays this assumption. Some of the lincRNAs are conserved for over 100 millions of years and therefore it seems more likely that they are functional (Chernikova et al., 2016). lncRNAs play a role in genomic imprinting, processes coupled with chromatin modifications and regulation of transcription (Mercer et al., 2009).

4.5.2. sncRNAs

As the function of lncRNAs is still a matter of current discussion, it is known that **sncRNAs** have well defined various functional roles. There has been described several classes and subclasses o sncRNAs, according to structure, function and context.

Snc-RNAs, sometimes referred to as small silencing RNAs, are expressed in the nucleus and function in epigenetic regulation of gene expression (Ghildiyal and Zamore, 2009). sncRNAs have specificity for DNA and mRNA. Several classes of sncRNAs have been described – **microRNAs (miRNAs), short interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs)** and others. One of the most significant mechanisms of controlling gene expression is **RNA interference (RNAi)**, a process in which miRNAs or siRNAs inhibit gene expression of certain genes based on complementary binding. Double-stranded siRNA or miRNA is unwound in single-strand RNA (ssRNA). One of the strands is randomly chosen by RNA-induced silencing complex (RISC) and bound to it. On the base of complementarity of the bound ssRNA, RISC complex is targeted to mRNA and destroys it by Argonaute protein, the catalytic component of RISC. By mRNA cleavage transcription is enabled. It is considered that there is one type of RISC complex and the modulation of function is caused by the sequence of RNA captured by the complex. The function of complex may be defined by the nature of pairing between sncRNA and mRNA (Liu et al., 2004).

miRNAs were the first class of sncRNA to be discovered (Lee et al., 1993): these are ~ 22 nucleotide long hairpin RNAs with imperfect complementarity to multiple targets, thousands of genes (Lewis et al., 2005). There are about 1000 miRNAs encoded in the human genome (Bentwich et al., 2005). miRNA silence mRNA (Guo et al., 2010) or non-coding transcripts trough

complementary base-pairing. One miRNA can control multiple mRNAs just as well as one mRNA can be a target for multiple miRNAs (Friedman et al., 2009). miRNAs are known to play a key role in cell development and differentiation (Cuellar and McManus, 2005). Levels of miRNA can be affected by the presence of competitive endogenous RNAs (ceRNAs) that are complementary to single miRNA or multiple miRNAs (Tay et al., 2014). Pseudogenes, long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs) were discovered to act as ceRNAs also known as miRNA "sponges". These RNA molecules interact creating a regulatory net (Sen et al., 2014). All the components of this ceRNA net can directly or indirectly affect each other: a small perturbation in the concentration of one component can have significant impact on the cellular state. miRNAs are generally highly conserved within species (Bentwich et al., 2005).

In contrast to miRNA, siRNA have perfect compatibility to targets (McManus and Sharp, 2002). These double-stranded 20-25 bp long molecules are known to have multiple functions, one of the functions is gene silencing through RNAi pathway and RNAi related processes. siRNAs also act as an antiviral mechanism or in shaping the chromatin structure. Exogenous dsRNA may be cleaved by an enzyme Dicer into siRNA molecules.

piRNAs are known to guide piwi-class of Argonaute proteins in germ cells (Aravin et al., 2006) and thus regulate silence transposon activity (Aravin et al., 2007). The activity in germline makes them remarkable candidates for TEI (Szyf, 2015). Within mammals, a class of piRNAs that are related to non-transposonal sequences were found (Ghildiyal and Zamore, 2009). piRNAs and siRNAs are involved in chromatin remodelling, transposon regulation, developmental gene regulation and in genome stability maintenance (Castel and Martienssen, 2013).

Furthermore piRNA and siRNA were identified to target specific loci for histone and DNA methylation (Volpe and Martienssen, 2011). Additionally, piRNA pathway was found to be crucial in de novo DNA methylation of one particular DMR within imprinting in mouse testes, not in other DMRs. This points to a function in sequence specific de novo methylation (Watanabe et al., 2011).

Even though all the processes through which ncRNAs operate have not been described yet, it has been known that ncRNAs affect gene expression in various ways. sncRNAs have an ability to be released from the cell of their origin and they may be systematically distributed (Mitchell et al., 2008; Creemers et al., 2012). sncRNAs may act in sequence specific manner and modulate other epigenetic processes. According to these properties the sncRNAs are best candidates for TEI (Szyf, 2015).

Noncoding transcription may open chromatin for transcription factors or further ncRNAs may compete with transcription factors (Hung et al., 2011). It has been proposed by some scientific groups that as long as we are not aware of the particular functions of highly abundant

diverse RNAs we should regard them as ‘junk’ (Palazzo and Lee, 2015). The current discussion on lncRNA function consults the issue of functionality vs. biochemical activity. Palazzo and Lee proclaim that considering every biochemical activity to be functional has an aspect of hyperadaptionism ideology. The biochemical activity has been put together with function by project ENCODE consortium (ENCODE Project Consortium, 2012). However, the deffinition of functionality appears to be the core of the problem.

4.6 Epigenetic modifications in metaphors:

Epigenetic modifications form another level of information that is not written in the DNA sequence. Cells operate dynamically in immediate reaction to extrinsic and interior signals, via the epigenetic control of processes of DNA usage by chromatin remodeling. Epigenetic processes influence cellular state through various ways. Epigenetic modifications formulate fundamental processes by which genome information is organized, adapted and interpreted (Tomizawa et al., 2012). They are a medium of cellular memory within an organism. Memory is constituted, based on events in time, constructing a backdrop for future processes; as such, memory can be gained, restored, altered, deleted or transmitted. These are the features of epigenetic modifications within organisms.

As I will demonstrate epigenetic modifications are also transmitted to next cellular generations. Besides regulation of cellular processes reactive to external environment, they form one of the basics of cellular differentiation and maintenance. Epigenetic processes enable to define cellular lineage identity and act as a highly important principle of development, organism plasticity and adaptability.

It seems that the most stable and heritable of epigenetic modifications is methylation of cytosine. The other epigenetic modifications function in a manner that is more reactive to recent actual environmental changes and cellular settings. ncRNAs and PTMs may change the methylation state. On the other hand, methylation alters the susceptibility to the establishment of the other epigenetic modifications. However, none of the epigenetic modifications can be considered apart from the other modifications, for the epigenetic state of a cell is a result of an overall co-option of all the modifications. Specific modifications influence the setting of the other modifications, resulting in a concrete chromatin state with certain permeability for internal or external signals.

Histone code is a methaphor. Barbieri explains any code to be a connection of two independent worlds. Due to his concept the two independent worlds DNA (RNA) and protein are concatenated through genetic code. Barbieris´code metaphor was assumed on the basis of our cultural experience with morse and is set on basis of structural simmilarity, empirically experienced

through our language structures (Švorcová, 2007). Nevertheless, the code metaphor might be applied to the histone PTMs which may grow on histones and together form a concrete pattern that might be understood by the reading enzymes that may eventually result in various cellular responses in terms of chromatin remodeling, genome use or other actions. An important think to denote is that such a histone code might be understand differently by different classes of readers-proteins which may lead to various cellular responses. One histone pattern might result in a several possible interpretations in dependency on the reader-enzyme molecule type and in dependence on the epigenetic state of neighboring regions. Such processes form the cellular fate that eventually affects cellular differentiation, during development the affection might result in altered organ morphology.

It makes a lot of sense that any alteration that gets involved into finely tuned and precisely settled ontogenesis may have a significant effect on the future fate of an individual. We can argue that cell fate is epigenetically defined based on the history of the cell. Cell lineages are defined by their own cellular history and by intracellular signaling. In a developing embryo, there are cells undergoing distinctive differentiations that give rise to subsequent cellular populations, tissues and organs. In these crucial moments, their fate may be fatally affected.

5. Germline

There are several manners in which an epigenetic trait can be transferred across generations. We should distinguish between parental effect and TEI through gametes. Further we can distinguish between in utero programming of F1 embryo and between life experience of an individual, that may result in an epigenetic transmission to the next generation. In case the inducing effect was present during the development of an embryo, it is the embryo itself that has been affected. When we consider TEI, we need to be aware when the affection of the germinal cells occurred. If the inducing effect acts during in utero development, there can be three generations exposed at once – mother, the embryo and the germ-cells of the embryo. The embryo and its gametes may acquire a change in epigenetic setting at once, induced by the same signal (phenomenon called parallel induction – already Weismann considered such possibility, Jablonka, Raz 2009). When an individual is exposed to the inducing signal later in life, his gametes may be influenced too, however these are only two generations. Considering the male line, only two generations are enough to observe the transgenerational epigenetic effect. If we consider female, we need to be aware if the inducing stimulus was present during pregnancy or not (Szyf, 2015). We can identify the actual transgenerational epigenetic transmission, which is unique, for the transmission happens truly by the germinal line without being solely an effect of environment.

Epigenetic state of sperm and oocyte is divergent from the one of somatic cells. Germ cells differ due to their purpose, lifestyle and status of highly specialized and phenotypically distinct cells. Once germ cells separate from the other somatic cells of an embryo, they keep their persistent state epigenetically defined. Germ cells are epigenetically programmed so that somatic fate is contradicted, and are strictly kept in their specific state. Extensive erasure of epigenetic modifications and activation of specific genes is required in sex-dependent manner (Leseva et al., 2015). It is essential for sperm and oocyte to promote totipotent cell state after fertilization. The reprogramming of parental epigenome after fertilization follows to retain totipotency and proper abilities for forthcoming development.

Zygote is the only totipotent cell of the embryo, because it is the only one that gives rise to all the other cells (Smallwood and Kelsey, 2012). Male and female gametes dispose of their own epigenetic landscapes formed by their history. The erasure of certain epigenetic settings during the process of epigenetic reprogramming is needed for totipotency and proper development; however, another setting of epigenetic landscapes is crucial for developing cell types, tissues and morphological bodily structures.

Sperm and oocyte are cells with overall hypermethylated genomes. Once fertilization happens, extensive reprogramming consisting of targeted demethylation and reorganization begins. The genome of the sperm is demethylated actively 6-8 hours after fertilization, before the onset of DNA replication, whereas the genome of the egg is gradually demethylated after several cleavage divisions by lack of maintenance methylation (Mayer et al., 2000; Oswald et al., 2000). This active demethylation of the paternal epigenome may be considered partly as a chromatin-remodeling process and also as a crucial step for the establishment of parent-specific developmental programs (Mayer et al., 2000).

5.1. Sperm

Sperm is a highly differentiated haploid cell with an epigenetic state dramatically different from all other cells. When sperm meets oocyte, fertilization happens. This very specific function requires epigenetic setting different from other cells. During the process of spermatogenesis, haploid round spermatids undergo crucial chromatin reorganization. The majority of sperm histones (90% in humans, 95% in mice) are evicted (Luense et al., 2016) and replaced, first by transition proteins and then by specific small arginine-rich proteins, protamines. These small proteins enable tighter compaction of chromatin which is essential for chromatin to fit into the small head of sperm and also lowers the mutability (Bao and Bedford, 2016).

The concrete transcription factors have been described that are crucial for the unique process of spermatogenesis. Nevertheless, co-opting epigenetic processes are fundamental for the process: there exists an intriguing hypothesis that histone PTMs could play a role in the epigenetic regulation of the embryo (Luense et al., 2016). There are special histone variants located in testes that were not found in somatic cells (Henikoff and Smith, 2015; Bao and Bedford, 2016). Testes-specific histones have special PTMs that trigger transcriptional factors on the base of histone code (Bao and Bedford, 2016). The histone PTMs have also a major function during the protamine replacement.

In men with normal spermatogenesis, the typical epigenetic state of sperm is observed: it includes localization of retained histones with bivalent histone modifications and hypomethylation of DNA; within those who show abnormal spermatogenesis or unexplained altered embryogenesis through in vitro fertilization (IVF), abnormal epigenetic states were registered (Carrell, 2012). Even though the very concrete processes are not precisely known yet, for they seem to be markedly complex, we can assume it as rather apparent that it is the very epigenetic state of the cell that identifies with the distinctively special cell type. Experimental study of human histone PTMs shows largely uniform histone PTMs signature in discrete individuals (Luense et al., 2016).

Not only are certain parts of histones with PTMs retained in sperm, even protamines were found to have their distinct PTMs (Brunner et al., 2014). Protamines also play a crucial role in male fertility and therefore it is considered that protamine sequence is shaped by postcopulatory sexual selection in mammals (Lüke et al., 2016). Sperm cells also contain divergent classes of snRNAs (Holt et al., 2016) that may be other candidates of transgenerational transmission. RNA present in seminal fluid together with a wide range of other molecules may affect the development of future embryo as well (Crean et al., 2016).

5.2. Oocyte

Oocytes also show differences in epigenetic landscape and their expression profiles like methylation of histones (Manosalva and González, 2009; Shao et al., 2015) and histone deacetylations differ according to the age of mother (Berg et al., 2011). Stillbirth and fetal malformations are higher in older mothers and with IVF (Ge et al., 2015) and the birth rate is lower in older female humans and mice.

During meiosis MI and MII, histone acetylations undergo deletion by histone deacetylase HDAC, which is crucial for the viability of embryo (Akiyama et al., 2006). Aneuploidy was caused in cells that did not undergo proper deacetylation, suggesting that histone acetylation is involved in chromosome distribution during meiosis. Function of deacetylase appears to decrease with time in mice (Akiyama et al., 2006).

Mammalian gametes undergo global epigenetic reprogramming at two crucial points – one is during their own differentiation within a developing embryo, when they divide from somatic cells, the second is the global deletion in the preimplantation of the embryo. During early stages of development (the day 8-9 in mice), primordial germ cells are globally demethylated. Certain areas – these areas tend to differ in between divergent species – escape these demethylations: intracisternal A-particles, LTR-ERV1 retroelements and single-copy sequences in mice; these areas escape global methylation erasure even later in the preimplantation of the embryo (Guibert et al., 2012). DNA metyhlation is re-established during maturation of the oocyte after birth.

There are two means by which demethylation can happen: passively, by lacking or insufficient of methylation on a nascent strand, and within subsequent cell divisions the methylation state is progressively diluted from the cell population over time; or actively, by oxidative conversion of 5-methylcytosine to 5-hydroxymethylcytosine and further derivatives, which is associated with enzyme ten-eleven translocation (Tet) family activity (Ficz et al., 2011). However, it seems that the process of active demethylation is Tet independent. Tet only prevents de novo DNA methylation activity in later zygotic stages (Messerschmidt, 2016).

In oocyte, epigenetic marks that define somatic cells are deleted in the early germ line stages during development, as mentioned earlier. It is unknown whether there is any other active turnover in DNA methylation in further specialization of oocytes (Tomizawa et al., 2012). However, the onset of a gradually emerging methylation profile, that the resultant oocyte will dispose of, begins. What methylation patterns are set is important, they can influence how genes are expressed in the oocyte and therefore the future of the developing embryo. This obviously happens within imprinted genes that fundamentally escape methylation erasure (Ferguson-Smith 2011).

5.3. Imprinted genes

Genomic imprinting is necessary for proper development in mammals. It is one of the first reported examples of the fact that epigenetic information can be transmitted across generations through germline (Ferguson-Smith, 2011). A small unique class of genes undergoes imprinting, in humans it is represented by only about 100 genes. In imprinted genes, usually only one allele is being expressed, which is epigenetically controlled by methylation of maternal or paternal variants of control regions of imprinted genes. The monoallelic methylation has its origin in the gametes and persists over the whole development and future life as most of the imprinted genes are methylated in the oocyte (Tomizawa et al., 2012). DNA methylation within imprinted genes is maintained during the whole development and persists through the life of the offspring. Some of the imprinted genes escape global deletion, whereas several methylated imprinted genes gain their methylation statuses later gradually during postnatal oocyte growth (Lucifero et al., 2004).

De-novo methylation is specific to some concrete genes and facultative to others. What concrete process is responsible for targeted methylation remains generally unclear; Lucifero et al., (2004) suggest that the accumulation of some kind of a regulatory molecule is needed, assuming so because the imprint establishment is related to oocyte diameter.

Some of the methylation statuses are crucial for the viability of the embryo. In case of using In Vitro Fertilization (IVF), the methylation status may be associated with human imprinting disorders (Cox et al., 2002; DeBaun et al., 2003; Gicquel et al., 2003). This can be caused by insufficient maternal imprinting in a developing oocyte or embryo (Cox et al., 2002). During implantation, the observed epigenetic switch is targeted to repress germline expression program (Borgel et al., 2010). De novo methylation in embryo is provided by Dnmt3b (Borgel et al., 2010).

Enzymes Dnmt3a and Dnmt3b methyltransferases together with a non-enzymatic protein are involved in de novo methylation of a mouse embryo (Hata et al., 2002). Dnmt3l does not dispose of enzymatic activity; it is postulated to be a regulator of maternal imprinting establishment in growing oocytes, not in primary oocytes (Lucifero et al., 2004). Dnmt3l may serve as a recruitment factor for methyltransferases, as Dnmt3a is, to DMRs of imprinted genes and direct methylation to these sites (Lucifero et al., 2004). It is considered a possibility that Dnmt3a in a complex with Dnmt3l respond to histone modification status of associated chromatin which is permissive for the methyltransferase complex (Tomizawa et al., 2012); Dnmt3l interacts with amino acid tail of H3 if H4 is not methylated (Ooi et al., 2007). It is considered a possibility that transcription events dictate the placement of histone modifications, and a result is the triggering of methylation enzymes (Tomizawa et al., 2012).

As an example of a maternally imprinted gene to which methylation is triggered by Dnmt3l, may serve the *SNRPN* (Small Nuclear Ribonucleoprotein Polypeptide N) gene. The centre of methylation is located to its 5´UTR area of the gene. *SNRPN* is a gene responsible for one of the polypeptides of a small nuclear ribonucleoprotein (snRNP) complex that is considered to be involved in pre-m-RNA processing, most notably in tissue specific alternative splicing events. The protein snRNP SMB/SMN family, to which snRPN belongs, is a family of proteins that are capable of recognizing specific nucleic acid sequences through RNA-RNA base pairing. The specific role of *SNRPN* is unknown, nevertheless if the gene is mutated or an imprinting failure of this gene occurs (and this is often observed in case of IVF), Angelman syndrome appears. Patients with systemic lupus erythematosus have autoantibodies against snRNPs. *SNRPN* gene protein product has itself two alternative-splicing forms. Various metabolic, morphological and physiological phenotypes in divergent tissues are linked with the miss-function of the gene or within its impaired imprinting.

5.4. Developmental epigenetic reprogramming

In early mammalian embryos, the epigenetic information undergoes reprogramming on a genome-wide scale (Reik et al., 2001; Smith et al., 2012). The reprogramming happens on the DNA-methylation level and on histone-modification level as well. During embryogenesis, deletion of DNA methylation patterns in early stages, and de novo methylation in the course of further development are required for proper ontogenesis (Okano et al., 1999).

A major epigenetic switch happens during implantation into the endometrial wall (Borgel et al., 2010). De-novo DNA methylation catalyzed by Dnmt3b represses the expression programs of the germline (Borgel et al., 2010). In the epiblast, DNA methylation is targeted to lineage-specific genes. The lineage specific genes are subsequently demethylated during terminal differentiation. Borgel et al., (2010) also identified non-imprinted genes that inherit promoter DNA methylation from parental gametes, which supports the hypothesis that certain parts of mouse genome may escape general DNA methylation reprogramming.

Later another general methylation reprogramming in mice appears in primordial germ cells (PGCs) (Lees-Murdock and Walsh, 2008): parental epimarks are erased and gender-specific imprinting patterns are established (Seisenberger et al., 2012). All the processes that are particularly involved are unknown so far; however, these are at least TET3 mediated oxydation of 5mC (Gu et al., 2011; Iqbal et al., 2011), ss-DNA breaks and also the activation of base-excision repair (Hajkova et al., 2010). These processes lead to open chromatin state of inner-cell mass (ICM), which is crucial for ICM pluripotency. The deletion of specific epigenetic marks is needed equally as a presence of certain epigenetic signaling during development (Sasaki and Matsui,

2008). Concrete deletions and markings are crucial in determining of the cell fate. This happens during the whole life, both in the somatic and the germinal line. However, in germ cells and during embryogenesis, the concrete programming is of a great significance. Epigenetic programming is involved in complex developmental processes and affects the whole organism, hence morphological or physiological changes may be observed on various levels. For example, sexual development and sex-specific ontogeny can be canalized by epigenetic marking established upon environmental stimuli. Furthermore, such epimarks can be partly transmitted through several generations, being sexually antagonistic. Such epimarks are beneficial for the individual in which they are established, but may be harmful for ontogenesis of the opposite sex when not deleted in the next generation (Rice et al., 2016).

"Upon union of these gametes, reprogramming of the new organism's epigenome is initiated, which eventually leads, through pluripotent cells, to the cell lineages required for proper embryonic development to a sexually mature adult. This never-ending cycle of birth and rebirth is accomplished through methylation and demethylation of specific genomic sites within the gametes and pluripotent cells of an organism." (Leseva et al., 2015)

6 Review of studies on trans-generational epigenetic inheritance in mammals

There has been growing evidence for certain characteristics to be transmitted across several generations from parents to offspring without a change in DNA sequence, e. g. the transmission seems to be mediated by epigenetic means. The transmitted characteristics appear to be affected mostly by parental lifestyle – diet, stress, infections or toxins. An assumption has even been made that some DNA methylation patterns could depend on the season of birth, resulting in different predisposition to allergies (Lockett et al., 2016). Lately, even an involvement of epigenetic processes in the development of auto-inflammatory disease has been stated: DNA methylation appears to be reactive to immunological signaling and plays a role in inflammatory processes in monocytes, and furthermore, immunological treatment is able to alter methylation (Vento-Tormo et al., 2016). It can be another chapter for DNA methylation, however, transgenerational effect has not been registered or studied in this case.

Studies on humans are limited in this area; a few ´natural experiments´ or, one can say, historical disasters, on which we possess sufficient documentation, may serve as the most useful material for research in this field. One of the main clues is that these unique historical events have further biological effect (on cellular and molecular level) on subsequent generations. Unique data sources represent Avon longitudinal study of parents and children (ALSPAC) in UK (designed for studying early development) and Överkalix cohort studies: in this particular area of Sweden,

historical records including harvests, food prices and parish registers were well managed and reperesent a uniqe data source for analyzing trangenerational effects. The other possible data emerged due to historical disasters – on people who experienced Dutch famine due to the geo-political situation of World War II in 1944 or on holocaust survivors.

The results of the large studies vary in recognizing certain possible mechanisms that could be responsible for trans-generational inheritance. Nevertheless, there mostly appears a pattern of sex-specific transmission of a trait that was induced by a certain input from the environment, and some authors therefore consider pre-evolved transgenerational response mechanism to be responsible (Pembrey, 2010). The sex-specific transfer happens to manifest mainly when the exposure to the environmental input happened before puberty, which indicates the involvement of gamete reprogramming (Szyf, 2015). Similarly, within the Avon longitudinal study of parents and children that was focused on an effect of smoking, the result shows that the effect on the offspring's growth was observed only if parental smoking started before puberty (Pembrey, 2010): this also points to the developmental stage of germinal cells.

It has been observed that intrauterine and neonatal environment has long-term or even permanent impact on health and phenotype in the later life of an individual. Intrauterine nutrition, toxins and infections influence mechanisms of epigenetic programming during ontogenesis. Furthermore, it has been suggested that these processes can affect the phenotype of subsequent generations.

6.1 Nutrition

Intrauterine nutrition is affected by the transfer capacity of placenta, the mother's food intake during gestation, and her food intake in her earlier life, which includes even her own intrauterine development.

The Dutch famine in 1944-45 is a unique event that enables us to study intrauterine starvation in humans: malnutrition during gestation had divergent effects on developing individuals; depending on the phase of gestation in which they were exposed to it, different health disorders were listed (Painter et al., 2005). Besides damages of organs and tissues, higher risk of schizophrenia was observed within those who underwent the famine *in utero* (Hoek et al., 1998).

The cohort study by (Painter et al., 2005) showed that individuals exposed to famine during early gestation showed a higher occurrence of coronary heart disease, raised lipids, altered clotting and more frequent obesity. Those who experienced the famine in mid gestation had a higher rate of obstructive airways disease and microalbuminuria in further life, and in those who went through the famine in the late gestational phase, decreased glucose tolerance was observed (Ravelli et al.,

1998). When obesity was tested (Ravelli et al., 1976), the results varied according the period of gestation during which the individuals underwent the famine. Exposure to the famine in the late gestational phase and during an early stage of life led to a lower obesity rate in adulthood. This period of development is crucial for the establishment of adipose-tissue cellularity. On the contrary, when the developing embryo underwent the famine during the first half of pregnancy, a correlation with higher occurrence of obesity in adulthood was listed. In these stages of development, hypothalamic centers regulating food intake and growth are differentiated (Ravelli et al., 1976). The epigenetic setting in these crucial stages may alter the future metabolism-related phenotype.for in this period of pregnancy the embry is developing tissues and organs and therefore such an event might gravely affect health of the developing organism.

The studies analyzing data from the human history of famine are supported by experimental studies on animal models on which experiments with malnutrition during gestation were executed. As experiments with malnutrition in rats during gestation were performed, it was shown that fetal exposure to a maternal low-protein diet impairs nephrogenesis, and therefore promotes hypertension (Langley-Evans et al., 1999; Yuasa et al., 2016); it also has an effect on the development of endocrine pancreas (Snoeck et al., 1990).

Sheep that undergo malnutrition in the early gestational phase exhibit reduced pituitary and adrenal responsiveness in the late gestational phase (Hawkins et al., 1999). Which organs or functions are affected depends on the timing of intrauterine undernutrition.
Concerning diet, it is not only a poor food intake of the mother that can alter ontogenesis: on the contrary, maternal overnutrition increases the risk of obesity and diabetes in later life (Huypens et al., 2016).

As mentioned above, when we concern maternal heritability, it is often difficult to distinguish between an epigenetic path through oocytes, and the effect of intrauterine environment on a developing individual that can also alter epigenetic modifications. As mouse-model study by Watson and Rakoczy (2016) claimtries to bypass the problem it is possible to bypass the problem by using in vitro fertilization, and the results of numerous studies suggests that the susceptibility of the offspring to certain metabolic disorders can be transmitted through the sperm and oocyte path and the oocyte-path heredity appears to have a huger effect (Watson and Rakoczy, 2016). A study in mice using IVF approach in mice, shows that parental high-fat diet increases the risk of obesity and diabetes in offspring (Huypens et al., 2016). To exclude maternal and intrauternine effects and seminal fluid Huypens et al. used sperm and eggs from F1 generation that had been exposed to high fat diet (HFD) and fused these with germcells from naive mice during IVF, resultant embryos were implanted into naive female. As a result F1 generation showed

obese phenotypea and glucose intolerance in sex-dependent manner (see The Table of TEI; Attachments). Even though the hereditary process apparently goes through germline, nevertheless the concrete molecule responsible for that was not found within the experiment (Huypens et al., 2016).

Pandemic of obesity is a theme that has been often discussed recently. The considered causes are maternal obesity during pregnancy and other environmental effects (Loche and Ozanne, 2016). Maternal obesity during pregnancy is most likely to lead to changes in methylation patterns of genes involved in cardio metabolic pathways which raise the risk of cardiovascular diseases later in the life of offspring (Guénard et al., 2013). DNA methylation changes and snRNAs are also assumed to be linked with obesity for changes in these epigenetic profiles were found in werespermatozoa of extremely obese men (Donkin et al., 2016).

6.2 Drugs

Drugs have an impact on behaviour, involving an impact on brain regions that are connected to reward-seeking and craving, addiction, withdrawal (which causes anxiety), learning and memory. Drugs of abuse activate the mesolimbic dopamine system. It is hypothesized that drugs usurp brain systems that control behaviour needed for survival and species continuity (Koob and Volkow, 2010). Drugs of abuse change neural circuits involved in reward and stress systems which results in a motivation for taking drugs.

The changes in neural system that lead to memory formation are highly complex and not described in detail yet. However, it is assumed that regulation of transcription in neuronal and also glial cells plays a key role in the process. Epigenetic processes that engage histone and PTMs adjustment are involved. As an example histone variant H3.3 and histone deacetylase inhibitors may serve (Albensi and Djordjevic, 2016).

Cocaine induces neuroadaptations, by i.e. regulation of gene expression, by inducing specific histone-modification that are acute, not chronic (Kumar et al., 2005). However, what is supposed to cause a chronic epigenetic change is the addictive behaviour by repeated drug intake. All rewarding drugs or activities increase dopaminergic transmission from Ventral Tegmental Area (VTA) to Nucleus Accumbens (NAc), involving different neural pathways. Drugs of abuse differ in ways in which the brain is influenced; however, brain regions mediating the lasting nature of addictive phenotype are generally engaged (Robison and Nestler, 2011).

6.2.1 Alcohol

Alcohol-use disorder (AUD) has been observed to be heritable, but there are difficulties

finding underlying gene specificities, certain gene variants of enzymes related to alcohol metabolism (heritable variants of alcohol (ADH) and acetaldehyde (ALDH) dehydrogenase) are associated with higher occurrence of AUD. Polymorphisms deactivating ALDH2 that are found almost exclusively in Asian populations are associated with decreased risk of developing AUD, whereas ADH1 and ADH7 single-nucleotide polymorphisms in European and African populations are related to higher risk for developing AUD. ADH1 polymorphism has been modulates vulnerability of developing fetal alcohol syndrome disorders during pregnancy (Finegersh and Homanics, 2014). Alcohol has been shown to act as an epi-mutagen in several types of tissue, including germline; transmissible epigenetic change linked with alcohol abuse has been suggested (Finegersh et al., 2015).

As sons of alcoholics show significantly less sensitivity to alcohol than normal control subjects, the biological effect on the subsequent generation has been shown (Pollock, 1992). In children of alcoholics, a lower increase in body sway after ethanol consumption was listed (Schuckit, 1985; Lex et al., 1988).

Alcohol related patterns of behavior are difficult to link together with genetic variants. Mutations in single genes for enzymes of alcohol metabolism have been discovered in humans. Alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) inactivation has been found in Asian populations, leading to a decreased risk for developing AUD (Higuchi et al., 1995). Paternal exposure to alcohol induces a spectrum of morphological and cognitive deficits in offspring (Finegersh et al., 2015; Ledig et al., 1998). An increased risk of psychiatric disorders was documented in children of fathers with AUD. Studies performed on humans mostly come to include social and environmental factors that are associated with the effect of being raised by an alcoholic father (Ervin et al., 1984; Ozkaragoz et al., 1997).

Experimental studies in rodents bypass this problem by avoiding offspring rearing by ethanol influenced sires; hence some evidence for the transmission of acquired effects of ethanol on subsequent generations may be provided. Low birth weight was observed in offspring of male rats exposed to 9 weeks of alcohol consumption. This paternal alcohol exposure affected cytosine methyltransferase mRNA levels in sperm, which can indicate altered epigenetic programming or imprinting by reduced DNA methylation that would lead to normally silent parental alleles (Bielawski et al., 2002).When male mice were chronically exposed to ethanol vapour, their male offspring had reduced ethanol consumption and preference, enhanced sensitivity to the anxiolytic and motor-enhancing effects of ethanol, and increased Bdnf expression in VTA and germ-cells. Hypomethylation and changes in gene expression persist in the brains of the offspring and the offspring show decreased ethanol consumption and preference, and increased sensitivity to it

(Finegersh and Homanics, 2014). Exposure of male mice to alcohol induces an attention deficit hyperactivity disorder-like phenotype, and epigenetic dysregulation of dopamine transporter expression in brain and germ cells of the offspring (Kim et al., 2014).

Fetal alcohol-exposed rats showed impaired function of neurons containing proopiomelanocortin[1] (POMC) derived peptides that control stress, metabolism, the immune system and the brain reward system. They also showed increased methylation at CpG dinucleotides of the proximal part of the *Pomc* promoter, and altered histone modifying proteins levels and DNA methyltransferase levels in POMC neurons. *Pomc* gene methylation persisted in the F2 and F3 male germline, and in sperm of F1 males, not in females (Govorko et al., 2012). Hypomethylation of imprinted genes in offspring was induced by maternal exposure to alcohol (Stouder et al., 2011) and by paternal pre-conception alcohol abuse in mice (Knezovich and Ramsay, 2012).

The results in rodents are opposite to those found in humans, rodent offspring generally avoid alcohol, whereas in human susceptibility to alcohol abuse is higher within those whose fathers were alcoholics. This could be caused, on the one hand, by the differing conditions in experiments in rodents and casual human abuse, or, on the other hand, by activation of divergent neuronal circuits in these two species. However, the effect on behavior appears to be transmitted through an epigenetic pathway in rodents, in humans it is difficult to exclude cultural inheritance and the effects of environment.

Long term repeat transposons (LTRs) are regulatory transposable elements that are silenced by methylation and are considered to have a regulatory epigenetic function (Slotkin and Martienssen, 2007). LTRs show decreased methylation and decreased DNMT1 activity was listed after chronic alcohol use in humans (Ponomarev et al., 2012). An experimental study in humans reveals that demethylation of usually hypermethylated imprinted genes in sperm correlates with chronic alcohol use in males, the authors conclude that this could result in fetal alcohol spectrum disorders (FASD) (Ouko et al., 2009). Alcohol affects male fertility and semen quality in humans which indicates that germ cells are sensitive to alcohol exposure (Muthusami and Chinnaswamy, 2005).

6.2.2. Opioids

Morphin, heroin and other opiates are drugs that stimulate opioid receptors in the nervous system. Opioid receptors mediate rewarding and analgesic effects in the nervous system. Female

1Proopiomelanocortin (POMC) is a peptide precursor that gives rise by cleavage to several peptide hormones – α-MSH, ACTH, β-endorphin and Met-enkephalin (β-endorphin and Met-enkephalin are endogenous opioids).

offspring of mouse dams exposed to morphine during puberty showed anxiety-like behaviour and morphine sensitization in adulthood. Male offspring exhibited morphine sensitization (Byrnes, 2005). The behavioral alterations were observed even in the F1 and F2 generations of females, together with an upregulated kappa opioid receptor and dopamine D2 receptor gene in NAC, which points to a transgenerational effect (Byrnes et al., 2013). However, a particular epigenetic process involved in the transmission has not been described yet. It seems as if system critical for motivated behaviour was affected in F2 generation and transgenerational transmission was induced. Such transmission may be of epigenetic character.

Morphine is known to alter methylation (Sun et al., 2012) and acetylation of histones (Sheng et al., 2011; Wang et al., 2015) of genes that are involved in neural circuits connected with addictive behaviour. Furthermore, morphine directly induces DNA methylation by modulating cellular oxidative stress (Trivedi et al., 2014).

Concerning germ cells, opioid receptors were found to be present in sperm (Albrizio et al., 2006) and in oocyte (Agirregoitia et al., 2012).

6.2.3 Nicotine

Transgenerationally transmitted changes in cognition and dopamine were observed in offspring of nicotine-exposed parents. Offspring of F0 mothers exposed to nicotine during pregnancy showed an ADHD-like behavior in the F2 and F3 generations (Zhu et al., 2014).

Nicotine alters DNA methylation patterns of genes involved in the metabolism of dopamine: decreased methylation of monoamine oxidase A (MAOA) (Philibert et al., 2010), increase in DNMT1 and methylation of glutamate decarboxylase (Satta et al., 2008). Smoking in pregnancy is associated with the brain-derived neurotrophic factor-6 in adolescents (Toledo-Rodriguez et al., 2010).

6.3 Stress

There has been some evidence that stress in parental lineage may alter the behavioral status of the offspring. These relations observed in humans are excessively complex, making it difficult to distinguish whether they were transmitted biologically, or by social learning (i.e. behaviorally transmitted). Growing preclinical and epidemiological evidence supports the idea of transgenerational manifestation, through behavioral changes in offspring, induced by parental stress without parenting interactions. This leads to an assumption that cannot be easily overlooked – the effect most considerably happens biologically, through the germline. After a certain environmental input is given to one generation, the resulting phenotype may be transmitted to up

to three generations in a sex-dependent manner (Franklin et al., 2010).

6.3.3 Maternal care during early life stages

It has been documented that variations in maternal care during early life stages may affect the phenotype of the offspring, e.g. their stress reactivity; these traits may be transmitted behaviorally in a non-genetic manner (Francis et al., 1999). Stress during gestation alters maternal care in the early life stages and the development of the offspring in rats (Champagne and Meaney, 2006). This is the effect of environmental adversity transmitted across generations through a nongenomic mechanism, i.e. maternal care. Furthermore, maternal care also influences maternal behavior of female offspring, leading to a significant effect of parental care in the mediation of the effects of environment on neural development (Meaney, 2001).

Maternal stress during pregnancy in rats may be transmitted to further generations in a sex-dependent manner: through the maternal line, a change in HPA axis regulation and anxiety-like behavior is observed in the F2 generation (Grundwald and Brunton, 2015).

Maternal maltreatment in early life has an effect on the methylation of the brain-derived neurotrophic factor (BDNF) gene in the central nervous system; the changes in methylation persist through life, which results in altered BDNF gene expression in the prefrontal cortex in adulthood. The offspring of females who experienced maltreatment show altered BDNF DNA methylation as well (Roth et al., 2009). Generally, early abuse or neglect results in changes in gene expression through the epigenetic molecular control. The environment and molecular mechanisms work together.

Maternal stress in gestation influences her care in early life of the progeny (Champagne and Meaney, 2006). Prenatal stress or glucocorticoid exposure during pregnancy reverse central prenatal programming, and are also associated with alteration of circulating sex steroids and changes in steroid metabolism in the brain of the F1 offspring (Grundwald and Brunton, 2015).

Maternal behavior influenced by mating with father, resulting in divergent care of the progeny in concordance with the paternal experience (Mashoodh et al., 2012).

Traumatic experience in early life can alter emotional and psychological disorders. This effect may be transmitted through several generations. An experimental study on a mice model shows that deprivation of maternal treatment leads to depressive-like behavior in the progeny, with some extent of manifestation in several subsequent generations. The transmission is not caused by parental interaction. The depressive-like beahavior phenotype is transmitted most probably by the germline with changes in DNA methylation at CpG islands of the MeCP2 (methylated CpG binding protein 2), CB1 (cannabinoid receptor 2), and CRFR2 (corticotrophin

release factor receptor 2) genes found in the sperm of early-life stressed males (Franklin et al., 2010).

The effect of maternal care through an epigenetic regulation of the glucocorticoid receptor gene, and on the hypothalamic-pituitary-adrenal (HPA) stress response of the offspring through epigenetic changes, has been shown in animal-model studies. To test this effect of fetal programming in humans, a study was conducted, focusing on maternal mood and its correlation with the methylation status of a CpG rich region of a promoter, and an exon 1F of the human GR gene (NR3C1) in newborns, and HPA stress reactivity at three months after birth. This testing leads to the conclusion that prenatal exposure to maternal stress or anxiety is associated with increased methylation of the gene NR3C1 and increased stress responses of the newborns (Oberlander et al., 2008).

6.3.4. Paternal stress

Not only motherly stress may affect future generations, paternal stress has been observed to affect sperm.Offspring of males who underwent a six-week period of stress displayed significantly reduced HPA axis stress responsivity; global transcriptional changes were noted in certain parts of the brain, indicating epigenetic change. Along with these findings, robust changes in sperm miRNA (miR) were documented: nine specific miRs were significantly increased in paternal stress groups (Rodgers et al., 2013).

6.3.5. Post-traumatic stress disorder

Epidemiological studies concerning post-traumatic stress disorder (PTSD) have been performed in several generations of humans. These studies are limited to “natural experiments”. Similarly to cases of famine that modulated metabolism in the whole generation, the effect and perturbance of post-traumatic stress disorder, can be shown on the example of holocaust survivors and their offspring.

In a study where the offspring of holocaust survivors with PTSD were compared for cortisol levels and other chronobiological alterations with a control group of offspring of holocaust survivors with no PTSD, and another controls selected from the random population with parents without PTSD. The result of this study showed that PTSD in parents had an effect of lowering cortisol levels in the progeny, especially following PTSD registered in mothers (Yehuda R et al., 2007). Concerning fathers, it has been proposed that effects of stress could be transmitted through sperm by microRNAs (Rodgers et al., 2015).

A similar effect was observed in a study on a mice model that examined the effect of stress

experienced in adults. Male and female offspring of distressed fathers exhibited an increased measure of depressive-like behavior and anxiety-like reactions in a sex-dependent manner (Dietz et al., 2011). Male descendants also displayed increased plasma corticosterone level and lower level of the endothelial growth factor, which is considered to play an important role in depression. Warner-Schmidt and Duman (2008) observed that the phenotype was more robust in males. In this study, only a very small transgenerational effect was shown when using in vitro fertilization, which would indicate that a transgenerational epigenetic transfer through the germline is limited; the authors suggest that the transfer was performed by the mother, who recognized the male as defective and therefore induced a specific phenotype by altered (insufficient) maternal care (Dietz et al., 2011).

An experimental study in mice suggests that corticosterone treatment in male mice induces an altered behavioral phenotype in the offspring (Short et al., 2016). Corticosterone treatment is supposed to simulate the effect of long-term stress on the parental generation, for higher levels of corticosteroid hormones are continuously released during long-term stress reaction. The corticosterone treatment was revealed to alter several generations in terms of behavioral changes such as anxiety or depressive-like behavior. These changes are characteristically manifested in subsequent generations in dependence on the sex of the offspring. In the F1 generation, male descendants exhibited hyperanxiety-like behavior, and the expression of the paternally imprinted gene *Igf2* in the hippo-campus was registered. F2 generation males and females showed a lower anxiety profile than controls; however, F2 males were seen to demonstrate depressive-like behavior. Along with these changes, specific microRNAs (miR-98, miR-144 and miR-190b) were found in parental sperm; these RNAs are predicted to interact with multiple growth factors such as Igf2 or Bdnf. Taken together, this data indicates that paternal corticosterone treatment alters anxiety and depressive-like behavior statuses in multiple subsequent generations, being transmitted through the male germline in a process that involves small non-coding RNAs (Short et al., 2016).

6.4. Fear conditioning

Animals behavior is species-specific and differs in-between individuals, furthermore the behavior depends on the actual situation and is affected by individuals´ history. The broad environmental contexts that affect the individual are emotionally, socially and motivationally mediated to the animal. Innate behavior such as mating, aggression or defense are controlled by the limbic system; the olfactory system in rodents represents the primary activation system of the neuronal circuits that control innate types of behaviour.To a certain extent, these types of

behaviour are gained by learning and experience, and to a certain extent it is innate: this distribution is species-specific (Sokolowski and Corbin, 2012).During the development of the nervous systems, neurons differentiate from progenitors that line the ventricular zone; the central nervous system arises from the neural tube (Huilgol and Tole, 2016). Cell migration is crucial for the development and functioning of the brain. Aberrant migration is linked with disorders such as epilepsy, schizophrenia, autism or severe learning disabilities and these processes are linked with epigenetic setting.

The developmental migration and setting is crucial for the future abilities of neuronal cells. The setting appears to create certain boundaries that differ in signals and transcription factors within developing system. Once such a boundary is crossed, it creates a novel trait to the cell. There is a hypothesis that neurons that differentiate from the same progenitor preferentially connect with each other in the neocortex (Yu et al., 2009); such a manner could be possible within the olfactory bulb. The olfactory system represents a structure essential for behavioral traits such as mating, fear or aggression. In rodents, there are two components – the main olfactory system, responsible for the sense of smell and vomeronasal system (the accesssory olfactory system), essential for pheromone based communication.

Processes in the brain are underlined by epigenetic changes. The first piece of evidence concerning fear-conditioned- reported acetylation of H3 in the hippocampus. The histone-associated change in the chromatin structure was associated with fear conditioning during long term memory formation (Levenson et al., 2004).

Further, it has been observed that the inhibittion of histone deacetylase HDAC enhances long term memory formation – including contextual, auditory, spatial memory and fear memory (Maddox and Schafe, 2011), whereas the inhibition of histone acetylase HAT impairs histone acetylation and long-term fear memory formation (Maddox et al., 2013). A most notable experimental study in mice reports that an effect of traumatic exposure to odour may be transmitted through generations; when acetophenone, which activates the odorant receptor Olfr151, was used to condition F0 mice, the behavioral sensitivity of F1 and F2 was complemented by an enhanced neuroanatomical representation of the Olfr151 pathway (Dias and Ressler, 2014). It has been shown that the sensory nervous structure and function induced by the experience of an individual may be transmitted onto the F1 and F2 generations through sperm – the effect was observed even with IVF. The neuroanatomical structure was altered in the progeny – the bulubus for acetophenon was enlarged compared to control, the animals showed enhanced startle after acetophenon perception and CpG hypomethylation of *Olfr151*, the olfactory receptor gene, has been found in sperm of F0 and F1. The effect was observed to appear through female germline

also. Social transmission was excluded by the observation of the same effect with IVF and within cross-fostering studies. This finding indicates that the neural development of the progeny has been affected due to parental experience by information stored in sperm: the information is most likely of epigenetic nature, and as such it gets involved in a concrete, specific developmental process within brain formation.

As far as the brain and cognitive capacity is concerned, mental and physical activity alters the risk of dementia and other psychological diseases in rodents. Even in cases of diseases that have classical Mendelian inheritance patterns, the effect of environment has been shown (Nithianantharajah and Hannan, 2009; Rodgers et al., 2015).

It is generally accepted that environment and lifestyle has an effect on the epigenetic setting of an organism. In the light of studies that examined the effects of living in an urban environment and its correlation with a higher rate of mental disorders, it has been proposed that changes in DNA methylation could be one of the candidates for the responsible underlying molecular process (Galea et al., 2011).

6.5. Temperature

The temperature of the environment is considered to be a strong selection factor, shaping phenotype within various species, in reptiles and fish temperature even plays its role in temperature-dependent sex determination. The effect of the enivronment's temperature on phenotype has been shown in plants, insects, corals, chicken and fish; it has been studied in mammals to some extent.

A study in wild guinea pigs (Cavia aperea) has been executed to reveal whether these wild animals react to environmental changes, in this case increased temperature. The males were exposed to increased ambient temperture for two months, which corresponds to the time required for complete spermatogenesis. Differently methylated regions were found in the liver, the main termoregulatory organ, when measured before and after the heat exposure. The epigenetic change was found even within the subjects' sons – in both the liver and the testes – which indicates the possibility of transmission to the F2 generation (Weyrich et al., 2016). The phenotypic plasticity, underlined in epigenetic change, was revealed to be in a certain manner responsive to environmental change.

6.6. Endocrine disruptors

Endocrine disruptors, such as vinclozolin or bisphenol A are, are chemicals known to interfere with endocrine (hormonal) system. These molecules mimic or inhibit the actions of

endogenous hormones. Hormones are signaling molecules that are transported through circulatory system into various tissues where a specific response is caused in dependency on the type of particular tissue or cell type. Hormonal regulation or signaling is crucial for the responsiveness of the body to the changing environmental and inner conditions. Hence the chemical molecules that interfere with hormones, or by any other mean disrupt endocrine signaling, represent a significant exemplary model for testing the effect of endocrine signaling and its´ effects on epigenetic setting of specific tissues.

It has been shown that within endocrine disruptor treatment the epigenetic profile gets affected. These experiments most significantly lead to developmental disturbances and disorders that persist across up to three generations. Hormonal regulation interacts through epigenetic setting of wide ranges of cell types including germinal cells. Epigenetic modifications, most notably methylation of DNA, may be affected by endocrine disruptors. This points to the phenomenon of DNA methylation being affected by hormonal signaling. Hormonal signaling permeates cells to change the epigenetic profile due to specific factors in reaction to environmental changes with consideration of own cellular history.

Vinclozolin is a common fungicide in agriculture while cultivation of fruits (vineyards) and vegetables. Two major metabolites of vinclozolin are antiandrogenic compounds.
Exposure to vinclozolin or to an extragenic compound methoxychlor during the period of gonadal sex determination induced decreased spermatogenetic capacity and increased incidence of male infertility in F1 generation. This phenotype was transmitted through male line across several generations, up to the generation F4. The occurrence of the induced phenotype was stated to correlate with altered DNA methylation patterns in the germ line (Anway et al., 2005).

However, further studies concerning endocrine disruptors and effects induced by the exposure to those has been executed. Vinclozolin was found to affect both sexes in mating preferences in a sex-dependent manner. Exposed female rat up to three generations (F3) after maternal (F0) exposure prefered male that were not exposed to the chemical. Males similarly imprinted do not show such a manner of preference. The authors of the study claim this phenomenon to have not solemnly the transgenerational effect but even a “transpopulational” effect. Further they claim this phenomenon to be a possible ´unappreciated force´ of sexual selection with a significant impact on the whole evolution of mammals (Crews et al., 2007).

Vinclozolin causes germline reprogramming and promotes induction of imprinted-like genes. Such an epigenetic profile is in a sex-dependent manner transmitted across generations and causes altered phenotype in the progeny. Not only embryonic exposure to endocrine disruptors is known to promote transgenerationally inherited changes that may lead to disease in adult life.

Vinclozolin has an ability to induce transgenerationally inherited epigenetic change in male germline. Exposure of F0 mother to vinclozolin leads to alterations of methylation that is registered in promoter regions of F3 sperm.

Also another potentionally indirectly induced genetic abnormalities such as Copy Number Variation[2] (CNV) have been proposed to be possibly responsible for phenotypic change in further generationsons (Guerrero-Bosagna et al., 2010).

Vinclozolin is not only affecting fertility and mating preferences, it has been stated that vinclozolin induces various epigenetically transmitted diseases that are also persisting up to generation F4 and that correlate with changes in methylation (Anway et al., 2006). The disease phenotype may appear to be one of the causes affecting mating preferences, however there are some other phenomenons that are proposed to be a part of the process (Crews et al., 2007).

"Normally, fertilization is possible only by mutual consent, with the interacting individuals being chosen by, as well as choosing, their partners. This consent is based not only on the internal milieu that motivates each individual to seek a partner but also on the satisfactory nature of the phenotypic traits the potential mate displays. The importance of self selection of mates has long been appreciated in animal husbandry, but the scientific study of this phenomenon has lagged, particularly in relation to mate choice. Yet experiments with flies, birds, and rodents have the common result that those individuals who are allowed to select and be selected by their mate enjoy greater reproductive success than force-paired animals. This Complementarity Principle, in which each partner participates in the mate selection process, has broad implications for all animals, regardless of their mode of reproduction. This principle has been extended to the genetic and now to the epigenetic levels." (Crews et al., 2007)

One of the parts of genome that undergoes transgenerationally transmitted altered methylation after vinclozolin exposure are CpG islands of major histocompatibility complex (MHC)[3] genes (Crews et al., 2007).

Other factor known to influence mating choices is odor that is being processed mainly through vomeronasal organ in rodents. There exist several peptides known to act as pheromones that are expressed in the facial area of mice. Exocrine gland-secreting peptide 1 (ESP1) is produced by males whereas ESP34 has been detected in females (Kimoto et al., 2005). The genes for ESP proteins are located in proximity to MHC genes (Kimoto et al., 2005).

2Copy Number Variation is a type of structural variation that affects a considerable number of base pairs. CNV affects phenotype and is heritable (McCarroll and Altshuler, 2007). It is considered to be one of the phenomenons operating during mammalian evolution as generating variation and disease phenotype (McCarroll and Altshuler, 2007).

3MHCs are molecules that are crucial for immune sytem. MHCs are present in various polymorphic allelic forms in vertebrates and are also known to affect mating preferences in order to maintain and spread large variability of their form across populations. MHC molecules differ in affinity to peptides that are presented thanks to MHCs on the cell surfaces to T cells that may induce immune reaction if recognized the peptide as unfamiliar.

Bisphenol A is an organic molecule with estrogenic like properties. It is widely used for making plastics that are used for example in fabrication of water bottles. In utero or early postnatal exposure may cause wide range of adverse effects on the development. Impaired brain development, sexual differentiation, behavior and immune functions may be affected. What is more, the effect may be transmitted to further generations (Kundakovic and Champagne, 2011). Bisphenol A was found to alter methylation patterns of certain geness (Anderson et al., 2016).

However, there has been a recent discussion on the topic of responsibolity of DNA methylation in trans-generational transfer of endocrine disruptors induced phenotypes. There has been a study in which the authors claim to effects of endocrine disruptors to be corrected in the germline (Iqbal et al., 2015). Nevertheless this interpretation of data has been criticized by other scientific groups as not plausible for refuting TEI (Sharma, 2015) (Guerrero-Bosagna, 2016).

7. Introduction to evolutionary theory in the context of epigenetic transmission

Growing evidence on epigenetic inheritance motivates us to study the newly emerging issues. Since the ways in which epigenetic changes take shape, in combination with environmental impacts, are highly complex, this field is rather intricate to study. However, it is clear on the face of it that we will have to use a series of simplifying models to capture the ways in which evolution operates. As we look backwards at the history of evolutionary thinking, the figure of Jean-Baptiste Lamarck often emerges, with his theory of the inheritance of acquired characters that has been the very first complex theory of evolution.

Very often this reference causes aversion to and rejection of both Lamarck and epigenetic inheritance, even in scientific circles. Connecting Lamarck with epigenetic inheritance seems to validate a denial of epigenetics in people’s minds. Lamarck’s theory of the inheritance of acquired characters was absolutely dismissed with the acceptance of Weismann theory of germ plasm and later also most ultimately with acceptance of neo-Darwinian model of evolution, which, back in the days, quickly made Lamarck into a symbol of fatal error. Lamarckism was later on associated with Lysenko, an infamous soviet pseudo-scientist, who inspired emotions such as repulsion and mockery in scientific circles. This association cast a shadow on the figure of Jean-Baptiste Lamarck and his thinking. Today the disdain for Lamarck is considered to be a historical injustice (Gissis and Jablonka, 2011). However, the repulsion for Lamarck or for any form of Lamarckism still has a relevant effect in general.

7.1. Natural selection as framing evolutionary principle that affects various levels of biological hierarchy

Since the acceptance of the Darwinian theory of evolution, biology became itself a historical science. Nature is undergoing change in time, according to unique events that have impact on it and on its means. Darwin´s theory of evolution by natural selection as such is interpreted as building stable structures by the rejection of the weak: as an evolution by competition, a struggle for life in between conspecifics. Being elegant in its own way, and also compatible with population genetics, it became the widely accepted theory of evolution in various circles, with an overall impact on the understanding of the theory of evolution – which means a great impact on the human society.

According to numerous authors, Darwin´s natural selection´s principle is framed in terms of a "struggle for existence" (Lewontin, 1970; Ryan, 2002), which is only an affective point of view that has been assumed by Darwin and influenced further generations. Not only is it the case that such a principle is inaccurate with respect to our observations (contrast the theory of symbiosis (Ryan, 2002) - such emotionally motivated expression may be misleading for further thinking.

The mechanism of natural selection is stated as a crucial principle of evolution. More importantly, one can say that the widely accepted concept of evolution is built upon this principle – a basic one, which, however, only emerges from the basic properties of life, e.g. living, reproduction, heredity and dying. It is a truism: that which survives, survives, what raises offspring, raises offspring, and what dies, dies. This is the true nature of evolution itself – there is no possible misinterpretation we can think of, for the definition defines itself. Here we can only argue about what the single terms mean. Whatever definitions we set for them, this simple principle remains. What is to be observed, is continuity. And as such, it is the continuity of the soma that enables life to persist, and change during the time of its persistence.

The particular processes through which nature and life operate are constantly being discovered. With the discovery of DNA and its function in the cells and living organisms, the application of natural selection on genes resulted in the interpretation of evolution as the result of genetic information hidden in the DNA code.

However, I would rather not separate natural selection from the other mechanisms. Taking natural selection to be somewhere above those processes, when we look at the crucial problem we conclude that natural selection is not any one particular process – actually, it is the basic principle of evolution, and as such it has an overall effect on biological processes on various levels of biological hierarchy, and also, as such, natural selection itself can be and is affected by global and holistic biological processes.

Natural selection is a term that covers or illustrates an abstract boundary between living forms that happened to survive, and proliferate, and the other forms that ended by death without any other evolutionary (or we can say biological) involvement. As I found myself unable to distinguish which forms or stages of life are possibly not involved in evolution or the biosphere, I find the only possible way in defining this boundary of natural selection as the boundary of death. For any organism can get involved in another life and change the environment in a manner affecting evolution.

7.2. Modern Synthesis, neo-Darwinism and the gene definition in the 21th century

By directing attention almost exclusively to genetic studies, evolutionary biology gained an elegant model of evolution, integrated within the conception of 'Modern Synthesis'. This conception, which puts together natural selection and statistics of population genetics, might be useful in dealing with a quantity of cases. However, the Modern Synthesis in its current state is unable to embrace not only certain experiments, but whole branches of biology such as epigenetics, developmental biology, or behavioral biology and evolutionary ecology as is well discused in anthology of Evolution, the Extended Synthesis (Pigliucci and Müller, 2010).

The use of genome-wide association studies that compare the occurrence of genetic markers and manifested phenotypes led to an impressive result: only a small portion of cases had a genome-related basis. Thus, all the hereditary traits could not be sufficiently explained by genetic inheritance alone.

The discussion on what actually is subject to evolution has been going on for decades. A classical piece by Richard Lewontine analyzes biological entities within the hierarchy of biological organization as the possible units of selection – i.e. genes, self-reproducing molecules, cells, organisms, groups or species (Lewontine, 1970). Richard Dawkins and G. C. Williams claim, it is the gene, or the information in the gene itself that is the unit of selection. Ernst Mayr considers the possibility that it is rather an 'avatar' of genomic information.

The concept of a gene itself is problematic as it underwent many changes of definitions that describe different phenomena (Gerstein et al., 2007). The idea that the unit of evolution is not the organism but the gene, firstly introduced by W. D. Hamilton (1975), became famous thanks to popularization of Richard Dawkins (Dawkins 1976). This point of view has widely spread around the world and affected biological thinking.

Lately, there has been some discussion on the redefinition of the concept of a gene. The gene definition went through a series of variants during the 20 thcentury. The current definition of a gene relies on the sequence view: thus a 'gene' was defined by the Human Genome

Nomenclature Organization as *"a DNA segment that contributes to phenotype/function. In the absence of demonstrated function a gene may be characterized by sequence, transcription or homology."* (Wain et al., 2002).

More recently, the Sequence Ontology Consortium reportedly called the gene a "*locatable region of genomic sequence, corresponding to a unit of inheritance, which is associated with regulatory regions, transcribed regions and/or other functional sequence regions*" (Pearson, 2006). When the whole human genome was to be sequenced in the Human Genome Project (HGP), there were huge expectations for revealing the linkage of all genes with their appropriate functions. HGP was completed in 2004 and the crucial findings were these: Human genome has approximately 20 500 protein-coding genes, which is only 1,5-% of the whole genomic sequence. The genome contains a large number of repetitive sequences. And less than 7% of the genes are vertebrate specific. The genes with no protein products were called the 'junk DNA' in the past. However, according to more recent views, these appear to be regulatory sequences rather than junk. The discussion on the topic of functionality is still being held.

7.3. Encode Project

After HGP, another project called Encyclopedia of DNA Elements (ENCODE) followed. ENCODE was supposed to identify all functional elements in human genome. The vast majority of genome was historically regarded as ´ junk DNA´, however, today it became more apparent that the ´junk DNA´ has a regulatory function. The changes in regulatory sequences are considered as leading to altered transcription, protein production and cellular processes. Revealing of regulatory regions and their function is crucial for understanding how the information in genome is used. However, it has been showed that regulation plays an important role in phenotype construction (Carroll, 2005). The genomic sequence should be viewed as a dynamic system in which one functional element is influenced by the concrete setting of other functional elements.. The expectation of connecting one specific functional element with particular phenotypic trait or function was not fulfilled.

As there is a present uncertainty of gene definition, we may rather use the term of functional elements (ENCODE Project Consortium, 2012). Functional elements vary in their nature, including all the distant regulatory sequences (promotors, silencers, enhancer etc.) and RNA coding sequences. In fact, some genes have been found to overlap with one another, sharing the same DNA sequence in different reading frames or on the opposite strand. Thanks to alternative splicing, there are numerous divergent gene products that share the same open reading frame (Gerstein et al., 2007).

Within the ENCODE project, a map of 3 millions of Dnase 1 hypersensitive sites was constituted. Dnase 1 hypersenitive sites indicate that underlying sequence is of a regulatory character, such sequences allow chemical factors to influence their expression (Thurman et al., 2012). Then a lexicon of 8,4 millions of distinct short DNA sequences that present recognition motifs for DNA-binding proteins was compiled; these elements are listed to be preferentially sheltered from methylation (Neph et al., 2012). It has been shown that somehow biologically active is at least 80% of the genome, this part appears to have mostly the regulatory function. Only 1% of the genome is protein coding ("An Integrated Encyclopedia of DNA Elements in the Human Genome," 2012).

Project ENCODE revealed that at least 80.4% of the human genome participates in at least one RNA or chromatin associated event in at least one cell type. 95% of the genome lies close (within 8kb) to regulatory events and 99% lies within 1,7 kb of at least one biochemical event.

Primate specific elements without detectable mammalian constraint show evidence of negative selection, thus there a supposition exists that primate specific elements might be functional.

It has been suggested that 399 124 regions dispose of enhancer-like features, 70 292 regions appear to have promoter-like features. And hundreds of thousands of quiescent regions were found in human genome (ENCODE Project Consortium, 2012).

In terms of chromatin marking, the authors also found 3 millions of different nucleosome types (which means each of the nucleosomes has differential histone modification pattern), but only 3700 of these 3 millions are in all 147 studied human cell types. This shows an enormous diversity within different cells.

It has been shown that it is possible to correlate transcription to RNA with chromatine marks and transcription factor bindings at promoters. Non-coding variants lie in functional regions, the number is at least the same as that of those that lie in protein coding genes.

Biologically active is 80% of the genome, this part appears to have mostly the regulatory function. Only 1% of the genome is protein coding ("An Integrated Encyclopedia of DNA Elements in the Human Genome," 2012)

Within the ENCODE project, a map of 3 millions of Dnase 1 hypersensitive sites was constituted. Dnase 1 hypersenitive sites indicate that underlying sequence is of a regulatory character, such sequences allow chemical factors to influence their expression (Thurman et al., 2012). Then a lexicon of 8.4 millions of distinct short DNA sequences that present recognition motifs for DNA-binding proteins was compiled; these elements are listed to be preferentially sheltered from methylation (Neph et al., 2012).

Transposable elements are considered as possible catalysts of evolution, for it is possible that transposable elements provide a major source for evolutionary novelty (Brosius, 1999) and transposable elements together with a vast majority of regulatory sequences does not show apparent sequence conservation. Nevertheless, lack of sequence conservation does not mean the sequence is not functional. It has been argued that cell specific transcription and splicing are rather more reliable indicators of genetic conservation (Mattick and Dinger, 2013).

As has been mentioned before, it had been shown that sequencing of the genome did not reveal much of the entire genomic function. Within Encode project while questioning the role of the genome, we can look at the "junk" DNA, which represents the majority of the genome, as much as it is DNA with regulatory function. The regulation of transcription is controlled by the overall setting of chromatin structure, where epigenetic modifications play an important role. The evolutionary conservation does not need to be directly viewed as a conservation of concrete rigid DNA sequences, it is considerable to peer at conservation of functionally cooperating regulatory functional elements. Epigenetic modifications represent general regulatory instrumental system that mediates adaptable genome usage. Today, in the post-Encode era large quanta, of epigenomic data are analyzed. For further information see DeepBlue Epigenomic Data Server (Albrecht et al., 2016).

7.4. Construction of phenotype: epigenetic processes in the context of heredity, development and evolution

DNA has been considered the carrier of biological information. Current scientific knowledge reveals that DNA is not the only medium for the transmission of information. Epigenetic inheritance can be observed as a form of transmitted information too, for it affects construction of phenotype; and it is thus that what Wagner and Danchin (2010) define as biological information, namely a factor that can affect the phenotype in ways that may influence fitness.
It is being debated where phenotypic variation originates. The idea that phenotype is a mere bodily reflection of the genotype has been disproved, nevertheless it is still widely spread within the general public opinion. Genotype is predetermining phenotype but not in a way of a mere genetic program. Genetic information represents prerequisite upon which phenotype is being constructed during the development, nevertheless the developmental processes, influenced by a wide range of environmental inputs, result in the phenotypic form (Oyama, 2000).

Epigenetic cellular profile defines the possibilities in a manner of epigenetic landscape which is responsive to the environmental changes. One signal can even lead to divergent reactions in divergent cells, thus the interpretation of a signal is context dependent. Epigenetic systems

represent definition of an interactive interface. The cellular settings are acquired due to the history of cellular signalling.

We can perceive epigenetic modifications as a tool that permeates the cell to work efficiently with information in DNA sequence due to the cellular history and in a way that is reactive to an actual state of the cell and to its environmental conditions. Epigenetic informing shapes further forming. Somatic epigenetic heredity enables repeating of cellular and intercellular structures and systems that are reactive to changes in environment.

"All biological systems are inherently dynamic and entail dynamic features such as pleiotropy, robustness and rewiring." (Hu et al., 2016).

Whitelaw considers a mechanism for phenotypic variation based on inheritance of transcription patterns by somatically active retrotransposons (Whitelaw and Martin, 2001). Epigenotype of retrotransposons is reset in every generation, these regions are abundantly epigenetically silenced through imprinting, and an incomplete resetting may lead to a heritable epigenetic effect. The stochastic nature of retrotransposon activity, and the very large number of genes that may be affected, produce subtle phenotypic variations even between genetically identical individuals, which may affect disease risk and be heritable in a non-mendelian fashion. Retrotransposons that are discussed as a possible source of evolutionary novelties undergo epigenetic silencing or activation which may also affect phenotypic construction during development (Whitelaw and Martin, 2001).

In the realm of DNA sequence, we can embrace the neo-Darwinian hereditary model as plausible - but concerning epigenetic inheritance, we should find another model and combine them. Russel Bonduriansky suggests that the concept of 'soft inheritance', previously rejected during the 20th century, differs fundamentally from the current concept of non-genetic inheritance (Bonduriansky, 2012). Bonduriansky claims that the concept of heredity as being mediated by a single, universal mechanism, is being replaced by an emerging pluralistic model of heredity based on a recognition of multiple, parallel mechanisms of inheritance. Heredity itself is not rigid varying in its means within species. There are novelties existing within heritability pathways.

In the conception of the Modern Synthesis, heredity is 'hard', which means that it is mediated by gene alleles impervious to environmental influence. Hard heredity is a model of heredity based on the transmission from parents to offspring, at conception, of a set of factors whose nature is unaffected by the environment or phenotype of the parents. Within this model, Lamarckian 'soft' heredity was rejected, according to which traits acquired during the lifetime of an individual are passed onto offspring. Bonduriansky claims that the triumph of hard Mendelian heredity has all the hallmarks of scientific revolution in the sense established by Kuhn (Kuhn,

1996).

Non-genetic inheritance processes operate along with genetic inheritance. It is clear that human cultural and linguistic variation is transmitted from parents onto offspring via non-mendelian mechanisms, that is, by cultural inheritance. The theory of cultural evolution stems in 1970´s, when the concept of gene-culture coevolution emerged. Cultural inheritance is further known to operate in non-human animals as well (Mundinger, 1980; Avital and Jablonka, 2000). The cultural inheritance is Lamarckian in its means, for the subsequent generation is taught what the parental one has learned (Gould, 2010). The common feature, according to Bonduriansky, of non-genetic mechanisms is that they are transmitted onto offspring by elements of the parent´s "extended phenotype" (a term taken from Dawkins, citace) – i.e. components of the parent's body, behavior or environment. Such processes are coined under the term ecological inheritance, niche construction theory (Odling-Smee et al., 1996) being one of the major concepts of it. Niche construction means that the activities of organisms lead to modifications of their environment, which affects selection and influences evolution. Organisms choose their habitats, and construct their environment, which has an impact on them and their progeny. Organisms not only adapt to environments, but they also construct them. The environment that the organisms are exposed to is shaped by their ancestors. A pluralistic theory of heredity represents an emerging consequent outcome of the cultural inheritance theory and niche construction theory acceptance.

It is possible to accept any of the levels of biological hierarchy as a subject to evolution, genes, functional elements, cells, germ cells, organs, tissues, traits, individual, societies, species, ecosystems or even evolution itself. However, in each of the possible variants, the effect is highly reductionist.

The DNA sequence without a cell does not appear to be able to perform any of its hereditary activities (Markoš and Švorcová, 2009). Thus the processes present in cells and whole bodies are substantial for evolution, and therefore, it is apparent that non-genetic inheritance cannot be overlooked. The neo-Darwinian model is more or less plausible concerning the genome, i.e. the DNA sequence. DNA base sequence is changed within random mutations, nevertheless there are processes that can enhance or suppress mutability directly to the site, as an example higher mutability at CpG sites may serve. However, the subject of evolution cannot be restricted to DNA base sequence only: it is not merely the sequence itself which undergoes natural selection, but it is rather the phenotype, which is modulated on the basis of the DNA sequence together with various biological molecular and intraorganismal levels. It is always the cell, at the minimum, that is able to live (Markoš and Švorcová, 2009). And the somatic continuity is severely unexceptionable.

As Bonduriansky points out, the rejection of “soft inheritance” was based on the premise that there is only one heredity pathway. Mendelian genetics, and its idea that heredity is mediated by a single, universal mechanism, appears outdated: there are so many means by which inheritance can be effectively provided – besides genetic inheritance, there is environmental, behavioral, cultural inheritance or possibly epigenetic effects. Furthermore, these all operate together, one trait being influenced by another, and we include genetic inheritance in the set. We cannot separate out one of the heritability tracks and expect to see the whole nature of evolution (evolutionary nature). Not a single one of these processes operates in detachment from the others. Mostly the ‘core’ is represented by the DNA sequence, which changes in the slowest way. However, the somatic continuity that never goes away is present, influenced by environment, different in every generation and as such forming the history of evolution. A cell or an organism, however, needs constraint for retake of development in every generation. This is provided by reprogramming in embryo that happens twice. One for all cells of the organism, second for the germinal cells that eventually give rise to the next generation. As is evident from the above noted molecular stories (chapter 4.), there are areas that escape this process, there are traits that do get transmitted. It is at least possible. Even if the smallest opportunity for escape were present, a large overall change of our conception emerges, and becomes more plausible than the previous model of predeterminant rigidity.

7.5. Rivoire and Liebler´s mathematical model of emerging hereditary traits

Rivoire and Leibler’s model for the generation and transmission of variations in evolution (Rivoire and Leibler, 2014) is a theoretical mathematical model of heredity that includes new hereditary traits produced through evolution which, however, does not prescribe the particular processes through which heredity operates. The mechanisms are unconstrained, being subject to natural selection themselves. The model allows variations to be inherited, randomly produced, or environmentally induced. The variations may or may not be transmitted during reproduction. According to Rivoire and Leibler (2014) in Origin of species Darwin states: “*Any variation which is not inherited is unimportant for us.*” This statement is questionable. We can imagine a situation in which a phenotype that is not transmitted to the next generation may influence the transmission of other phenotypic alterations, and so it becomes a part of evolution itself.

The mathematical model highlights that under some environmental circumstances, nontransmitted variations may be more beneficial than transmitted (Rivoire and Leibler, 2014).An analysis of the model proposes that different hereditary traits are favored in dependence on the

fluctuating environment. Multiple inheritance traits may operate in a parallel manner: each of the traits may be a potential subject to a different selection pressure, and it may be selected for those parallel hereditary traits.

Information is transmitted across generations through different pathways, with divergent topology and with various dynamics, and the topologies of hereditary traits are themselves subject to selection.

8 Discussion

Epigenetic processes enable cell to define its own state and identity through regulation of genomic information usage. The genome sequence represents most preserved information that changes due to sparse random mutations. Nevertheless, the chromatin state, mediated by epigenetic modifications, impacts the rate of mutability in DNA sequence. The DNA that is used and manipulated with, is more prone to mutations – this concerns highly transcribed genes and their neighboring regions.

In 20th century, within modern synthesis approach, novel variations arised from random mutations in genetic information and natural selection was to choose from the genome-rised variants. The genetic sequence was predetermining the rest of the levels of biological hiearchy and developmental and heritability processes were explained as a consequence. Loads of molecules (chromatin modifying enzymes and RNA molecules) are involved within gene regulation, and these molecules are encoded in the genome themseves. Nevertheless, these molecules are crucial even for genetic sequence maintenance and furthermore the regulatory molecules choose what is going to be translated, therefore we can not simply consider the genome to be the predeterminant to all bodily and evolutionary processes because we might see the genome as a consequence of the processes as well. Methylated cytosine can spontaneously hydroloytically deaminate into uracil and uracil might be´corrected´by corecting enzymes to thymin, which alternatively may lead to the change of a base pair from GC to AT (Turner 2009). And as has been mentioned before (chapter 4.3.) hypermethylation was stated to correlate with higher mutability in the underlying sequence (Hernando-Herrarez et al., 2015). These phenomenons reveal that mutability in genetic sequence might be affected by methylation profile.

On the other hand, the sequences of genes that are crucial are evolutionary fixed, the severe changes would not go through natural selection, and the sequences that are not used, even if epigenetically silenced, may gain new mutations over time and alternatively a new function. The epigenetic change specifies the usage of genome. The epigenetic change is influenced by environmental factors, inner signals, and also by the history of the cell or the organism. The

epigenetic state of a cell is partly inherited, in somatic cells, from one generation by another. In the germline, the epigenetic change has certain limitations, as is previously noted. Yet, germ cells are influenced by their environment just as well as somatic cells are, and changes in the epigenome also appear during their lifetime. The core problem of transgenerational epigenetic inheritance lies in the resetting of the germ cell epigenome, which this lineage undergoes during embryogenesis. For the deletion is incomplete (as is previously described in chapter 5.4.) and there are epigenetic modifications that are passed through germline to the next generation – we have to consider the posibility of the effect of epigenetic modifications seriously (further discussed in chapter 8.4.).

8.1. What supports acceptance of TEI

1. **TEI is theoretically acceptable as one of the evolutionary hereditary traits.** There is more than one hereditary pathway in evolution (genetic inheritance, cultural inheritance, learning, environmental inheritance) and TEI may be one of the hereditary pathways that operate all together. The modern synthesis paradigm was based on the presupposition of only one hereditary trait (Bonduriansky, 2012). Evolution is not limited to one hereditary trait only, as such TEI may be one of the co-opting evolutionary traits. It can be theoretically selected for a model of evolution that accepts more hereditary pathways (Rivoire and Leibler, 2014).

What is more, **TEI has already been accepted**, concerning plants. When looking at evolution, we have to look at all the branches of life in which evolution is supposedly operating simultaneously in various ways. As far as we assume the life in a manner of current biological paradigm, we presume it to have one common origin. The issue to be discussed is not whether TEI exists within evolution but whether it operates within wider range of species, not only plants.So the question is the following: Is TEI operating within mammals?

2. **There are phenotypic traits that are inherited across generations in a non-Mendelian manner,** as a lot of studies, mentioned also in this work, (Chapter 6.; The Table of TEI, Attachments) reveal. The phenotypic traits are environmentally induced and are transmitted through germline to subsequent generations in sex-dependent manner. T**h**ere is evidence from experiments (Franklin et al., 2010; Huypens et al., 2016) and cohort studies that supports TEI (Pembrey, 2010; Yehuda R et al., 2007). The hereditary manner appears to be sex-dependent and the hereditary manner appears to be ‘soft’; after several generations, the phenotype is observed to disappear in case that inducing stimuli is no longer present in the environment. Such TEI-like hereditary patterns were observed within experiments on rodents (Anway et al., 2006; Franklin et al., 2010) and further in human studies, the effects of trans-generational transmission that point to possible TEI are observed too (Pembrey, 2010; Yehuda R et al., 2007).

3. **There are molecular epigenetic processes found to underlie the non-Mendelian acquired phenotypic hereditary traits in mammals.** As such TEI may be possible through germline. Inheritance of methylation patterns is possible and imprinting represents the strong evidence for such a hereditary pathway. Further it has been shown that other methylation patterns escape general deletions in the development, SncRNAs have been found in sperm and in oocyte. Histone PTMs may supposedly alter chromatine structure of the germline and snRNAs coding regions were found at the locations that escape deletion (Weigmann, 2014).

Further-more, the fact that epigenetic chromosomal state is inherited does not necessarily mean that a concrete molecule must be responsible for that (Boffelli and Martin, 2012), for chromatin is a considerably complex structure and all the processes operating within have not been identified yet.

"The finding of germline methylation is tantalizing: it raises the possibility that a substantial portion of the epigenome is heritable, but it does not present a clear path to clarify the relative importance of genomically encoded states versus purely epigenetic states. If one considers inheritance as a chromosomal phenomenon, it is possible that purely epigenetic variants associated with DNA, but not strictly dependent on its sequence, carry heritable information. However, while the presence of epigenetic states in the germline is a requirement for their inheritance, it does not demonstrate that they are inherited. The mechanisms by which epigenetic states are maintained in the germline are not clear: their molecular complexity makes it possible that some components are removed, but re-established based on information that is retained; elucidation of these processes will be necessary if we are to understand the contribution of pure epigenetic inheritance to species evolution." (Boffelli and Martin, 2012)

There are pathways through which the molecular signals are transmitted to the next generations; the transmission is happening as far as empirical data are concerned (chapter 6.; The Table of TEI, Attachments), even though not all of the processes involved are known yet. Even if we consider the transmission of such complex traits as merely a stochastic escape, that has appeared within evolution, we might conclude the transmission to be a subject to natural selection itself.

4. **Molecular processes that alter epigenetic modifications in germline are influenced by environmental stimuli.** Germline is responsive to environmental conditions, the presupposition that the germline stays intact is a misleading simplification. Sperm and egg is influenced by hormonal and endocrine signalization that may alter epigenetic state of the cells. SnRNAs have an ability to circulate through blood stream and be targeted, so these molecules are theoretical candidates for soma-germline communication.

5. **There are biological phenomena that are not possible to be satisfactory explained by genetic inheritance only.** Non-Mendelian hereditary phenomenona, fast evolution in domestication experiments, inheritance of acquired traits and explanation of emerge of such an evolutionary phenomenon as instinct is, have not been sufficiently explained by neo-Darwinian hereditary model to date, acceptance of TEI might elucidate some of the processes involved within those phenomena.

The arguments against TEI acceptance

There is not enough data to support TEI acceptance. This argument relies on a matter of extent. Either there must be more experiments to support TEI or to refute it. However, the data that exist explain phenomena that are hardly to be explained by genetic inheritance, the inheritance of environmentally induced phenotypes shows in a sex-dependent non-mendelian hereditary manner. In future there is hope for more data to support TEI, as the amount of data supporting TEI is growing by the time. The branch of knowledge is relatively new and so is the data. Whitelaw argues that the data that would indicate that there is no epigenetic transmission are scarcely published, therefore it is difficult to publish negative experimental results and thus current literature lacks studies that would deny the idea of TEI (Whitelaw, 2015).

However, some of the authors denote the data to be sufficient (Jablonka and Raz, 2009; Szyf, 2015) and allege that epigenetic inheritance plays an important role in plant evolution. It is more than considerable to question if related processes might play any role in evolution of animals, possibly mammals.

The influence of TEI is minor. This argument is a matter of specifying what means to be minor. However, even if we assume the genetic sequence to play the 'core' informational role, the 'reading' of the genome is still crucial and it is the process through which the phenotype is constructed. Even a 'small' perturbation on an epigenetic level may influence the development and affect the phenotype (see Angelmans´syndrome, chapter 5.3.).

The epigenetic reprogramming during development resets the epigenetic state to a naive form, most of the epigenetic settings gained during the life are eliminated. This has been historically the strongest argument against TEI. To date it has been shown that not all the acquired epigenetic changes are deleted, hence there is a possible way for the transmission. Now the argument relies on a matter of extent. Some authors (Heard and Martienssen, 2014) argue that TEI has an important impact on the evolution of plants and even some animals such as nematodes, but that it is most unlikely to manifest with any great importance in mammals. The main argument against TEI in mammals are global epigenetic deletions in the germ line. However, looking at molecular

processes, the escapes of epigenetic modifications from deletion has been observed (Guerrero-Bosagna et al., 2010; Guibert et al., 2012; Manikkam et al., 2012).

8.3. All the main arguments against TEI rely on a matter of extent

As biology is a historical science itself (Pigliucci, 2010) what needs to be respected is that a unique historical event may affect the future paths of life. As well as single division of a cell or a cellular fusion may be of a significant importance, the generation and extinction of species are concerned to be historical events and are widely accepted to have an impact on the whole biosphere.

In mammals, one generation is at certain point represented by the germinal cells which is the core point when the single cellular events meet the whole organismal level. In this case, we are aware of the importance of change in cellular signalization that is possibly capable of altering the development of the whole organism. This point I consider to be crucial and I would recommend to be very careful with refuting the phenomenon of any biological process on the base of quantitative proportions generally. For we see, looking at various examples from molecular biology and biochemistry, wide range of signalization cascades that are able to multiple a signal exponentially. Such a ´minor´ signal may have an impact on the cellular fate. In case a single cell represents one generation of an organism, we have to be aware of the possibility the ´minor´ signal to have an impact on the whole organism. Thinking further, one organism may have an importance on the scale of species. There are no such phenomenons in biology we could recklessly consider to be minor. I refute the arguments for refuting TEI on basis of being ´minor´ as unjustifiably simplistic even wrong.

TEI may appear ´minor´ in comparison to genetic inheritance. However, this does not mean it would not be a part of evolution and would not affect the whole process of life continuity. TEI must be considered as a possible evolutionary pathway through which an amount of information is passed to the next generations, affecting the whole evolutionary process.

8.4. The problem of TEI acceptance – the collision of paradigms

One of the core problems why TEI in mammals is so often highly refuted is that when once accepted, the change in paradigm that is till current time held by modern synthesis approach must come. Nevertheless, TEI is not the only reason that would support the call for the change of the evolutionary paradigm, there are further reasons and whole branches of biology that have pointed to the change of evolutionary paradigm. Such topics are widely discussed in anthology named Evolution, the Extended Synthesis (Pigliucci and Müller, 2010). As James Griesemer states in

Transformations of Lamarckism anthology, in a chapter dedicated to philosophical consideration of the relative significance of epigenetic inheritance in evolution, the acceptance of TEI is not colliding with molecular biology whereas with quantitative evolutionary specialities (Griesemer in Transformations of Lamarckism, Gissis and Jablonka, 2011, p. 331).

Acceptance of TEI in mammals has become a delicate topic in current biology. For the change of current quantitative evolutionary sciences paradigm follows the acceptance of TEI. Looking at molecular biology, there is no discrepancy in TEI acceptance. On the molecular level TEI in mammals represents only one extension of currently accepted molecular phenomena, hence the theory is only specified by this extension. As far as we concern quantitative evolutionary sciences headed by modern synthesis paradigm, a radical theoretical change is required within TEI acceptance.

As James Griesemer states (Gissis and Jablonka, 2011) the structure of causal narratives is assembled ad hoc in act of explaining phenomena within molecular sciences. There is no contradiction in TEI acceptance here. Whereas the structure of causal naratives of quantitative evolutionary sciences is constructed in terms of expression of the theory and TEI acceptance does not correspond with the theory, therefore a radical theoretical or even paradigmatic change is required within.

However, TEI acceptance may explain and enable us to understand numerous biological phenomena (transmission of environmentally induced phenotypes, fast evolution in domestication or emerge of instinct) that had to be dismissed previously in order to keep the existing modern synthesis paradigm. Knowledge from various biological branches were impossible to be fused while keeping the elegant paradigm of modern synthesis. Keeping the simplistic modern synthesis theory as the only evolutionary pathway is no longer possible in such a situation. Eventhough quantitative genetics of modern synthesis was very elegant for mathematical modeling. The mathematical models previously used within genetic-heritability-only concept are not possible to be applied on epigenetic inharitance manners, for TEI hereditary pathways are significantly different. Nevertheless, with certain adjustment the mathematical modeling of quantitative genetics can be still applied on gene sequence but not for all the phenotypic traits anymore.

8.5. What follows the acceptance of TEI in mammals?

Firstly, the possible transmission of epigenetic modifications supports a higher variability in the next generation. When such a trait appears in evolution, it is possible that the process enabling the higher variability will be selected for. The offspring from one individual may vary not only in the more or less randomly divergent genomes but even in epigenetic profiles that may be established

according to different environmental conditions at different times of parental life. On the contrary whole generation may be affected by a particular environmental stimuli. Even though the epigenetic changes of one generation will represent an epigenetic reaction for one environmental stimuli, the epigenetic profiles might vary among themselves. If any epigenetic traits are transmitted to the next generation, the effect of plasticity is indisputable. The whole generation may be immediately affected in various responses to the changing environment. Such a model is further more plastic than the one of ´waiting-for-the-suitable-random-mutation´ concept.

The gain from variability within TEI acceptance is obvious. The intriguing question is: are these traits some pre-set adaptations, or are they rather randomly arisen variations? Does evolution have some routine pre-setted epigenetic states that are ´turned on or off´ in reaction to certain environmental stimuli?

It has been observed that it is possible to choose between two preexisting phenotypes in dependency on the environment into which the new generation is born. Such an example may be the case of mice that are stressed and develop depressive-like behavior, this behavioral phenotype trait is inherited epigenetically across generations and represents one of the two possibilities of the behavior. The transmission of the behavioral phenotype is also transmitted or induced by motherly care.

If any epigenetic traits are transmitted to the next generation, the effect of plasticity is indisputable. The whole generation may be immediately affected in various responses to the changing environment. Such a model is further more plastic than the one of ´waiting-for-the-suitable-random-mutation´ concept.

It has been discussed whether TEI might be eventually adaptable. In case we consider epigenetic inheritance only as a random process, we have to acknowledge that such a process that persists through generations is itself a subject to evolution. Now the matter in question is at which stage the process of epigenetic inheritance in mammals exists, what particular means it uses, and how robust the epigenetic inheritance effects are.

For example I would only point to the fact that a lot of transpozons (potentially harmful) are often epigenetically silenced. This might be considered as an adaptive trait (Grossniklaus et al., 2013). Additionaly, Whitelaw considers a mechanism for phenotypic variation based on inheritance of transcription patterns by somatically active retrotransposons (Whitelaw and Martin, 2001). Epigenotype of retrotransposons is reset in every generation, these regions are abundantly epigenetically silenced through imprinting, and an incomplete resetting may lead to a heritable epigenetic effect. The stochastic nature of retrotransposon activity, and the very large number of

genes that may be affected, produce subtle phenotypic variations even between genetically identical individuals, which may affect disease risk and be heritable in a non-mendelian fashion.

However, TEI might not be adaptable in every case. I would argue that this depends on particular cases. Sometimes there are consequences of a direct physiological effect – e. g. malnutrition during gestation that alters development is rather caused by the impaired development of particular organs, as shown by studies in humans (Ravelli et al., 1976, 1998; Painter et al., 2005). The effect of malnutrition in those cases affected the organs that were in the crucial developmental stage at the time of the famine. In those cases, we can scarcely consider adaptation or pre-adaptation. Matters are different with fear conditioning. Here we can claim the transmission to be adaptive, shaping behavior of next generations in reaction to parental experience of changed environment. This represents an epigenetic rewiring in an adaptive manner, where the linkage of certain odour with avoidance was inherited across generations. We can consider that such an emerge of a typically Pavlovian reflex could possibly be connected with a different odour, or on the contrary with a different behavior. Olfactory sensations are known to direct behavior of rat, mice and other mammalian species, although, the extent of the effect on behavior differs among them. Within olfactory stimulation that led to heritable effect on behavioral phenotype, we can conclude that a pre-adaptation in terms of linkage of certain olfactory perception with a behavioral pattern might exist. Such a pre-adaptation would enable an emerge of a new phenotypic form in response to the environment that has been experienced by parental generation. And the new phenotypic form would be likely to be transmitted epigenetically to the subsequent generations.

The epigenetic change is reactive to the genome usage and therefore might be ‘directed’ to the site. Within somatic inheritance the genome usage is defined by the epigenetic profile and on the contrary the epigenetic profile might be affected by genome usage. The signals that are considered to affect germinal cells (snRNAs or hormones) are affected by the processes in the somatic cells and might be more or less specific. Further genetic mutability is affected by epigenetic setting and genome usage. Therefore epigenetic change may alter mutation rate in a manner that is directed to the site.

Are epigenetically activated modes fixed on the evolutionary level or are epigenetic processes only stochastic events that randomly escape general epigenetic reprogramming? Both of the possibilities appear to function, DNA methylation is known to be stochastic to a certain extent, nevertheless, in other cases is directed to the site. Might there be a nouvel phenotypic trait that emerges on the base of differential reading of the genome? The development might be quickly affected on a regulatory level, hence a new phenotypic variant may emerge from change in

developmental programming. Some epigenetically activated phenotypes might be a result of a stochastic escape that might eventually lead to new phenotypic forms. Some might be evolutionary fixed responses that directly follow particular environmental stimuli., or are there emerging new modes within new generations with their unique experience?

Epigenetic change may focus natural selection on a particular trait. Phenotypically plastic responses (phenotypic accommodation, West-Eberhard 2003) are of epigenetic nature and such may facilitate production of genetic change via Baldwin effect or Waddingtonian genetic assimilation. When a particular organ is affected during the developmental epigenetic changes in the whole generation, then the natural selection is focused on that particular organ and therefore the selection might be focused on that organ. The epigenetic affection by famine represents such a case. It is not a predisposed reaction, it has been set due to environmental affection of developmental processes, nevertheless it got involved in the particular organ development. Thus, a change in epigenome is raised based on environmental "restriction". Such a change may not be considered usefull or intentionally targeted or evolutionary constrained as a process of preadaptation. In this case, we can consider epigenetic change as directed to the site due to impaired developmental processes. Such a change, being unintended, may be observed rather as a mismatch. As a result, this event focuses natural selection by lowered function and ability of the particular organs of the generation. Also a rise of variation thanks to divergent damages appears in the generation. In each individual the effect of mismatched epigenetic setting of development has a divergent effect. The epigenetic setting, being partly transmitted to the next generations modulates also the phenotype of subsequent generations. This may serve as a response to the environmental change, where focusing on an affected organ enables natural selection to be directed to the particular traits that had been affected due to environmental change. A similar process may be involved focusing on a behavioral trait. Such epigenetically emerged phenotype which focuses natural selection might be physiological, morphologi cal or behavioral.

TEI does not have to be deterministic. Epigenetic profiles are further more susceptible to changes than genomes. Therefore a particular epigenetic profile might change during the lifetime of an individual and therefore result in divergent phenotypes dependently on the environment or history. There is a possibility of 'going back'. An epigenetic profile might be deleted, and in a great digree it is deleted during embryogenesis, as has been discussed. This enables evolution to test novelties, keeping basic settings working. If the novelties appear not to be particulary suitable, they may quickly fade away.

Here emerges a new capacity of being reverted to an earlier state – this possibility evolution lacked when relying merely on previous modern synthesis evolutionary concept. As

Rivoire and Leibler, (2014) say, it might be advantageous not to transmit everything, certain phenotypic variations are better to be erased, eventhough once involved in evolution. Within neo-darwinian model a disappearance of a trait was possible through accumulation of mutations or by natural selection – by death of the carriers of the ´undesirable´ trait.

Overall the evolution might be more quick and plastic adaptively responding to the change of conditions. If the environmental change was supposed to continue during the time, the phenotypes of organisms in subsequent generations have to adapt to it, or become extinct. A non-deterministic and reversible change in variability represents a plastic manner for adaptive change. TEI may help us explain such phenomena of ´fast evolution´ that were listed during domestication experiments and their ability to reverse quicky in a few generations into wild-like phenotype – these phenomena were highly unlikely to be explained by genetic inheritance (8.10.)

Stress might be considered as a factor stimulating variability, enhancing epigenetic, consequently genetic changes. The level of stress might modulate the pace of evolutionary changes (8.10). TEI might explain the phenomena of innate reflexes that were impossible to be sufficiently explained through genetic inheritance.

8.7. Parental effects influence evolution

There has been quite strict criteria set to distinguish TEI from other epigenetic hereditary phenomena as parental effects are. TEI represents the hereditary pathway of transmission of an acquired or induced trait that can be transmitted regardless on cultural or environmental setting or on parenting (defintion chapter 5.) TEI therefore represents the biological molecular pathway for environmentally induced traits that might be inherited. Nevertheless, other phenomena, as parental effects or intrauterine exposure, are involved in evolutionary process. Phenotypes are shaped through these pathways importantly. Parental effects may induce epigenetic changes that alter behavior, may alter environment and further parental care and so on and so on. The parental effects may even result in TEI (Franklin et al., 2010), therefore we shall consider the processes to be highly intertwined in nature, which may identify their effects. Further intrauterine effects are of considerably high importance because the developing embryo is susceptible to environmental inputs that might affect the epigenetics of developmental programming and such effects are very likely to have impact on the whole organism (including its germline) and potentially might result in TEI as well.

8.8. Shall we include cultural transmission within the whole concept of evolution?

Learning or maternal care play an important role within the wide range of mammalian

species. The cultural traits have an effect on phenotype, which also alters evolution. Phenotype has an effect on what is selected.

TEI represent a phenomenon that concatenates nature and culture. The environmental stimuli that include historical events formed against a cultural background, as was famine during World War II, induce epigenetic changes that may be transmitted to the next generation either biologically - through epigenetic inheritance – or behaviorally, which is part of culture, at least in terms of human evolution. These chains of mutual involvement of culture, behaviour, the environment and biological processes that include epigenetic transmission of information have together an effect on the course of evolution, which means that all these phenomena, operating together, affect natural selection on the genetic level. Changes in the genome are changing in the slowest pace, compared to epigenetic, cultural or behavioral changes. Genome represents the most stable structure and information that is not subjected to environmental changes, once when such a change in genome is established it is most likely to be preserved. The other hereditary traits represent a change that reacts to immediate environmental changes and may be easily eliminated in the following generations. This makes the organisms plastic and adaptable, yet firmly ingrained in their nature.

Within TEI we observe the cultural events to have an impact molecular biological processes that affect germline and further generations in the means of phenotypic variation. Hence we see that nature and culture are not possible to be separated, looking at the evolutionary implications. Cultural inheritance plays an important role in evolution. The inheritance through epigenetic processes represents a biological molecular medium for transmission of traits that are induced by events on an organismal level. As such, here comes the union of nature and nurture, nature and culture, nature and history (similar interpretation may be also found in Markoš 2002).

8.9. Is TEI necessarily beneficial?

What we understand as being beneficial appears to be our point of view, when looking at the history of life. As an evolutionary mean TEI might be beneficial but not necessarily. In case TEI is of stochastic origin or emerged due to developmental impairment, we conclude it not to be immediately beneficial. However, within further generations it might be beneficial for the focusing of natural selection and therefore it might be selected for the beneficial traits quickly.

A mathematical model, similar to the one of Rivoire and Liebler, models a situation of incomplete resetting in dependency on environment in a haploid asexually reproducing organism. The modeling results in statement that selection favours incomplete resseting in the germline in case the environmental conditions persist stable for longer time and matternal error rate is

sufficiently high (specificity of matternal cue is low) (Uller et al., 2015). Here comes the criteria of extent again. The theoretical model also states that incomplete resetting is favoured only within a period of four generations. According to the authors it is uncertain whether TEI could possibly be adaptive. The theoretical model is indicative of an explanation that incomplete resetting is more likely to evolve *"when the strength of selection against mismatched phenotypes is weak relative to the rate of environmental change"* (Uller et al., 2015). According to this model, the possible heritability is dependent on the environmental change, which is the point that emerges from wider range of biological concepts. The authors in their interpretation claim that incomplete resetting might be adaptable in case of slow environmental change.

8.10. TEI and behavior: thine ice of Lamarckian philosophy

Eva Jablonka argues that it is possible that the need to be plastic and adaptable is gained in plants by their lack of the Weissman barrier, whereas in animals it is represented by their learning ability. Learning, being itself a kind of behavior, enables mammals (and birds too) to acquire new behavioral traits. Abilities acquired through learning are transmitted by a large proportion culturally (we can presume the ability to learn to be innate). Within learning the informational change is happening within epigenetic structures of somatic cells. As behavior is rather a complex of various traits, we can still partly distinguish between innate behavior and acquired behavior. Innate behavior is supposedly inherited biologically, not within cultural transmission. Mammalian behavior is a complex of variable adaptive traits that emerge through development and is established during the whole life. The behavior is generally triggered by emotions, e. g. feelings, and thinking. Very generally the feelings might be divided on positive and negative – positive triggering in response to phenomena needed for life maintenance, and negative for the elimination of life threatening events. The brain's plasticity represents the space where the behavioral patterns may be rewired. Good or bad feelings stimulate mammals to a certain form of behavior, such a connection is supposedly evolutionary fixed for it has served as a form of behavior that has generally functioned for life preservation. Many behaviours are considered to be innate and lots of mammalian innate behaviors are related to odor perception. Odors of the opposite sex induce reward and attraction, on the contrary, the evolutionary experience favoured avoidance of certain stimuli that seems unpleasant: predators´ odor induces fear and aversion (Sokolowski and Corbin, 2012). Within mammals such innate behavior plays an important role for survival. Behavior is regulated by nervous system, limbic system controls responses to social or emotional saliance (Sokolowski and Corbin, 2012).

Lamarck considered behavior to be ´the engine of animal evolution´ (Ginsburg in

Transformations of Lamarckism, Gissis and Jablonka, 2011). The ability to feel represents a necessary condition for thinking according to Lamarck, he conceived that the ability to feel results from a new organization in the nervous system which ´leads to a new overall sensory state´. And for Lamarck it is the whole animal rather than a part of it that dispose of the overall sensory state of an inner feeling that gives rise to various emotions during the evolution Ginsburg in Transformations of Lamarckism states (Gissis and Jablonka, 2011). Such an overall sensory state might be affected by hormones, molecules that affect various cell types, induce epigenetic changes, modulate behavior and participate on emotional feeling.

Behavior represents the most plastic compound of organismal integrity. An animal is able to change its behavior easier than its morphology and further more easier than the genome. Considering learned behavior we can assume the underlying molecular processes to be of epigenetic nature. Nevertheless, what molecular basis is responsible for innate behaviors?

The possible candidates for transmission of innate behavior is either genome or epigenome. It has been difficult to explain behavioral patterns by neo-Darwinian approach, however, it has been thought that such specific innate behaviors are able to emerge only due to natural selection (Szyf, 2014). However, the behavioral traits induced in one generation may be transmitted to the next, looking at TEI experiments, it has been observed that certain behavioral patterns or phenotypes might be transmitted in a non-Mendelian manner through several generations after parental exposure to inducing environmental stimuli. As examples inheritance of depressive-like behavior, drug-related behaviors or fear conditioning, may serve. Within transgenerational epigenetic transmission of depressive-like behavior it is quite questionable wheather we can talk about adaptivity. Nevertheless, I think that depressive-like behavior might be adaptable in certain situations as higher occurrence of predator might be. The mice that are stressed by presence of predator incline to stay in their holes and do not go exploring other places where they could find a better place with no predators possibly, but we are not sure of this alternative, for their progeny happened to inherit their depressive-like behavior phenotype and never went out. The behavior might not be beneficial, in terms the mice suffered under the stress, however it might appeared adaptable in a way. What is more, the mice that went exploring and those of them who did not got caught by the predator are possibly living somewhere happily ever after with no transgenerationally inherited depressive-like behavior. Environment induces certain behavior that can be further transmitted and this is perfectly shown within the studies of drug exposure. After several exposures to a drug of abuse the neural system gains an epigenetic rewiring that modulates neural circuitry and affects behavior resulting in addictive behavior. As has been previously mentioned (chapter 6.2.) drugs are hypothesised to usurp brain systems that are

evolutionary set for life preservation and continuity (Koob and Volkow, 2010), the effects the drugs provide are rather fast for these are molecules that directly affect neuronal system and hormonal release and some of the drugs (alcohol, opiates, nicotine) are known to affect epigenetic modifications directly, as has been discussed (chapter 6.2). The addictive phenotype and other behavioral changes have been observed to display within subsequent generations. We can consider a pre-adaptation to these phenomena in terms of preexisting neural circuitries and behavioral responses that has been evolutionary fixed for life preservation. Such pre-set behavioral responses might originated as triggering animals to food, sex-partners or prey, possibly, and drugs of abuse might have wedged into these evolutionary structures.

Looking at examples of drug-related behaviors, we can interpret the data in a manner of information for the particular behavioral pattern residing in the genome (or epigenome) and being ´activated´ epigenetically by the drug directly or by the hormonal release after the intake. A similar linkage might be also considered within endocrine disruptors and the heritable effects connected within. In this case the endocrine disruptor molecules provide a mismatch in regularly functioning hormonally controlled processes related to reproduction and result in damages within these processes with a trangenerationally heritable effect.

The things are even more intricate with fear conditioning. Within the experiments by Dias and Ressler (2014) parental olfactory stimulation led to change in behavior and neural structures in subsequent generations. Here we presuppose the change not to be only on a level of ´turning on or off´pre-existing evolutionary responses. And the environmental inputs are not solemnly molecules that would directly affect pre-existing structures, in the case of fear conditioning a nouvel behavioral response acquired during adult life has been transmitted to its progeny. The parental behavioral experience was transmitted through germline into subsequent generations. The progeny showed enhanced behavioral response to odor to which their parents were exposed in association with pain that led to behavioral response in terms of avoidance in generation exposed and in the progeny. The neuroanatomical structure was changed, the olfactory bulbus for acetophenon was enlarged in the progeny which means the neural development was affected. The gene sequence for acetophenon receptor was noted to be undermethylated in sperm which indicates that the transmission was of epigenetic nature. Behavioral transmission was excluded.

The induced phenotype lead to epigenetic changes in sperm that lead to altered development in brain structure of the progeny resulting in behavioral response adaptive due to parental experience. It means an emerge of a new heritable pattern on epigenetic basis. This appears to be an evolutionary pathway for behavior shaping. And as mammalian behavior is often stimulated by olfactory response and is also shown to be transmitted epigenetically we would

rather not take it as a coincidence. Most notably the process of such a transmission must be quite complex for it enables the information from parental phenotypic change to be transmitted through germline and result in changed neural structures formation. The emerge of such a specific behavioral pattern indicates that the heritable epigenetic rewiring might be of a great importance in the case. This experiment points to an emerge of an instinct, a phenomenon that has been previously very difficult to explain through neo-Darwinian evolutionary model.

Is it possible for epigentically gained novelties to be evolutionary conserved for a long time or are these only genetical changes that might persist beyond a lifetime of a specie? Looking at instincts there is a possible explanation that those emerged by epigenetic means therefore, by certain extent might be inherited epigenetically. Is it necessary for an instinct to be fixed genetically? We do not know whether innate behaviors are stored in genetic or epigenetic information pool. In a case that instincts were stored in epigenetic information pool the possibility of their loss in a few generations could support the presupposition that instincts are inherited through epigenetic means. To proof the possibility that some features might be conserved epigenetically an experiment forcing to a loss or a change of such an innate behavior could support the idea. However, there has already been a study that points in this direction and show a radical change of innate behavior in a few generations.

A most excuisite experiment was executed by Dmitri Belyaev in Novosibirsk. In 1958 Laboratory of Evolutionary Genetics started to model animal domestication for studying artificial selection (term by Darwin). Even though there is supposedly the same processes underlying natural and artificial selection, artificial selection may eventually lead to reduction in fitness as the animals struggle with the stress of artificial selection regime. Black foxes (*vulpes vulpes*) previously held in cages were supposed to follow the path that the wolves took when becoming dogs. Belyaev believed that "*reconstructing the early steps of animal domestication could shed light on the evolutionary reorganization that occurs in the stressful conditions accompanying strong selection for behavior*" (Markel and Trut in Transformations of Lamarckism, Gissis and Jablonka, 2011). Domestication involves profound changes in behavior and therefore the foxes were selected for weak aggressiveness and decreased fear of humans. Within several generations they became doglike in many ways: followed human tracks, sought interaction with humans, emitted positive vocalization, wagged their tails and responded to their names. A great increase in variability emerged within the foxes, and the variability embraced morphological and physiological traits and functions that appeared unimportant for their behavior. Furthermore, the foxes resembled dogs within their appearance of floppy ears as pups, shorter and curled tails, changes in skeleton, shorter legs and piebaldnes (a white star on the head). The piebaldnes occurred within

generations in frequencies too high to be attributed to spontaneous mutations. The emerged star had a dominant-like inheritance trait, so the possibility that it has been caused by a rare recessive allele became homozygous was ruled out and therefore it might be considered that the molecular basis is of epigenetic character (Markel and Trut in Transformations of Lamarckism, Gissis and Jablonka, 2011). The high variability, also observed previously within domestication of other species, has been deduced to origin in stress acting as a destabilizing evolutionary agent. Belyaev hypothesized stress to function through neuronal regulatory system, destabilizing homeostasis and enhancing mutational rate (Markel and Trut in Transformations of Lamarckism, Gissis and Jablonka, 2011). Today glucocorticoid stress hormones are known regulate DNA mehylation (6.3.) therefore the processes linked with variability burst might be concerned as being of an epigenetic character.

Stress that occures when organisms meet an unusual environment is considered as a factor inducing variability upon which the subsequent selection pressure may act and lead to an adaptable change (Markel and Trut in Transformations of Lamarckism, Gissis and Jablonka, 2011). Theoretically the change in behavior that is observed within the foxes might be of an epigenetic information source. And as it has been conserved within a specie forming its nature, this at least could mean that the epigenetic effects might be of a huger effect than only focusing evolution through natural selection in a direction cooperatively with environmental conditions. An important behavioral trait might be of an epigenetic character, inherited inside (maybe even across) species and maybe even beyond species diversification (from the fox and dog common ancestor). This would mean epigenetic traits could go beyond one specie and supposedly could even play a role in speciation. However, we do not know what all the particular hereditary processes are involved within inheritance of behaviors. Supposedly these are combinations of hereditary pathways that function in heredity of behavior, however, it seems plausible to embrace epigenetic hereditary pathways in the set.

"..´experience´ may help to bridge the gap between learning and development by including all aspects of environmental stimuli that lead to long-term adaptive changes of behavior, ..."(Stotz, 2014).

Stotz argues that not only learning and instinct should frame our understanding of behavioral development.

"Nature and nurture don't interact as if they were separated entities, with nature as the a priori plan being separated from concrete living and nurture being the means for modifying nature's plan through experience. Instead, every trait develops out of the nonlinear interaction between arange of very diverse developmental resources that cannot be usefully divided into genetic and non-genetic resources." (Stotz, 2014)

This is why I thing all the particular traits as learning and inheritance (genetic, epigenetic, cultural) may be fused under a term of memory. Memory as being essential for life, for its ability to reproduce the developmental process again and again, in different times and context, permeating the emerge of new phenotypes. What is crucial for our understanding of evolution are the means through which memory is passed and such vary in their nature. From learning to genetic inheritance, this all may be percieved as a manner through which life uses its experience.

Animals are plastic in their behavior which is affected by environmental factors and the traits that form the behavioral phenotype might be partially passed through molecular epigenetic processes into subsequent generations where the predisposition to certain behavior meets new consequences. Environment alters behavior as well as behavior alters the environment and further the behavior of the next generations and so on in perpetuum.

8.11. Erasing as a manner of evolution

TEI as a hereditary pathway is so different from genetic heredity. TEI operates together with various heritability pathways: modulates the genome usage and is intertwined within cultural and behavioral hereditary pathways, as has been discussed previously. Nevertheless, there is a very important property evolutionary therory lacked before TEI acceptance and it is an epigenetic erasure of a trait. However previous data generally support the idea that not all the epigenetic marks are erased within a new generation, once we accept TEI, we might find mostly interesting the fact that a heritable trait might be possibly deleted.

At the molecular level, epigenetic change within the continuity of life represents a pathway that has been chosen at a particular time in reaction to a situation. Having attributes of memory, these changes can be undone in several manners – firstly within the organism itself, where these changes are influenced by other parallel interorganismal processes, secondly in the generation of a new individual (see epigenetic reprogramming), and thirdly in subsequent generations. Deletion of a trait or avoidance of a trait that would lead to death may have the very same importance as learning for survival.

In neo-Darwinian model of heredity, the possible way for disappearance of a trait was either due to subsequent accumulation of mutations in a gene essential for the trait or due to natural selection that would not allow the carriers of the trait to reproduce. Nevertheless, in case a heritable trait might be of an epigenetic character, it is possible it is deleted. The epigenetic heritable changes might emerge and also disappear, which represents a great tool for evolution to test novelties.

9 Conclusion

Epigenetics is a domain of study that concerns cellular and physiological variations that are not caused by direct changes in DNA sequence. Epigenetic processes are known to be responsible for variation within cellular lineages in one body. The organisms' or cells' fate is not predetermined simply by their genomic sequence, but it is being further influenced by environmental factors or by set conditions of the cell or organism itself. Transgenerational epigenetic inheritance was historically regarded as impossible due to the Weismann barrier paradigm, nevertheless, it has been shown that sperm and oocyte are influenced by environmental factors.
In genuine process of TEI epigenetic information is transmitted through germline to the next generation.

Epigenetic modifications regulate transcriptional activation or inactivation of specific genes, sets of genes, genomic domains or chromosomes.

DNA methylation profiles are established during development and further life. Methylation on cytosines in the body of a gene generally enhances gene activity whereas in the immediate proximity of trancriptional start site causes gene silencing, hypermethylation at CpG sites enhances change in DNA sequence of neighboring regions. DNA methylation or hydroxymethylation differs among specific cell types and is essential in cellular differentiation. Methylation regulates transcription by interfering with binding of transcription factors or by triggering factors that recruit histone deacetylases or histone methyltransferases.
The hypermethylated DNA regions have patterns of histone PTMs than hypomethylated DNA regions.

Histone PTMs are crucial in regulation of chromatine structure and the structure of chromatin regulates the accessibility of genomic sequences, affecting replication, transcription or DNA repair. Histone PTMs are involved in processes of protein degradation, gene transcription, DNA repair and replication, intracellular trafficking or virus particle budding. Acetylation on lysine is the only histone PTM known to function due to its´chemical properties – being negatively charged causes loosening of chromatin structure. The other histone PTMs enhance or supress transcription depending on which effector protein is being recruited. Histone PTMs appear to function in a manner of a code and specific patterns of histone PTMs trigger specific proteins. Nevertheles, a particular histone PTMs pattern might be recognized by divergent reading molecules whith divergent functions, therefore the effect of a histone PTMs pattern results from the concentration of reading molecules present in the cell at the time.

Histone PTMs are established by specific enzymes that react to the immediate change of

conditions as a dietary change is. Generally, histone PTMs tend to be more reversible than DNA methylation which guides the cell's differentiation and acts within cellular heritability. However, these processes operate together, one affecting another.

Chromatin structure packages into domains differently accessible for transcription due to the state of epigenetic modifications, this structure is inherited within cellular lineage even though not all epigenetic modifications that induced the structure formation are transmitted. Chromatine domains might be conserved even after the original inducing stimulus faded away in dependency on the length of time that the chromatin has spent in the particular state.

Chromatin structure is also regulated by RNAs: sncRNAs (miRNAs, siRNAs, piRNAs) and lncRNAs control genome-related processes on the basis of complementarity. lncRNAs play key roles in the control of pluripotency in ESCs, in regulation of cell differentiation, cellular cycle and in genomic imprinting. lncRNAs activate or repress genes as being precursors for miRNAs and piRNAs, through regulation of histone methylation or by acting as ceRNAs.

piRNAs and siRNAs target specific loci for histone and DNA methylation and are involved in chromatin remodeling, transposon regulation, developmental gene regulation and in genome stability maintanence. miRNAs (highly conserved within species, imperfect compatibility to targets) or siRNAs (perfect compatibility to targets) inhibit gene expression of certain genes due to complementary binding in RNAi process. ceRNAs (lncRNAs, pseudogenes and circRNAs) also known as miRNA "sponges" interact creating a regulatory net by complementary binding to miRNAs, siRNAs and ceRNAs. Hence ceRNAs may cancel or compensate function of the snRNA molecule bound. All the components of ceRNA net can directly or indirectly affect each other and a small perturbation in the concentration of one component can have a significant impact on the cellular state.. piRNAs regulate silence transposon activity in germline and are crucial in de novo DNA methylation in genome imprinting. Most importantly snRNAs have an ability to be released from the cell of their origin and be systematically distributed. Taken together with their ability to modulate other epigenetic processes in sequence specific manner snRNAs are best candidates for TEI.

Epigenetic setting of the germline is crucial for gene expression in the developement. Some of the methylation statuses are crucial for the viability of the embryo. In case of using IVF the methylation status may be affected and eventually lead to disease. Epigenetic processes in the course of development are needed for proper ontogenesis. Important epigenetic processes are two global deletions of DNA methylation – in early embryo after fertilization, the genome of the sperm is actively demethylated before the onset of DNA replication and the genome of the egg is gradually demethylated after several cleavage divisions by lack of maintenance methylation, then

during implantation to the endometrial wall within division of primordial germ cells the second demethylation happens in the cells that are to become germline. The germline therefore undergoes the both of the deletions whereas the somatic line undergoes just the first in a lifetime.

However, the deletion as incomplete, there are regions that escape general deletion: imprinted genes, transposable elements and certain single copy sequences as well. If the deletions of methylation were complete, it would be impossible to carry any information across generations.

The best evidence for transmission of epigenetic modifications is shown within imprinted genes. The imprinted genes are necessary for proper development in mammals and usually only one allele (maternal or paternal) is being expressed while the other one is silenced. The imprinted genes either escape global methylation erasure or their methylation profile is induced in a sequence specific manner and is gradually established by de-novo methylation during postnatal oocyte growth. The imprinted genes, being inherited from mother or from father result in monoallelic methylation in somatic line and persists over the whole development and future life. As the epigenetic modifications are gained during the life in response to environmental conditions and form the phenotype and some of them are transmitted throughout germline a most important question emerges here:

> Is it possible for these molecules to enable germinal transgenerational transfer of information that would be affected by history of their parents?

In other words: Does transgenerational epigenetic inheritance in mammals exist?

If yes, we would suppose phenotypic effects induced by parental experience to show in the offspring. The development and further ontogenesis affected by environmental conditions (diet, stress, infections, toxins, season of birth) create phenotypic form of an organism.
And truly, certain characteristics, which appear to be affected mostly by parental lifestyle were observed to be transmitted across several generations from parents to offspring without a change in DNA sequence within mammalian species. Such transmission that seems to be mediated by epigenetic means has been observed even in humans, however we can rarely exclude the effects of cultural transmission in the case.

Within experimental studies in animal models we can identify the actual unique TEI for there are experiments that had been designed so that we can tell the transmission to happen truly through germinal line, not through cultural or parental effects.

Considering male line, within second generation we are able to observe transgenerational epigenetic effect. The situation is different within female line, where we need to be aware if the inducing stimulus was present during gestation or not. In case the female exposed to the inducing environmental stimulus was pregnant during the exposure, we have to consider the embryo might

be affected and its germinal cells possibly as well directly by environmental effect. Therefore in the case of exposed pregnant female TEI is not showed untill the phenotype persists to generation F3. Numerous experiments were performed on exposed gestating females. During ontogenesis the environmental stimuli that affect epigenome may result in altered development of whole organs and other bodily structures.

The results of the large cohort studies in humans vary in recognizing possibly responsible processes for TEI. Nevertheless, there mostly appears a pattern of sex-specific transmission of the environmentally induced trait and the transfer happens to manifest mainly when the exposure to the environmental input happened before puberty, which indicates the involvement of gamete programming. Historical disasters that are well documented represent data source for observation of transgenerational hereditary effects in humans: malnutrition during gestation had divergent effects on developing individuals depending on the phase of gestation in which they were exposed to it, various health disorders, damages of organs and tissues and higher risk of schizophrenia was observed within those who underwent the famine in utero and further effects were observed within offspring.

Maternal starvation or overnutrition leads to alteration of metabolism in the progeny. Experiments on mice using IVF showed that metabolic disorders can be transmitted through the sperm and oocyte path, parental high-fat diet increases the risk of obesity and diabetes in offspring, obese phenotype and glucose intolerance showed in sex-dependent manner up to F2 generation.

Animals behavior is species-specific and differs in-between individuals, furthermore the behavior depends on the actual situation and is affected by individuals´ history. The behavior is triggered by emotions that are provided by hormonal release. Hormones are transported through circulatory system into various tissues where a specific response is caused in dependency on the type of particular tissue or cell type.

Endocrine disruptors, chemicals interfering with endocrine system, mimic or inhibit the actions of endogenous hormones. Experimental testing on animal models revealed that exposure to endocrine disruptors induces transgenerationally inherited epigenetic change in germline and may result in disease, infertility and altered mating choice in the progeny.

All rewarding activities increase dopaminergic transmission from VTA to NAc involving different neural pathways. Drugs have an impact on behaviour, activate the mesolimbic dopamine system and change neural circuits involved in reward and stress systems which results in a motivation for taking drugs. Addictive phenotype is underlined by changes in brain regions that are connected to reward-seeking, craving, withdrawal, anxiety, learning and memory. Histone PTMs

are involved in regulation of transcription in neuronal and glial cells in the processes of memory formation. It has been hypothesized that drugs usurp brain systems that control behaviour needed for survival and species continuity (Koob and Volkow, 2010).

Drugs, as alcohol, opiates or nicotine, induce changes in behavior that are transmitted through germline together with epigenetic changes related to genes for molecules involved in dopaminergic pathways and the behavioral and epigenetic changes show up to F2 generation.

PTSD in parents, especially mothers, was correlated with lowered cortisol levels in the progeny, as a study on holocaust survivors revealed. Stress or corticosterone treatment induce TEI of depressive-like behavior. A most notable experiment concerning TEI was performed within fear conditioning. Traumatic exposure to acetophenone lead to avoidance when the mice were exposed to the odorant later on. This behavior has been transmitted across generations through germline together with changed neuroanatomical structure – enlarged olfactory bulb for acetophenon, together with these findings hypomethylation of odorant receptor gene in sperm was found.

As far as we know, there are not any biological processes known to disprove the possible TEI, the cohort studies and experiments confirm TEI.

TEI exists in mammals as one of herditary pathways, TEI operates together with genetic inheritance, behavioral inheritance, cultural inheritance and environmental inheritance.
There is a little of a collision with modern synthesis paradigm.
Nevertheless, because TEI explains phenomena (transgenerational heredity of induced phenotypes in a sex-dependent manner), observed and experimentally tested, that are not able to be sufficiently explained by genetic inheritance only, the acceptance of TEI in mammals is required.
Experience may lead to an epigenetic emerge of a heritable phenotypic trait which may be passed through germline on the progeny. Such an epigenetically emerged phenotypic trait might be deleted in further generations.
TEI mediates phenotypic traits that might be adaptable or not.
TEI is often observed to transmit behavioral traits.
Mammalian behavior is either innate or acquired through experience of an individual.
As the acquired behavior is inherited, it becomes an innate behavior for the novel organism (e. g. mammal).

The acquired behavior that has become an innate is emotionally triggered.
Emotions are rather complex, however, very generally might be divided on positive and negative – may result in avoidance or attraction to a particular stimulus.
The connection of a particular stimulus with an emotion is affected due to experience. In case of

innate behavior the emotional connection might be affected by ancestor´ s experience.
Such an emotionally triggered behavior might be adaptale or not.
Mammalian evolution is affected by emotions, e. g. feelings. Lamarck was right at this point.
Epigenetically underlined behavioral or other phenotypic traits may disappear within epigenetic deletion or compensation even within one generation. If epigenetically underlined traits pass through germline to the next generation, these traits may be deleted in subsequent generations if not fixed.

The more people undertake a study, the more they find that there is no stable structure, no plan: everything is changing in time, and even the smallest and most apparently irrelevant events have an effect. The biological structure is, furthermore, intensely complex for us even to find causes for one particular being. However, what persists is memory, an imprint of history that has passed, and with particular memory settings, organisms interact with the environment and among themselves. Memory is stored in every single cell, and as such, every cell can alter or influence the whole biosphere. Memory itself is not rigid, it is truly plastic: not only may it rely on one particular certain input, but it keeps revising itself in time and in the end it may be deleted.

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