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The effect of the environment on bacterial DNA topology and gene expression

Vliv vnějšího prostředí na topologii DNA a genovou expresi u bakterií

Bachelor´s thesis

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Prague, 2020

Declaration:

I hereby declare that I have compiled this Thesis independently, using the listed literature and resources only. Content of the Thesis or any part of it has not been used to gain any other academic title.

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Prohlašuji, že jsem závěrečnou práci zpracovala samostatně 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.

V Praze, 04.06.2020

Podpis

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Abstract

Biological processes in the cell are affected by DNA topology, *i. e.* by DNA structure and shape. An important topological parameter is the level of supercoiling – additional twisting of DNA is relieved by positive (twisting in the same direction as the helix turns) or negative (twisting in the opposite direction) supercoils. In this Thesis I review the role of supercoiling in gene expression regulation. I describe how supercoiling is involved in homeostatic mechanisms that control the transcriptional output from some genes. Environmental changes such as shifts in temperature, oxidative stress, extreme pH and antibiotics and other inhibitors affect the level of DNA supercoiling. DNA supercoiling then affects the expression of enzymes, which influence DNA topology, as well as some other genes/proteins. In summary, this Thesis describes how changes in the environment influence bacterial DNA topology and gene expression with a brief mention of this type of regulation in eukaryotes.

Key words: DNA, DNA topology, gene expression, RNA polymerase, influence of environment, bacteria

Abstrakt

Biologické procesy v buňce jsou ovlivněny DNA topologií, tedy strukturou a tvarem DNA. Důležitý topologický ukazatel je úroveň nadšroubovicového vinutí DNA – otáčky DNA navíc jsou uvolňovány pozitivními (otáčení po směru otáčení dvoušroubovice) nebo negativními (otáčení v protisměru) nadobrátkami. V této práci se pojednává o úloze nadšroubovicového vinutí v regulaci genové exprese. Popisuji, jaké je uplatnění nadšroubovicového vinutí v homeostatických mechanismech, které ovládají produkci některých genů na úrovni transkripce. Změny prostředí, jako například změny teploty, oxidativní stres, extrémní pH a antibiotika a jiné inhibitory, ovlivňují úroveň nadšroubovicového vinutí DNA. Nadšroubovicové vinutí DNA poté ovlivňuje expresi enzymů, které ovlivňují DNA topologii, a také další geny a proteiny. Shrnuto, tato práce popisuje, jak změny vnějšího prostředí ovlivňuje DNA topologii a genovou expresi u bakterií se stručnou zmínkou této regulace u eukaryot.

Klíčová slova: DNA, topologie DNA, genová exprese, RNA polymeráza, vliv prostředí, bakterie

List of frequently used abbreviations

DNA - Deoxyribonucleic acid

RNA - Ribonucleic acid

RNAP - RNA polymerase

NAP - Nucleoid associated proteins

Topo I - Topoisomerase I

Topo IV - Topoisomerase IV

HU - Heat unstable protein

H-NS - Histone-like nucleoid structuring protein

FIS - Factor for inversion stimulation

IHF - Integration host factor

Lrp - Leucine responsive protein

App[NH]p - Adenyl-5'-imidodiphosphate

ATP - Adenosine triphosphate

Wt - Wild type

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1 Introduction

DNA is typically a double stranded helix composed of nucleotides. Nucleotides contain a base, a sugar and a phosphate. The bases provide DNA strands with the ability to pair specifically because they can form hydrogen bonds with other bases and therefore form base pairs. This is also known as the Watson-Crick pairing and plays an important role in the formation of the double helix of the DNA through the complementarity of the bases. The complementary bases in DNA are cytosine with guanine, and adenine with thymine (in RNA adenine with uracil) (Watson & Crick, 1953a). The most common conformations of DNA are A and B. The Z conformation is relatively rare. In some environments DNA and RNA rich in guanine can also form one of the G-quadruplex conformations where guanines are stacked into G-quartets (reviewed in Keniry, 2000).

DNA is involved in processes such as replication and transcription, and these processes alter DNA topology. Among these alterations belongs the introduction of supercoiling into the double helix. DNA supercoiling may be either positive or negative and can be characterised by the linking number (reviewed in Deweese, Osheroff, et al., 2008). In bacteria, DNA is kept mostly in a negatively supercoiled state. Positive supercoiling is over-twisting of the helix, while negative supercoiling is under-twisting of the helix.

Enzymes called DNA topoisomerases have the ability to change the DNA topology (reviewed in Schvartzman & Stasiak, 2004). Alterations in DNA supercoiling caused by topoisomerase mutations influence the expression of many genes because they affect transcription (M Gellert, 1981; Gmuender, Kuratli, et al., 2001; Jovanovich & Lebowitz, 1987; Steck, Franco, et al., 1993; James C. Wang & Lynch, 1993).

DNA topology and gene expression may be influenced also by other factors. These factors include specific DNA binding proteins and nucleoid associated proteins. The nucleoid is the compact structure that bacterial chromosomal DNA is folded into (Dame, 2005). Nucleoid associated proteins play an important role in the formation of the nucleoid along with DNA supercoiling and macromolecular crowding. The presence of various nucleoid-associated proteins depends on the conditions, which affect the nucleoid. H-NS-histone-like nucleoid structuring protein- and HU the heat unstable protein are examples of important DNA-associating proteins (Varshavsky, Nedospasov, et al., 1977).

The change in DNA topology in the cell may alter the properties of promoter sequences with respect to their abilities to bind RNA polymerase (RNAP) and/or transcription factors. Also, torsional stress in underwound DNA - negative supercoiling - may cause hydrogen bonds between base pairs to break. This helps transcription because it leads to the formation of single-stranded bubbles, which is crucial for transcription to occur (Yan, Ding, et al., 2018).

The environment influences gene expression too because a change in the environment can cause a different level of expression of various DNA topoisomerases, which in turn affects the supercoiling of the DNA.

The aim of this Thesis is to describe how changes in the environment can influence DNA topology and how DNA topology affects gene expression. Most of the Thesis deals with research done in bacteria but a small portion focuses also on this type of regulation in eukaryotes. The Thesis shows how expression of DNA topoisomerases I, IV, and DNA gyrase is regulated and influenced by various factors/environmental conditions such as pH, temperature, antibiotics and other inhibitors and how these enzymes can then, in turn, influence their own expression as well as the expression of other stress related proteins. Finally, the Thesis shows how DNA supercoiling can affect some promoters while other promoters are not affected at all.

2 Gene expression

Gene expression is part of the central dogma of molecular biology. It can be divided into two distinct steps. The first step is transcription, which copies DNA into RNA, and the second step is translation that turns the information contained in RNA into specific proteins (Crick, 1970). Gene expression can be influenced by many different factors. The change in the environment can activate certain proteins, which then in turn regulate gene expression in a specific way that leads to the synthesis of a crucial protein or possibly a small regulatory RNA needed for the survival of the bacteria in those specific conditions. The regulation of gene expression may take place at different stages, however most frequently it occurs during transcription initiation (reviewed in Browning & Busby, 2004; Crick, 1970; Helmann & DeHaseth, 1999).

2.1 RNA polymerase

The enzyme responsible for transcription is DNA dependent RNA polymerase (RNAP) and bacteria have only one type of this enzyme (unlike eukaryotes that have three types). The RNAP core enzyme in bacteria is made up of five subunits α_2 ββ´ω and the bacterial RNAP holoenzyme may have multiple forms. The variety of the holoenzymes is caused by various σ-factors that associate with the core. Association with the different σ-factors depends on the conditions the bacteria are in (reviewed in Browning & Busby, 2004; Helmann & DeHaseth, 1999). The α dimer serves as a scaffold for the catalytic subunits β and β´. These subunits assemble on this scaffold with the assistance of the ω subunit, which helps bind the subunits to the α dimer (Minakhin, Bhagat, et al., 2000). In general, the bacterial core enzyme structure is similar to the archaeal RNAP as well as the eukaryotic RNAPs (Cramer, Bushnell, et al., 2001; Hirata, Klein, et al., 2008; Zhang, Campbell, et al., 1999). The largest differences are located on the surface of the whole complex. The core and the holoenzyme structures resemble a crab claw, where β is one pincer and β´ is the other pincer. Within the holoenzyme-DNA complex the (C)-terminal domain and the (N)-terminal domain of the σ subunit are oriented according to the promoter. The (C)-terminal domain, specifically its σ region 4.2, binds to the -35 element of the promoter, while the σ region 2.3 - 2.4 binds to the -10 element of the promoter (see next Chapter for definition). The σ factor that is used most often mainly for housekeeping genes in bacterial cells is known as σ^{70} in *E. coli* and is responsible for the recognition of many promoters in the cell. In other species the σ^{70} is called σ^{A} (Helmann & DeHaseth, 1999; Murakami, Masuda, et al., 2002). The exact interaction between the promoter and σ^{70} is depicted in Fig. 1.

RNAP contains two important channels that are bifurcated by the bridge helix. The bridge helix is a metastable α-helix in the β' subunit of RNAP, which spans the main channel of RNAP downstream of the active site. Its role is to coordinate the movements of modules in RNAP (Hein & Landick, 2010). Promoter DNA passes through the main channel, which is the cleft between the β and β´ pincers. This led to the proposal that the narrower secondary channel serves as a route for nucleoside 5´- triphosphates

and therefore enables their access to the active site. Furthermore, it was discovered that the secondary channel may serve also as a channel for small molecules and proteins to reach the RNAP active site and this may have various effects on transcription itself (Landick, 2005).

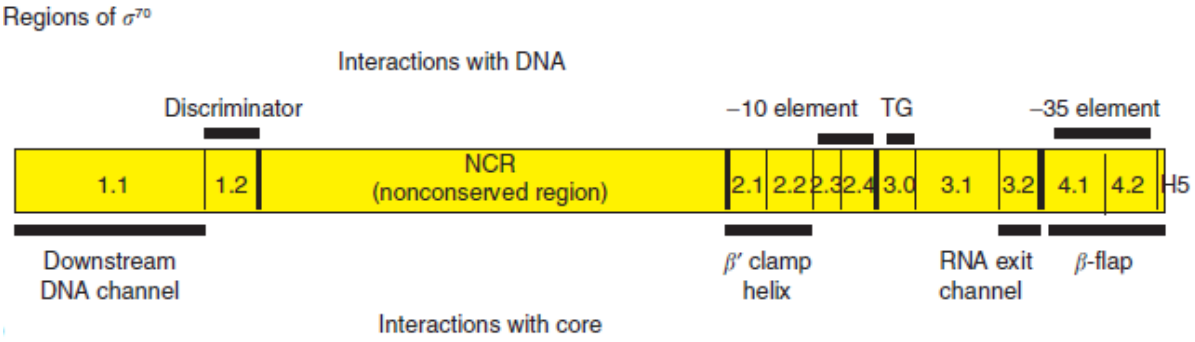

Figure 1. The interaction of σ^{70} with the promoter elements -10 and -35. The main interactions of promoter with DNA are shown on the top and interactions with the core are shown on the bottom. Adapted and modified from (Hinton, 2016).

2.2 Promoter

During studies of DNA sequences, two salient promoter elements were found. These elements are -10 and -35 (numbering relative to +1, the transcription start site) which interact with bacterial RNAP based on their sequence (Helmann & DeHaseth, 1999). The -35 and -10 sequences are hexamers separated by the spacer region, which also has an important role. It contributes to the promoter strength (Lozinski, Adrych-Rozek, et al., 1991).

The activity of a promoter is usually regulated by activators and repressors. Activators increase the activity of the promoter, whereas repressors decrease it. Activators and repressors are proteins which can bind to certain DNA sequences that are near to or possibly overlapping the RNAP binding site or to RNAP itself. This means that the regulators with the ability to bind to DNA sequences may only affect promoters, which have the transcription-factor binding sites (Barne, Bown, et al., 1997; reviewed in Browning & Busby, 2004).

Furthermore, the activity of promoters can also be influenced by proteins, which bind to RNAP and affect the kinetics of initiation for example ppGpp (nucleotides tetraphosphate and pentaphosphate) and DksA (Paul, Berkmen, et al., 2005).

2.3 Transcription

Transcription plays a part in gene expression and leads to protein synthesis. It is an important step because with the help of RNAP it transforms one DNA strand of the double helix into RNA, which can then be translated into a sequence of amino acids and finally become a protein. The process itself is divided into three steps: initiation, elongation and termination. For further review see (Browning & Busby, 2004; Helmann & DeHaseth, 1999). These steps are schematically illustrated in Fig. 2.

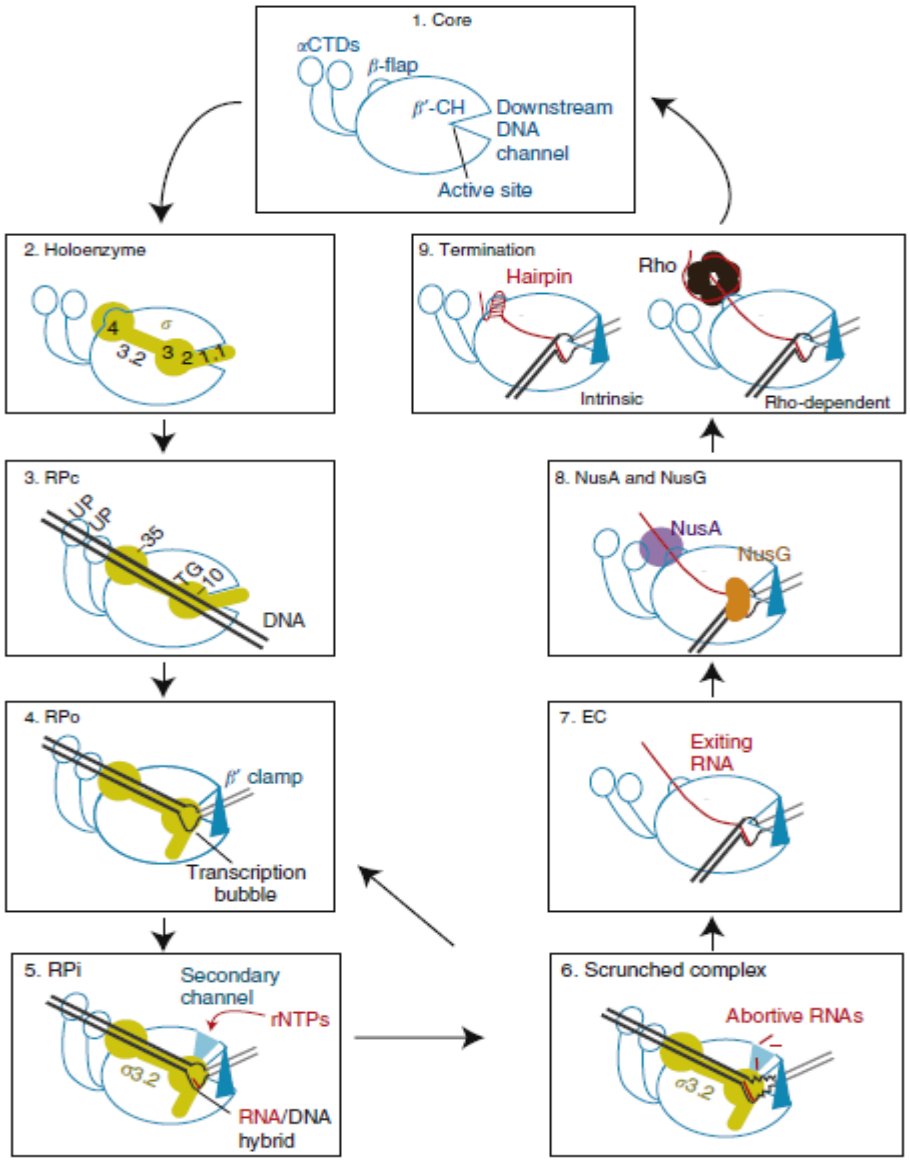

Figure 2. The structure of RNAP and stages of transcription. (1) The core RNA polymerase. Positions of the αCTDs, β-flap, β′ -CH (clamp helix), the downstream DNA channel, and active site. (2) Holoenzyme. σ is illustrated in yellow; core is illustrated in cyan. Positions of σ Regions 1.1, 2, 3, 3.2 and 4 are shown. (3) Closed complex (RPc). Promoter DNA lies across RNAP. Positions of promoter elements (UP, -35, '$^{-15}$TG^{-14}', and -10) are shown. (4) Open complex (RPo). Isomerization of RNAP along with bending, unwinding of the DNA generates the transcription bubble around the start site and the β' clamp. This secures the DNA within the downstream DNA channel. (5) Initiating complex (RPi). The rNTPs enter through the secondary channel. The rNTPs start RNA synthesis. The initially transcribed RNA stays annealed to the DNA template. This creates an RNA/DNA hybrid of 8–9 bp. (6) Scrunched complex. RNA is made as RNAP moves forward and pulls the DNA into the active site, but the promoter is not released. The collapse of the scrunch complex is caused by the release of small abortive transcripts. The complex returns to RPo. (7) Elongating complex (EC). RNA exits through the RNA exit channel. This channel displaces σ Regions 3.2 and 4, eventually results in the loss of σ. (8) Elongation factors NusA and NusG. NusA interaction with β-flap, αCTD, and RNA. NusG interaction with the β' -CH. It extends over the DNA upstream of the transcription bubble to hold the DNA more securely. (9) Termination. The RNAP/DNA/RNA complex is disturbed either by a terminating hairpin (intrinsic termination) or a hexamer of Rho protein (Rho-dependent termination). Adapted from (Hinton, 2016).

2.3.1 Initiation

The promoter DNA -10 and -35 elements are recognized by bacterial RNAP in complex with a σ factor, the holoenzyme. In order to initiate transcription, a transient complex must form and undergo multiple conformations and kinetic energy changes. It is thought that there exists a mechanism, which is common for all promoters. RNAP bound with the promoter (RP) forms three intermediate complexes, which are closed (RP_C), intermediate (RP_I) and open (RP_O) complex. For the conformational changes between these complexes to occur the complex must undergo certain changes - isomerizations. We can describe the processes as "binding, nucleating, and melting". The transcriptional machinery and its movements along the way to initiation are driven by binding free energy derived from the conformations and interfaces of earlier intermediates, not by the hydrolysis of ATP. In the closed complex the two strands of DNA are not yet separated; however once the RNAP isomerizes it forms the open complex where the transcription bubble forms. Throughout initiation short abortive RNA products can be produced or slippage of RNAP may occur and these events may have regulatory functions (Barne, Bown, et al., 1997; Helmann & DeHaseth, 1999).

2.3.2 Elongation

A mature elongation complex may form only once RNAP breaks contact with the promoter and the σ factor is released. RNAP can then catalyse the addition of nucleotide triphosphates complementary to the template DNA and start forming RNA. A DNA-RNA hybrid is formed, and the complementary RNA continues to be synthesized. The RNA exits through the RNA exiting channel in the RNAP. For further review see (Uptain, Kane, et al., 1997).

2.3.3 Termination

Bacterial transcription is normally terminated by: intrinsic termination and Rho-dependent termination. In special cases, when RNAP is stalled/inactive on DNA, transcription can be terminated for example by Mfd (transcription-repair coupling factor)-dependent termination or by RNase J1 (Šiková, Wiedermannová, et al., 2020).

Intrinsic termination consists of the activity of the core RNAP and a sequence encoding an RNA hairpin with a uridine-rich terminal segment. Formation of the hairpin affects the conformation of RNAP, releases the RNA-DNA hybrid within the U-tract and dissociates RNAP from nucleic acids.

Rho-dependent termination uses the enzyme ATP-dependent RNA translocase Rho. Rho releases RNA by changing the structure of the elongating complex.

Mfd-dependent termination targets the elongation complex. The elongation complex is dissociated by the enzymatic activity of the ATP-dependent translocase. For further review see (Mitra, Ghosh, et al., 2017; Peters, Vangeloff, et al., 2011; J. Roberts & Park, 2004; J. W. Roberts, 2019).

RNase J1 then targets stalled transcription complexes and is able to disassemble them, preventing thus transcription-replication collisions, which might have deleterious consequences for the cell (Šiková, Wiedermannová, et al., 2020; Svetlov & Nudler, 2020).

3 DNA topology

DNA consists of two complementary strands, which are interwound and form a double helical structure. The double helix can be either left-handed or right-handed. The most common form is B-DNA, which is right-handed with 10.5 base pairs (bp) per turn in physiological conditions. The less common form is A-DNA, which is also right-handed but more compact than B DNA (Franklin & Gosling, 1953; Watson & Crick, 1953a).

A circular-closed DNA duplex is characterized by the linking number (Lk). The linking number is the number of times the duplex is intertwined. The linking number is the sum of the number of twists (Tw) and writhes (Wr). Twist is the number of double helical turns, where the right-handed twist of the Watson-Crick structure is given a positive value. Writhe is the amount of times the helix crosses itself in space as is displayed in Fig. 3.

$$Lk = Tw + Wr$$

The linking number can be changed only by the breakage of strands and their subsequent rejoining but the proportional amount of twist and writhe can differ depending on the conditions of the solution (Maxwell & Gellert, 1986).

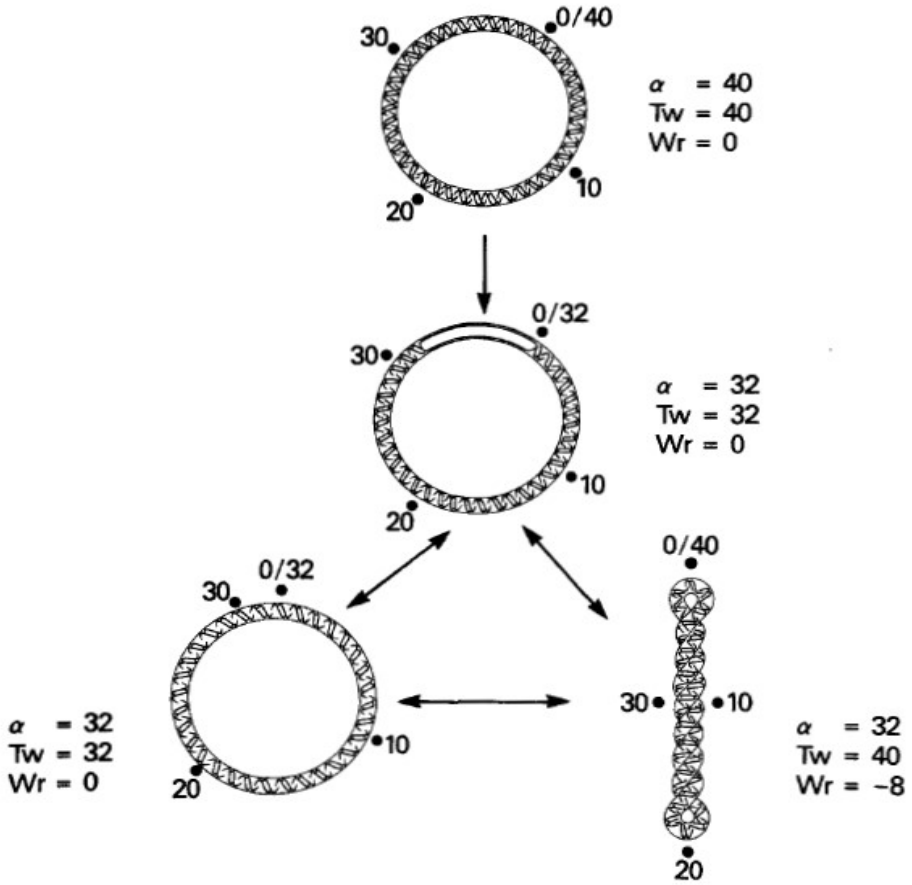

Figure 3. The connection between the Linking number, twist and writhe. Linking number in this figure is represented by α, twist by Tw and writhe by Wr. The first illustration, from top to bottom, depicts a relaxed closed-circular DNA. The numbers next to the molecules symbolize the counting of twists. The molecules are not two dimensional; therefore, the writhe number is always zero. Adapted from (Maxwell & Gellert, 1986).

Binding sites in the molecules can be brought closer together by writhing and looping of the DNA. When this occurs, it may result in inhibition or enhancement of the formation of the open complexes or the isomerization to open complexes (Yan, Ding, et al., 2018).

3.1 Conformation of DNA

The most common form in which we can find DNA is in a double helix, which can be found in different conformations. This double helix has more conformations besides A and B. The conformation it assumes is mainly influenced by the conditions it is in; typically, it depends on the presence of water. When DNA or RNA contains a large amount of guanine, they can form G-quadruplexes. There are many other alternative structures DNA can adopt; examples are Z-DNA, bubbles, slipped loops and cruciforms, H-DNA as well as four-stranded structures. H-DNA is a triple helix; therefore, it contains three DNA strands in which the third strand forms Hoogsteen base pairing with a double strand containing Watson-Crick pairing (Harvey, Luo, et al., 1988; Htun & Dahlberg, 1988; Lyamichev, Mirkin, et al., 1985; Sundquist & Klug, 1989; Watson & Crick, 1953b).

A double helix in an A-DNA conformation is stiffer than when it is in a B-DNA conformation. Therefore, distortion is less energetically favourable in A-type double helixes than in B-type double helixes. This aspect indicates that B-DNA should be the structure preferred for any packaging with tight DNA bending (Hormeño, Ibarra, et al., 2011). B-DNA is more stable in high humidity, while A-DNA forms for example in crystals that contain small amounts of water,. (Franklin & Gosling, 1953). Another factor affecting the equilibrium between A-DNA and B-DNA, along with the concentration of water, is base-type The substitution of bases can affect the presence and placement of the purine 2-amino group of guanine in the minor groove. This can then in turn affect the intrinsic curvature and DNA bendability.(Bailly, Waring, et al., 1995).

3.2 DNA supercoiling

The double helical structure poses an obstacle for processes that it must undergo. These processes need a short- or long-term separation of complementary strands of the double helix. The duplexity of the DNA inevitably results in a topological by-product, for example the formation of supercoils. The alteration in the topology is resolved by DNA topoisomerases (reviewed in Bates & Maxwell, 2005; Deweese, Osheroff, et al., 2008; Vos, Tretter, et al., 2011). DNA topoisomerases bind to DNA where they cleave one or both strands of the double helix and pass the other strand of the helix or a different double strand through the break and conclude by resealing the DNA phosphate backbone. In order to cleave DNA a transient phosphodiester bond must form between one end of the broken strand and a tyrosine in the active site of the topoisomerase. Several topoisomerases need divalent metal ions as cofactors to cleave and religate DNA (Ghilarov & Shkundina, 2012; Sissi & Palumbo, 2010; reviewed in James C. Wang, 2009).

DNA gyrase (belongs to subclass Type II topoisomerases) along with type I DNA topoisomerases (reviewed in Champoux, 2001) determine the amount of supercoiling of the DNA and resolve various topological problems caused by different biological processes such as DNA replication, transcription, repair, recombination and decatenation (Drlica, 1984; Forterre, Gribaldo, et al., 2007; M Gellert, 1981).

Two types of DNA supercoiling exist: positive or negative. Positive supercoiling means over-twisting of the helix, while negative supercoiling is under-twisting of the helix. Negative supercoiling allows DNA binding proteins to bind to the DNA and the initiation of replication of bacterial plasmids depends on it as well. It is required because it leads to the unwinding of the origin sequence (reviewed in Schvartzman & Stasiak, 2004).

Alteration of DNA topology may also be caused by transcription. DNA may rotate around its own axis so that RNAP can follow the helical path of the DNA strands (L. F. Liu & Wang, 1987). This rotation may result in the formation of positive supercoils in front of the transcription complex in the process of elongation and negative supercoils behind it. The enzymes DNA gyrase, topoisomerase IV and topoisomerase I can eliminate these supercoils in prokaryotes. The positive supercoils are removed by DNA gyrase, while topoisomerase I relaxes the negative supercoils (Lockshon & Morrs, 1983; G. J. Pruss & Drlica, 1986; E. L. Zechiedrich & Cozzarelli, 1995). It has been shown that the amount of supercoiling in the bacterial cell can influence the transcription of various genes (Dorman, 2008; Lilley, Chen, et al., 1996). Supercoiling affects the binding of DNA to RNAP and other proteins, which can repress or activate transcription. Transcription of genes encoding topoisomerase I (*topA*) and DNA gyrase (*gyrA* and *gyrB*) is affected by the amount of supercoiling in what is believed to be a homeostatic mechanism to control the extent of supercoiling in the cell. The increase of negative supercoiling results in the increased transcription of *topA* and decreased transcription of *gyrA* and *gyrB*. The main role of topoisomerase IV is supposedly in decatenation but it has been demonstrated that it affects DNA relaxation as well. This means topoisomerase IV plays a role in the level of supercoiling along with topoisomerase I and DNA gyrase (Deibler, Rahmati, et al., 2001; Menzel & Gellert, 1983; E. Lynn Zechiedrich, Khodursky, et al., 2000).

Furthermore, supercoiling of the DNA plays a role not only in transcription elongation but also in transcription initiation. It has been established that the function of the bacterial promoter may be strongly affected by the degree of supercoiling in the DNA template. Usually negative supercoiling of the DNA favours melting of the promoter; however, the response of promoters to alterations in DNA supercoiling may be different due to a variation in the promoter sequence. This means that some promoters may be strongly affected by the change in supercoiling caused by a *topA* mutation or possibly by the inhibition of DNA gyrase whereas some promoters seem to be unaffected in the same conditions (Dorman, 1995; Gmuender, Kuratli, et al., 2001; Jovanovich & Lebowitz, 1987; Gail J. Pruss & Drlica, 1989; Steck, Franco, et al., 1993; J C Wang, 1985; James C. Wang & Lynch, 1993).

3.3 Enzymes with influence on DNA topology

This Chapter describes in detail enzymes that influence DNA topology. A large group of these enzymes are DNA topoisomerases. We can divide these DNA topoisomerases into two groups type I topoisomerases and type II topoisomerases. Type I can generate transient single-strand DNA breaks, while type II can create double-strand DNA breaks (Leroy F. Liu, Liu, et al., 1980). Topoisomerases I and III and reverse gyrase belong amongst type I topoisomerases, whereas topoisomerases II, IV, V and VI along with DNA gyrase are type II topoisomerases. The reactions catalysed by DNA topoisomerases and DNA gyrase through which they control the amount of supercoiling can be seen in Fig. 4 and in Fig. 5.

3.3.1 Topoisomerase I

Topoisomerase I (Topo I) is a type I topoisomerase and it was previously known as the ω protein. It was the first ever discovered topoisomerase (James C. Wang, 1971, 2009). It is present in both eukaryotes and prokaryotes and is able to relax negatively supercoiled DNA and catenate and decatenate nicked DNA. The Topo I enzymes in bacteria are only able to relax negatively supercoiled DNA, while the Topo I enzymes in eukaryotes are capable of relaxing both positively and negatively supercoiled DNA. Topo I in bacteria is encoded by *topA* and relaxes negatively supercoiled DNA through an ATP-independent reaction (James C. Wang, 1971). If there is a loss in the activity of Topo I it leads to an increase in the negative supercoiling of the DNA (Jaworski, Higgins, et al., 1991; Gail J. Pruss, 1985; Gail J. Pruss, Manes, et al., 1982).

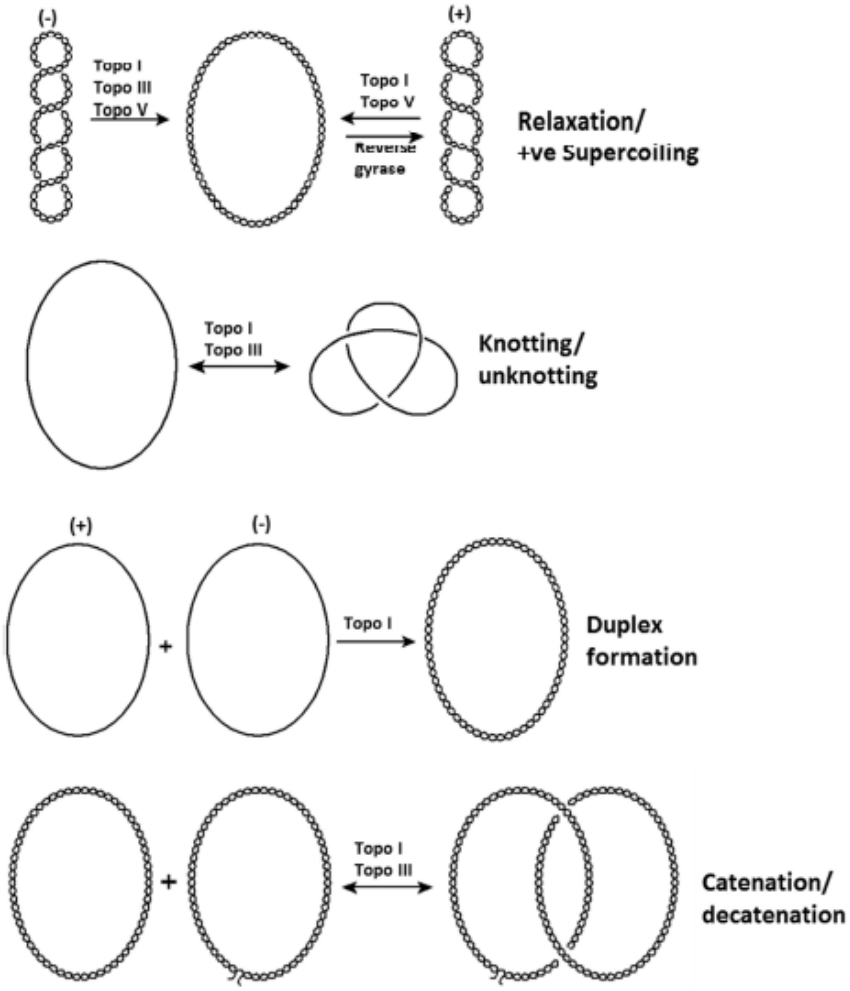

Figure 4. Topoisomerase type I reactions. The topoisomerases catalysing the reactions are shown above the arrows. In the case of the decatenation/catenation reaction, the non-nicked plasmid could be supercoiled before decatenation/catenation even though here it has been drawn as relaxed. Adapted from (Maxwell, Bush, et al., 2015) previously modified from (Maxwell & Gellert, 1986).

3.3.2 DNA gyrase

DNA gyrase can be found in bacteria and it has been mainly studied in *E. coli* where it consists of two 97-kDa GyrA subunits and two 90-kDa GyrB subunits These subunits form a tetramer. The GyrA subunit is encoded by the *gyrA* gene, while the subunit GyrB is encoded by the *gyrB* gene (Adachi, Mizuuchi, et al., 1987; Klevan & Wang, 1980; Akio Sugino, Higgins, et al., 1980; Swanberg & Wang, 1987).

DNA gyrase is a type II topoisomerase and can introduce negative supercoils into covalently closed double-stranded DNA with the use of ATP (Martin Gellert, Mizuuchi, et al., 1976). It can relax positive supercoiling of DNA using ATP hydrolysis (M Gellert, O'Dea, et al., 1976; A Sugino, Higgins, et al., 1978). It was previously discovered that it can perform decatenation and knotting reactions when ATP is present. There are assumptions that it is also able to catenate and knot DNA (Kreuzer & Cozzarelli, 1980; Leroy F. Liu, Liu, et al., 1980; Marians, 1987; Mizuuchi, Fisher, et al., 1980). DNA

gyrase is also capable of relaxing negative supercoils through an ATP-independent reaction (M Gellert, Fisher, et al., 1979; Higgins, Peebles, et al., 1978). In eukaryotes DNA gyrase seems to be absent. Therefore, this bacterial enzyme is a *bona fide* target of antibacterial drugs, such as novobiocin and coumermycin where it inhibits their ATPase activities and this causes a decrease in negative supercoiling of DNA (M Gellert, O'Dea, et al., 1976).

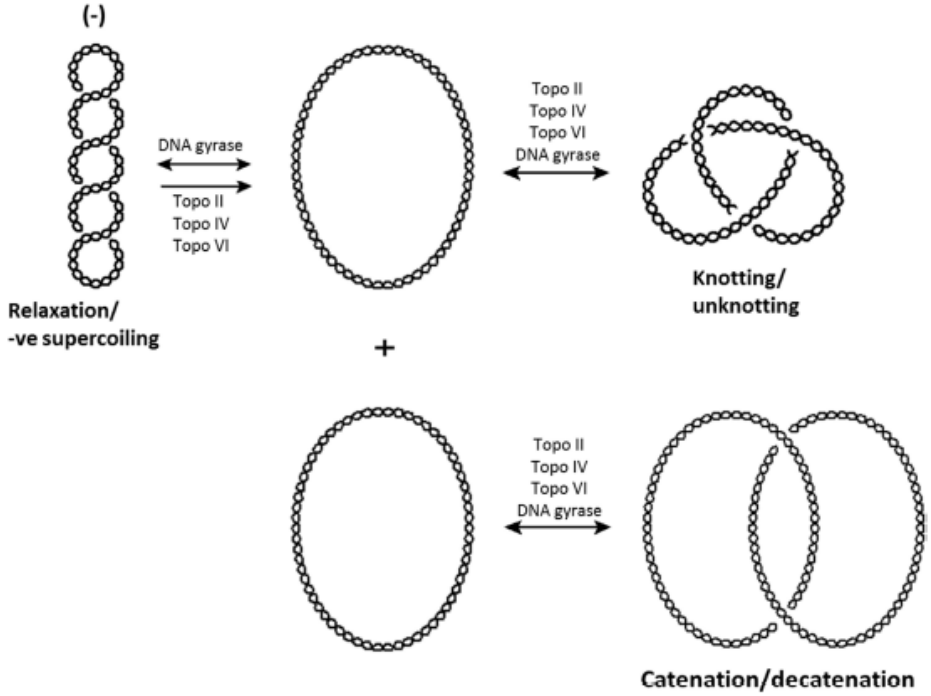

Figure 5. Topoisomerase type II reactions. The topoisomerases catalysing the reactions are shown above the arrows. As was stated in the previous Fig. 4 the plasmids in the decatenation/catenation reaction could be supercoiled but are shown as relaxed here. All type II topoisomerases are able to relax positively supercoiled DNA as well as negatively supercoiled DNA even though only negatively supercoiled DNA is illustrated in this figure. Adapted from (Maxwell, Bush, et al., 2015) and previously modified from (Maxwell & Gellert, 1986).

3.3.3 Topoisomerase IV

Topoisomerase IV (Topo IV) is one of the type II topoisomerases found in bacteria (reviewed in Levine, Hiasa, et al., 1998). It can decatenate the products of replication, relax positively and negatively supercoiled DNA as well as knot and unknot DNA (Crisona, Strick, et al., 2000; Deibler, Rahmati, et al., 2001; López, Martinez-Robles, et al., 2012; H Peng & Marians, 1993). Topo IV uses the hydrolysis of ATP for these reactions. Topo IV consists of two subunits. They are encoded by the *parC* and *parE* genes in *E. coli* and *Salmonella* but in some other organisms the same genes are known as *grlA* and *grlB* (Baba, Kuwahara-Arai, et al., 2009; Ferrero, Cameron, et al., 1994; Takami, Takaki, et al., 2002). The ParC subunit is homologous with the GyrA subunit and the ParE subunit is homologous with GyrB. Subunits GyrA and GyrB are found in DNA gyrase, however there are some differences between gyrase and Topo IV; for example Topo IV cannot introduce negative supercoils into DNA while DNA gyrase can (Kato, Nishimura, et al., 1990; H. Peng & Marians, 1995). In *E. coli, in vivo*, Topo IV was shown

to be 100x more active at decatenation than DNA gyrase (E. L. Zechiedrich & Cozzarelli, 1995). Previous findings seem to imply that the compaction of DNA by DNA gyrase is crucial for the activity of Topo IV (Ullsperger & Cozzarelli, 1996), suggesting that one of the roles of DNA gyrase is to supercoil DNA catenanes in order for them to be better substrates for Topo IV.

3.4 Nucleoid associated proteins

All organisms need the means to compact their DNA because they rely on its storage and consequent replication to survive. This is mediated by nucleoid associated proteins (NAPs), which modify the shape of the DNA. The compaction of DNA can then influence transcription. (Delius & Worcel, 1974; reviewed in Luijsterburg, White, et al., 2008).

3.4.1 Bacteria

Bacteria contain chromosomal DNA folded into a compact structure - nucleoid. DNA supercoiling, macromolecular crowding and nucleoid associated proteins play important roles in defining the shape and size of the nucleoid. Certain NAPs can be associated with the nucleoid when found in particular conditions. The first NAPs to be isolated were H-NS (histone-like nucleoid structuring protein) and HU (heat unstable protein) (Varshavsky, Nedospasov, et al., 1977). Later on, Murphy and Zimmerman (1997) discovered two other proteins in the nucleoid: Fis (factor for inversion stimulation) and IHF (integration host factor) (Murphy & Zimmerman, 1997). Due to the high abundance of these four NAPs they are believed to be the most important factors in nucleoid compaction. Other new proteins have been discovered: the protein Lrp (leucine responsive protein) (Tapias, López, et al., 2000) and MukBEF (P. L. Graumann, 2001). For other important NAPs see Table 1 below.

3.4.2 Eukaryotes

Histones are proteins that modify DNA to form a more compact structure in eukaryotes. Histones affect the structure of chromatin and may influence transcription as well. The core histones are H2A, H2B, H3 and H4 and the linker histones are H1 and H5. For other NAPs see Table 1 below. For further review see (Luijsterburg, White, et al., 2008).

3.4.3 Archaea

Archaea can be divided from this point of view into two groups: Euryarchaeota and Crenarchaeota. The chromatin in Euryarchaeota has similarities with the chromatin in Eukaryotes, while Crenarchaeota show similarities with bacterial DNA looping with major roles of DNA bridgers and benders and usually lack histones. For specific examples of NAPs of Archaea see Table 1. For further review see (Luijsterburg, White, et al., 2008).

Table 1. Key nucleoid associated proteins of Eukaryotes, Bacteria and Archaea. Adapted and modified from (Luijsterburg, White, et al., 2008).

		wrappers	*benders*	*bridgers*
Eukaryotes		H2A, H2B, H3, H4 (core histones)	HMG	H1 (linker histone)
				BAF
				SMC
Archaea	Crenarchaea	*Lrp**	Cren7	Alba
			Sul7	Lrp*
				SMC
	Euryarchaea	HMfA and HMfB (histones)	MC1	Alba
		*Lrp**	HU	Lrp*
				SMC
Bacteria	Gram positive	*Lrp**	HU	Lrp*
				Lsr2 (H-NS-like)
				SMC
	Gram negative	*HU**	HU*	H-NS
		*Lrp**	IHF	Lrp*
			Fis	*Fis**
				SMC
				H1-like proteins

*these proteins have been proposed to exhibit dual architectural properties, that are likely dependent on protein concentration or DNA binding sequence. The secondary binding mode is indicated in italic.

4 Environmental change and its influence on DNA topology and gene expression

Environmental change influences gene expression through affecting the level of DNA topoisomerases and DNA gyrase (L. F. Liu & Wang, 1987). The accumulation of negative supercoiling occurs when Topo I is absent. During stress many genes have to be induced rapidly to enable survival of the organism. This is ensured when transcription-driven supercoiling is removed by higher levels of topoisomerase and therefore is important in stress response (Massé & Drolet, 1999; G. J. Pruss & Drlica, 1986). Throughout the response to environmental change the DNA supercoiling can be influenced by NAPs like FIS, IHF, HU and HNS. This occurs through the effects that the NAPs have on the level of expression and activity of DNA gyrase and Topo I (Schneider, Travers, et al., 1999). Different environmental changes may affect different promoters and regulons and use different RNAP sigma factors as is mentioned further on (Eisenstark, Calcutt, et al., 1996). However, the overall process is always controlled through the amount of DNA supercoiling, which depends on the activity of various DNA topoisomerases, the binding of NAPs (Eisenstark, Calcutt, et al., 1996; Menzel & Gellert, 1983; J.-Y. Wang & Syvanen, 1992) and on polyamines. Polyamines are metabolites, which are topologically active. The content of these metabolites considerably changes when the bacterial cell is under stress (Tkachenko, Salakhetdinova, et al., 1997).

In *E. coli*, DNA supercoiling regulates DNA gyrase and topoisomerase I and this contributes to the overall homeostatic regulation of its DNA supercoiling. Transcription of the plasmid-borne *topA* promoter fusions *in vitro* is less efficient on relaxed DNA templates and more efficient on DNA templates with negative supercoiling (Y C Tse-Dinh & Beran, 1988; Tse-Dinh & Yuk-Ching, 1985). Nevertheless, chromosomal *topA* transcription is quite insensitive when it comes to fluctuations of the DNA supercoiling levels in wild-type (wt) cells (Snoep, van der Weijden, et al., 2002). On the other hand, the relaxation of the DNA template stimulates transcription of *gyrA* and *gyrB* (Menzel & Gellert, 1983, 1987) and their transcription is more sensitive to the regulation by wt. levels of DNA supercoiling (Snoep, van der Weijden, et al., 2002). In *Haemophilus influenzae*, when a DNA gyrase inhibitor was added (novobiocin), transcription of *gyrA* and *gyrB* was found to be upregulated, while *topA* was downregulated. This corresponds with the homeostatic control mechanism through the amount of DNA supercoiling. DNA gyrase synthesis was shown to have been activated by the relaxation of DNA in the gram-negative *Haemophilus influenzae* (Gmuender, Kuratli, et al., 2001), gram-positive *Streptomyces sphaeroides* (Thiara & Cundliffe, 1989), gram-positive *Mycobacterium smegmatis* (Unniraman & Nagaraja, 1999) and neither gram-positive or negative *Mycobacterium tuberculosis* (Unniraman, Chatterji, et al., 2002).

In *E. coli* the transcription of *topA* is directed by four promoters. These promoters are P1, P2, P4 and Px1. The main sigma factor of RNAP in *E. coli* σ^{70} recognizes promoters P2 and P4. Promoters

P1 and Px1 are recognized by different sigma factors. P1 is directed by σ^{32}, while Px1 is controlled by σ^{S}. Sigma-32 is involved in the heat shock response (Lesley, Jovanovich, et al., 1990) and σ^{S} is active throughout the stationary stage of growth and in different stress conditions. Even though the nucleotide sequence of the P3 promoter appears similar to the σ^{70} consensus recognition sequence, its use in the transcription initiation of chromosomal of *topA* has not yet been discovered. The sum of activities from these various promoters controls the level of expression of *topA*. During the shift between exponential phase and stationary phase the level of Topo I protein remains relatively constant, due to the increased activity from the promoter Px1 and the decreased activity from the adjacent promoter P4 (H. Qi, Menzel, et al., 1997). A similar occurrence happens during heat shock (L. Qi, Menzel, et al., 1996). The sequences of promoters P1, P2, P3, P4 and Px1 are shown in Fig. 6.

Figure 6. The promoters of the *topA* gene P1, P2, P3, P4 and Px1 located at the 5′ control region in *E. coli*. Binding sites of NAPs IHF and Fis are also illustrated (H. Qi, Menzel, et al., 1997; Y C Tse-Dinh & Beran, 1988; Weinstein-Fischer, Elgrably-weiss, et al., 2000). The starting ATG is in bold in the bottom left corner of the illustration. Redrawn according to (Rui & Tse-Dinh, 2003).

In Eukaryotes the environment plays a role too. Eukaryotes react to different kinds of stress factors in ways that also lead to remodelling of chromatin as well as altering transcriptional and translational regulation. A major role in the response to environmental stress is played by Stress Granules (SGs). Stress Granules are dynamic aggregates of ribonucleoproteins. Eukaryotes deal with stress by decreasing the expression of housekeeping genes and increasing the expression of genes that are stress-induced and repair damage caused by stress. The result of this translational inhibition is the formation of Stress Granules, which form for example in response to heat shock or oxidative stress. For further review see (Atkinson & Walden, 1985; Palangi, Samuel, et al., 2017).

4.1 High and low temperature

Both high and low temperature can affect bacterial gene expression. This occurs usually through heat shock proteins at high temperatures and cold shock proteins at low temperatures. These proteins ensure transcriptional changes so that the bacterial cell can adjust and survive these conditions. The modifications of transcription through topological changes result in the expression of stress-induced genes. Previous studies where mesophilic bacteria were compared with hyperthermophilic archaea reveal that the activity of DNA topoisomerases and protein binding are both influenced by temperature. They are, therefore, major factors in the control of DNA topology during stress response. Topological changes can be caused by heat shock and cold shock proteins. However, it is possible that the overall change in gene expression is caused by the combined effect of these proteins and the effect of the temperature on cellular components, which causes transient changes and modifies the DNA topology itself (López-García & Forterre, 2000). As was mentioned before, the promoters of the *topA* gene encoding topoisomerase I play a role in the change in DNA topology caused by thermal stress. In *E. coli* in reaction to a shift in temperature to 42°C the activity from promoter P1 increases, while the activity from other *topA* promoters decreases (L. Qi, Menzel, et al., 1996).

When temperature of 42°C is applied to *E. coli,* a sudden heat shock occurs and causes a transient relaxation of plasmid DNA after 2 minutes. This transient relaxation increases the linking number Lk (Mizushima, Natori, et al., 1993). The change of plasmid topology by heat shock is illustrated in Fig. 9. After detailed studies of *E. coli*, it has been concluded that Topo I and DNA gyrase are both involved in the relaxation of DNA. This has been proven through experiments with *topA* mutants and with DNA gyrase inhibitors like the antibiotic novobiocin (M Gellert, O'Dea, et al., 1976; Ogata, Mizushima, et al., 1994). After a longer period of time after the application of 42°C, DnaK, a heat shock protein, has a stimulating effect on the supercoiling activity of DNA gyrase. This results in the re-supercoiling of DNA (Ogata, Mizushima, et al., 1996). Another necessary component for re-supercoiling of the DNA is the NAP HU; this was shown through observing the effects of mutations in the genes encoding this protein (Ogata, Inoue, et al., 1997). HU supposedly also facilitates the activity of DNA gyrase. Other NAPs do not seem to be involved in the response to heat shock (Malik, Bensaid, et al., 1996). The main proteins that affect the supercoiling of DNA and play a role during heat shock in the mesophilic *Escherichia coli* are summarised below in Fig. 7.

Escherichia coli

	Protein *(gene/s)*	Main features	Role during thermal stress
DNA topoisomerases			
Type I[a]	Topoisomerase I *(topA)*	ATP-independent relaxation of negative supercoils	Immediate relaxation after heat shock; Recovery of normal topology after cold shock (?)
Type II[b]	Gyrase *(gyrA, gyrB)*	ATP-dependent Lk decrease (with introduction of negative supercoils)[8] or Lk increase[21]	Relaxation and resupercoiling after heat shock; Negative supercoiling increase during cold shock
DNA-binding proteins	HU[c] *(hupA, hupB)*	Bending and unwinding	Recovery of negative supercoiling after heat shock; Negative supercoiling increase suring cold shock
Chaperones	DnaK *(dnaK)*	Protein folding and stabilization (Hsp70 family)	Recovery of normal supercoiling after prolonged heat shock by stimulating gyrase

Figure 7. Proteins that control the supercoiling of DNA in *Escherichia coli* during heat shock. (a) Topo III found in bacteria and archaea does not have a defined function. It appears to have a role in replication and is an RNA topoisomerase (J C Wang, 1985). (b) Another type II topoisomerase – topo IV- is found in *E.coli* with a major role in chromosome decatenation, but a minor role in DNA relaxation(Deibler, Rahmati, et al., 2001). (c) Other NAPs do not seem to be involved in the response to heat shock (Kataoka, Mizushima, et al., 1996; Malik, Bensaid, et al., 1996). Adapted and modified from (López-García & Forterre, 2000).

In other species, it has been shown that DNA supercoiling changes *in vivo* due to high temperature through experiments with reporter plasmids in the gram-negative *Salmonella typhimurium*, gram-positive *Bacillus subtilis* and gram-negative *Yersinia enterocolitica* (Dorman, Bhriain, et al., 1990; Grau, Gardiol, et al., 1994; Rohde, Fox, et al., 1994).

Cold shock has an opposite effect on DNA supercoiling than heat shock. In *E. coli* cold shock decreases the linking number (Lk) through an increase of transient negative supercoiling. The change of plasmid topology induced by cold shock is shown in Fig. 9. The protein HU and DNA gyrase are both involved in the response to cold shock and therefore in the transient change in the supercoiling of the DNA. Another difference (compared to heat shock) is that in order for the DNA supercoiling to return to normal, no protein synthesis is necessary (Mizushima, Kataoka, et al., 1997). The transient changes in DNA supercoiling induce the production of cold induced proteins (Cips) (P. Graumann & Marahiel, 1996; Sangita Phadtare, 2004). The production of these proteins increases depending on the severity of the cold shock (Hébraud & Potier, 1999).

Usually cold shock occurs when there is a decrease in temperature from 37°C to approximately 10-8°C. When this happens, protein synthesis of most proteins is stopped and after 2 hours only 28

proteins are synthesized; among these proteins belongs the cold shock protein CspA. After the lag period caused by the decrease in temperature the protein synthesis of additional 50 proteins occurs. These 50 proteins can be found in the cell even in normal physiological conditions. Only 13 proteins out of the total number of proteins (78 proteins) are synthesized at a faster rate during cold shock than at normal physiological temperatures (P. Graumann & Marahiel, 1996; P. G. Jones, VanBogelen, et al., 1987; Pamela G. Jones & Inouye, 1994). During the lag period, induced by cold shock the rate of synthesis of the protein CspA increases about 100 times (Goldstein, Pollitt, et al., 1990). Giuliodori *et al.* discovered that the cspA mRNA from *E. coli* undergoes a structural change when it is exposed to low temperatures. This results in more efficient translation of *cspA* mRNA. This cspA mRNA is not as likely to be degraded when compared with the same mRNA at 37 °C due to a change in its structure (Giuliodori, Di Pietro, et al., 2010). In *E. coli* a binding site for CspA had been discovered in the regulatory region of the *gyrA* gene, which encodes the α subunit of DNA gyrase (Pamela G Jones, Krah, et al., 1992). The ATTGG motif is found in the promoter region of the *gyrA* gene in three copies, therefore, the α subunit of DNA gyrase can be considered a cold shock protein. Its synthesis is activated by the presence of multiple binding sites for CspA. It is highly possible, that the decrease in activity of DNA gyrase at low temperatures is compensated by its higher synthesis. In this way a certain level of DNA supercoiling is maintained so that its transcription is ensured. On the other hand, the β subunit of DNA gyrase in *E. coli* is synthesized at an identically low level at both 37°C and 10°C. There are no binding sites for CspA, therefore it is not needed for the synthesis of the β subunit (Pamela G Jones, Krah, et al., 1992).

The CspA protein belongs to a family of nine homologous proteins (CspA to CspI); the genes encoding them and their functions in *E. coli* can be seen in Table 2 (Yamanaka, Fang, et al., 1998). Only five of these proteins are synthesized specifically during cold shock - CspA, B, CspE, G and CspI (Etchegaray, Jones, et al., 1996; Nakashima, Kanamaru, et al., 1996; Uppal, Rao Akkipeddi, et al., 2008; N. Wang, Yamanaka, et al., 1999). It was discovered by Xia *et al.* (2001) that four genes - *cspA*, *cspB*, *cspE* and *cspG* - when deleted in *E. coli* cause the cell to become sensitive to cold. Furthermore, if one or two *csp* genes were deleted it resulted in an increased and prolonged synthesis of the remaining *csp* genes induced by cold. This suggests that the proteins of the CspA family overlap in function and can potentially compensate for each other (Xia, Ke, et al., 2001). Graumann *et al.* (1997) discovered the same concept in *Bacillus subtilis* (P. Graumann, Wendrich, et al., 1997). CspA has recognition and binding sites for DNA and RNA (Yamanaka, Fang, et al., 1998). This suggests that it could be an RNA chaperone (Jiang, Hou, et al., 1997). Because of the chaperone function of Csps they can also work as transcriptional antiterminators, for example by inhibiting the hairpin structure, which causes transcriptional termination or act as pause sites at low temperatures in the target RNA (Bae, Xia, et al., 2000; Sangita Phadtare, Inouye, et al., 2002). CspA is involved in the transcription of certain proteins for example H-NS (Brandi, Pon, et al., 1994) or CspA itself, condensation of chromosomes and in the control of DNA gyrase.

Furthermore, it could also be involved in the stabilisation of different RNA at low temperatures due to its ability to bind RNA (Nakashima, Kanamaru, et al., 1996; Yamanaka, Fang, et al., 1998).

. **Table 2. The genes of the cold shock proteins and their functions in *E. coli*.** Adapted from and rewritten according to (Keto-Timonen R., Hietala N., Palonen E. & Lindström M., 2016).

csp genes of *E. coli*	**Reported function**	**References**
cspA	Induced by cold shock Major cold shock protein of *E. coli*	(Goldstein, Pollitt, et al., 1990; P. G. Jones, VanBogelen, et al., 1987)
cspB	Induced by cold	(Etchegaray, Jones, et al., 1996)
cspC	Involved in regulation of expression of stress response proteins RpoS and UspA. Involved in the regulation of growth	(Cohen-Or, Shenhar, et al., 2010; S. Phadtare & Inouye, 2001; Rath & Jawali, 2006; Shenhar, Biran, et al., 2012)
cspD	Induced by stationary phase growth and nutrient starvation Involved in persister cell formation and biofilm development Inhibits DNA replication Overproduction of cspD is toxic for the cell	(Kim, Wang, et al., 2010; Kim & Wood, 2010; Xia, Ke, et al., 2001; Yamanaka & Inouye, 1997; Yamanaka, Zheng, et al., 2001)
cspE	Induced by cold Involved in the regulation of expression of stress response proteins RpoS and UspA CspE constitutively produced at 37°C, increase in production during the lag phase	(Bae, Phadtare, et al., 1999; Czapski & Trun, 2014; S. Phadtare & Inouye, 2001; Shenhar, Biran, et al., 2012)
cspF	Expressed at very low level, no protein has been detected from the gene. Function unknown.	(Czapski & Trun, 2014)
cspG	Induced by cold	(Nakashima, Kanamaru, et al., 1996)
cspH	Expressed at very low level, no protein has been detected from the gene. Function unknown.	(Czapski & Trun, 2014)
cspI	Induced by cold.	(N. Wang, Yamanaka, et al., 1999)

Amongst the cold shock proteins also belong for example the initiator of DNA replication on *OriC* DnaA (Atlung & Hansen, 1999), the recombination and repair protein RecA, the transcription regulating protein NusA, the DNA translation initiation factors IF2α and IF2β, the enzyme polynucleotide phosphorylase, the enzymes of oxidative decarboxylation of pyruvate (P. Graumann & Marahiel, 1996; P. G. Jones, VanBogelen, et al., 1987; Pamela G. Jones & Inouye, 1994), the histone-like protein H-NS (Brandi, Pon, et al., 1994), the ribosome associated CsdA (Pamela G. Jones & Inouye, 1996), the trigger factor (TF) (Kandror & Goldberg, 1997), the molecular chaperone Hsc66 (Lelivelt & Kawula, 1995), the ribosomal protein RbfA (Pamela G. Jones & Inouye, 1996), the desaturase of membrane lipids (Aguilar, Cronan, et al., 1998) and the enzyme of the glutathione metabolism γ-Glutamyltranspeptidase (Ray, Kumar, et al., 1998).

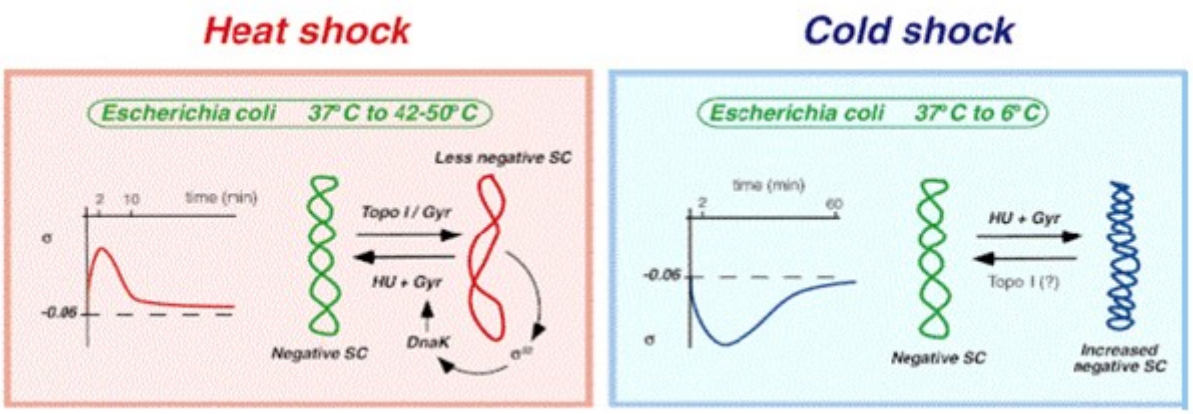

Figure 9. Heat shock and cold shock and their effect on plasmid topology in Escherichia coli. The variation of plasmid specific linking difference ($\sigma = \Delta Lk/Lk_O$) is shown to the left of each illustration along with the time of exposure to the certain temperature. The right side of each illustration shows the topological changes caused by either heat or cold shock along with the proteins that regulate the change. SC – supercoiling; Topo – topoisomerase; Gyr – DNA gyrase. Adapted and modified from (López-García & Forterre, 2000).

4.2 Oxidative stress

Oxidative stress is caused by active oxygen molecules and can damage various molecules. It can cause protein oxidation, lipid peroxidation, the cross-linkage of protein and DNA and finally DNA strand breaks (Ananthaswamy & Eisenstark, 1977; Halliwell, 1978). This damage can lead to bacterial cell death but also to mutations (Levin, Hollstein, et al., 1982). According to Weinstein-Fischer et al. in *E. coli* a transient relaxation of negatively supercoiled reporter plasmid DNA can be seen when treated with hydrogen peroxide after 2 minutes. The consequent re-supercoiling is finished after additional 30 minutes.

Oxidative stress also affects transcription of the *topA* gene in *E. coli*. When exponential phase *E. coli* was treated with hydrogen peroxide (or N-ethylmaleimide) the transcription from promoter P1 of *topA* increased in a significant way. Promoter P1 is also induced when stationary phase cells are treated with N-ethylmaleimide and transcription from the other promoters decreases. However, the treatment of stationary phase with hydrogen peroxide results in an increase of transcription of *topA* from the P2 promoter but not from the P1 promoter. This adjustment may occur because promoter P1 requires the NAP FIS to induce the transcription of *topA* in order to respond to hydrogen peroxide (Yuk Ching Tse-Dinh, 2000; Weinstein-Fischer, Elgrably-weiss, et al., 2000). Furthermore, during exponential phase the level of the FIS protein is high, while during stationary phase its level is low (Azam, Iwata, et al., 1999; Ball, Osuna, et al., 1992). Near the P1 promoter - in the 5' regulatory region of the *topA* gene in *E. coli* - binding sites for the NAP FIS have been found. It was also suggested that the FIS-dependent induction of the *topA* P1 promoter is responsible for the transient relaxation. The re-supercoiling of the DNA can be delayed when mutations in the genes *oxyR* and *katG* compromise the OxyR-dependent response to

hydrogen peroxide. OxyR is the transcription factor that controls the antiperoxide regulon that is primarily active in exponential-phase and controls the defence mechanisms to oxidative stress caused by hydrogen peroxide (González-Flecha & Demple, 1997).

The alternative σ^S subunit of RNAP (controls the RpoS regulon) then controls the defence mechanism against hydrogen peroxide throughout the transition from exponential-phase to stationary phase and to starvation (Eisenstark, Calcutt, et al., 1996).

Furthermore, oxidative stress can also be caused by the superoxide radical and in this case, the defence mechanism in *E. coli* is controlled only by the SoxRS regulon in all physiological cultures. However, in both cases, the adaptive mechanisms are regulated primarily at the level of transcription through the activity of the promoter, which is controlled by DNA topology as well as transcription regulating factors(J.-Y. Wang & Syvanen, 1992).

Earlier on, it was discovered by Horiuchi *et al.* (1984) that oxidative stress relaxed plasmid DNA in *E. coli*. In their study they used light irradiation, in the presence of a photosensitising dye (toluidine blue) and molecular oxygen and hydroxyperoxides (tert-butyl hydroperoxide, cumene hydroperoxide, and hydrogen peroxide). The relaxation of plasmid DNA could also be seen in the *topA* deficient strain DM800 when treated with toluidine blue and light or with tert-butyl hydroperoxide. This led to the belief that the presence of topoisomerase I is seemingly unnecessary for the relaxation of DNA in these cases. Moreover, it is possible that when these severe conditions are applied, the DNA relaxation could be caused by a decrease in the activity of DNA gyrase. The decrease in the activity of DNA gyrase is due to the low concentration of cellular ATP (Horiuchi, Takagi, et al., 1984).

Even though changes in supercoiling of chromosomal DNA have not been directly detected it is possible that certain parts are indeed affected in the same way the plasmid DNA is. This leads to the damage of the mechanism of replication, transcription and recombination of DNA (M Gellert, 1981). The treatment with the conditions mentioned above can therefore lead to cell death of the bacteria (Horiuchi, Takagi, et al., 1984).

Salakhetdinova *et al.* (2000) studied how DNA topology in growing and starving *E. coli* cells treated by hydrogen peroxide to induce oxidative stress depends on the putrescine content in the cell and its transport activity and the dependence on the energetic state of the cells. Putrescine is a polyamine and a topologically active metabolite. The content of putrescine in the cell changes significantly in stress conditions (Tkachenko, Salakhetdinova, et al., 1997). The authors concluded that indeed different systems are involved in the defence against oxidative stress in starving and in exponentially growing *E. coli*. When an *E. coli* starving culture contains hydrogen peroxide and an energy source is added it results in a second stress reaction (Fig. 10). Furthermore, they state that the dissociation and the binding

of putrescine to DNA seem to influence the topological state of DNA, which then in turn controls the expression of the genes that help the cell adapt (Salakhetdinova, Pshenichnov, et al., 2000).

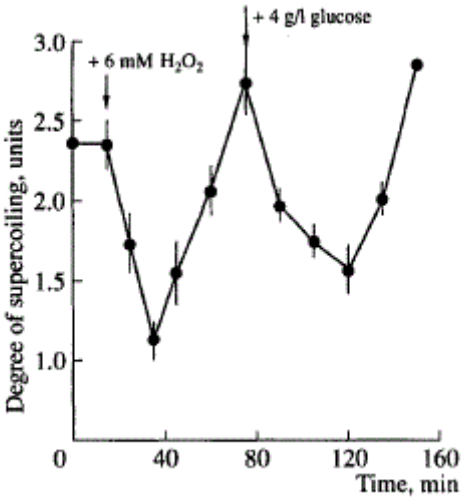

Figure 10. The effect oxidative stress has on the DNA topology in a starving culture of *E. coli* and after the addition of an energy source -glucose. Adapted from (Salakhetdinova, Pshenichnov, et al., 2000).

4.3 Extreme pH

To survive some bacteria must be able to tolerate very extreme pH values. In some cases, such as *Salmonella typhimurium* an enteric pathogen, acidic pH greatly influences the pathogenesis of this bacterium. Enteric pathogens need to survive the low pH of the stomach in order to enter the intestine and cause disease. Therefore, the ability of the bacteria to adjust to the acidic conditions is reflected in the infectious dose needed to cause disease. The ways enteric bacteria can protect themselves are preventing protons from entering the cell and pumping protons out of the cell, repair damage caused by the lowering of the internal pH or synthesize special isoforms of important enzymes with the optimal pH being acidic so that they are able to work in lower pH when needed (reviewed in Blaser & Newman, 1982; Boyd, 1995).

Furthermore, Dong *et al.* (2001) discovered by differential display PCR that one of the genes induced by long exposure to acid was *topA*. This was observed in *Helicobacter pylori*, which is a bacterium that is exposed to acidic pH repeatedly (Dong, Hyde, et al., 2001). Earlier on, it had been observed by Karem and Foster.(1993) that when *E. coli* and *Salmonella typhimurium* were grown in acidic conditions (pH 5-6) the subsequently isolated plasmid DNA had decreased negative supercoiling in comparison to plasmid DNA extracted from cells, which were grown at a higher pH of 7-8. In other words, acidic pH influences the DNA topology by decreasing the negative supercoiling, therefore causing the DNA to relax and the Lk to increase, while alkaline conditions cause an increase in negative supercoiling and a decrease in Lk. Karem and Foster (1993) also discovered that this change in the supercoiling of DNA can modify the pH-controlled expression. This was shown by using a mutant of

Salmonella typhimurium, where the *topA* gene had been deleted, which caused an increase by 31% in negative supercoiling when compared with wild-type and at pH 6 decreased the activity of the pH-regulated locus *aniG*. The *aniG* locus is made up of two genes *amiA* and *amiB*, which are responsible for acid and mannose induction (reviewed in Brown, Falla, et al., 1995). The locus *AniG* is regulated by to two genes *earA* and *earB* (Foster & Aliabadi, 1989). The pH-controlled *aniG* locus is also controlled by various other conditions among which belong for example osmolarity and oxygen. When novobiocin, a DNA gyrase inhibitor, was added it caused a decrease in negative supercoiling (Lk increase) due to the influence of the pH-regulated gene expression (Bang, Audia, et al., 2002; Karem & Foster, 1993). In *E. coli* and *Salmonella typhimurium* the NAP protein H-NS also plays an important part in the pH-regulated gene expression (Shi, Waasdorp, et al., 1993; Soutourina, Krin, et al., 2002). When *Salmonella typhimurium* is in non-lethal stress conditions (acidic pH > 4) it synthesizes proteins in the acid tolerance response, which then allow it to be more resistant to more lethal pH conditions (< pH 3) (Foster & Hall, 1990).

4.4 Antibiotics and other inhibitors

Some antibiotics also affect gene expression. For example quinolines and coumarins. In this case the alteration of gene expression is primarily caused by the inhibition of various DNA topoisomerases as well as DNA gyrase. DNA topoisomerase inhibitors can be split into multiple classes of antibiotics. This division includes antitumor drugs, which affect the eukaryotic topoisomerase II. These enzymes affect DNA supercoiling, which in turn can influence the gene expression of various genes (reviewed in Champoux, 2001). This change in DNA supercoiling can affect replication, transcription, repair as well as recombination (Drlica, 1984; Drlica & Franco, 1988; Forterre, Gribaldo, et al., 2007; M Gellert, 1981).

In previous publications, three reactions that involved energy transduction of the whole reaction, which generates negative supercoiling reactions, were studied. These are (i) the ATPase reaction, (ii) App[NH]p-induced supercoiling, and (iii) ATP- or App[NH]p- driven cleavage site reorganization (App[NH]p is a nonhydrolyzable analogue of ATP). It has been concluded that both DNA gyrase subunits A and B in *Escherichia coli* are necessary for all three reactions and are inhibited by low concentrations of novobiocin and coumermycin A_1. According to Sugino *et al*. (1978), the same antibiotics influenced the movement of DNA gyrase along the DNA from one cleavage site (induced by ATP or App[NH]p) to another.. The inhibition was due to the antibiotics preventing the binding of ATP to DNA gyrase. Therefore, the sensitive step is a step in energy transduction occurring before ATP hydrolysis. This step is the binding of ATP to DNA gyrase. Novobiocin, coumermycin A_1 and App[NH]p are competitors of ATP. They inhibit the catalysis of supercoiling and ATPase in the cell. Sugino *et al.* (1978) also states that ATP or APP[NH]p allosterically affect conformational change of

DNA gyrase and this then leads to one round of supercoiling; the hydrolysis of ATP is necessary for the enzyme turnover (A Sugino, Higgins, et al., 1978).

Earlier on, Gellert *et al*. (1976) established that when *E. coli* is treated with novobiocin or coumermycin the supercoiling of DNA is inhibited and colicin E1 DNA and phage λ DNA remain relaxed. The inhibition of supercoiling leads to the inhibition of replication in *E. coli* (M Gellert, O'Dea, et al., 1976). Furthermore, later it was observed by Peng and Marians (1993) that the genes encoding the two subunits of Topo IV *parC* and *parE* had to be complete without any shortening. Otherwise Topo IV did not reconstitute. Moreover, Topo IV is also ATP dependent and is supported only by ATP or dATP. Topo IV was inhibited by coumarin and quinolone antibiotics; however the concentrations needed for its activity to be inhibited by 50% were 3-30 times higher than the concentrations necessary for the inhibition of DNA gyrase. Therefore, DNA gyrase is more sensitive to these antibiotics. The difference in sensitivity may be due to the replication fork being able to overcome the frozen Topo IV-DNA complex or the complex can be repaired by a mechanism that does not take place for the gyrase DNA-complex. Another cause might be that Topo IV has restricted access to the chromosome possibly through the competition for binding sites with DNA gyrase. Topo IV inhibition of relaxation by the stated antibiotics is illustrated below in Fig. 11 (H Peng & Marians, 1993). The Topo IV inhibition of relaxation by these antibiotics along with controls without inhibition are illustrated below in Fig. 11. Subunits ParC and ParE are to some extent homologous with the subunits GyrA and GyrB of DNA gyrase. However, there is a difference in the assembly of Topo IV and DNA gyrase. Topo IV, unlike DNA gyrase, does not require ATP nor DNA. Moreover, the difference in organization of the subunits of both enzymes may possibly reflect in their primary tasks performed in the cell. DNA gyrase may play a role in regulating the density of supercoiling while Topo IV may be involved in the segregation of daughter chromosomes (Hong Peng & Marians, 1993).

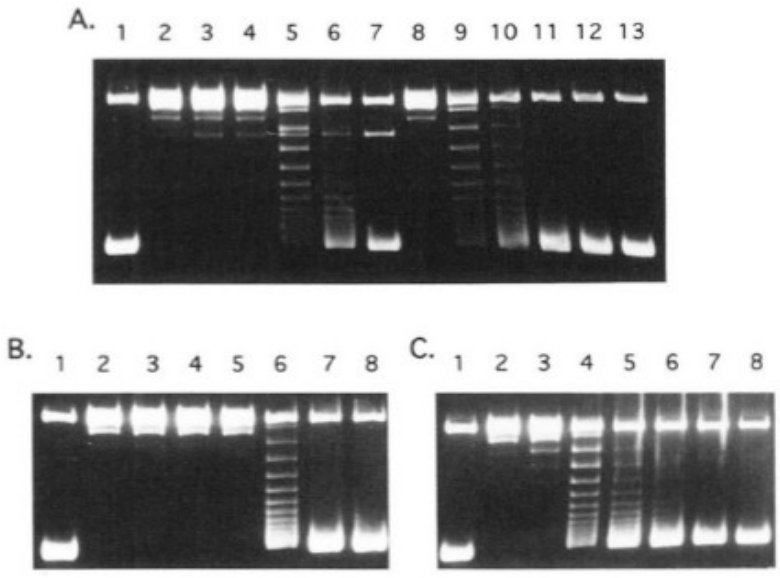

Figure 11: The inhibition of the Topo IV catalysed relaxation by quinolones and coumarins. A: Shows the inhibition by norfloxacin or coumermycin. Standard reaction mixtures, where lane 1 contains no enzyme and all the other lanes contain 280 fmol of Topo IV. Lanes 2 and 8 contain no antibiotic, while lanes 3 - 7 contain concentrations 7.8, 15.6, 31.3, 62.5 and 125μM of norfloxacin (in order) and lanes 9-13 contain concentrations 22.5, 45, 90, 180 and 360 nM of coumermycin (in order).
B: Shows the inhibition by novobiocin. Standard reaction mixtures, where lane 1 contains no enzyme and all other lanes contain 280 fmol of Topo IV. Lane 2 contains no antibiotic and the other lanes contain concentrations 0.31, 0.63, 1.25, 2.5, 5, or 10 μM.C: Shows the inhibition by nalidixic acid. Standard reaction mixtures, where lane 1 contains no enzyme and all the other lanes conatin 280 fmol of Topo IV. Lane 2 contains no antibiotic and the other lanes contain concentrations 0.1, 0.21, 0.43,0.85, 1.7, and 3.4 mM of nalidixic acid. Adapted from (H Peng & Marians, 1993).

Sioud *et al*. (2009), performed various experiments on *B. subtilis* and *B. thuringiensis* using the DNA gyrase inhibitor novobiocin and etoposide, which specifically inhibits the eukaryotic type II topoisomerase. They found that that two strains of *Bacillus thuringiensis* BMG1.7 and HD9 are DNA gyrase inhibitor sensitive as well as etoposide VP16 sensitive. When inhibitory and subinhibitory concentrations of VP16 were added it resulted in a drug-tolerant response. The results seem to indicate that etoposide VP16 modestly influences transcription and gene expression of the *gyrA, gyrB* and *topA* genes in *B. thuringiensis* BMG1.7. Furthermore, in *Bacillus subtilis* 168 the inhibition of DNA gyrase ATPase activity by novobiocin lead to the activation of 80 genes and the repression of 89 genes 20 minutes after the addition of novobiocin. The genes which had been altered belonged to categories with different functions; however, they were dominated by the products of genes that were responsible for transport of low molecular-weight compounds, maintaining DNA integrity and finally the genes responsible for the SOS response. These genes along with the correlation with the time of the experiment are illustrated below in Fig. 12. When the effects of novobiocin in *B. subtilis* and *thuringiensis* were compared by quantitative RT-PCR it was revealed that the effects on gene expression were comparable. On the contrary, when they were treated with VP16, a moderate effect on the gene expression of *gyrA* and *gyrB* as well as *topA* was revealed. Nevertheless, the genes *dinB* and *lexA*, which contribute to the SOS response, when compared with untreated cells showed a relatively high level of transcription. Moreover, when Sioud *et. al* (2009) extracted a small plasmid DNA from *B. thuringiensis* BMG 1.7 it contained a partially relaxed topoisomer ladder, whereas when its counterpart was extracted from cells, which had been treated with VP16, its electrophoretic mobility remained unchanged (Sioud, Boudabous, et al., 2009).

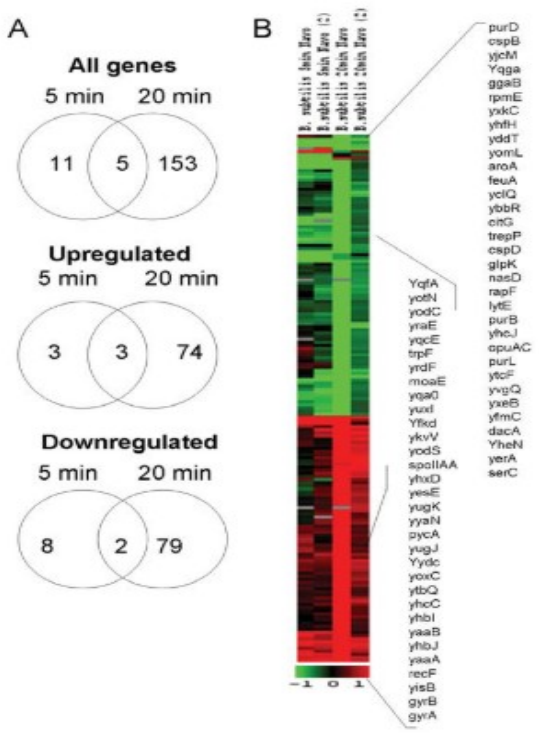

Figure 12. The analysis of the change in gene expression in *B. subtillis* 168 when treated with novobiocin. A: Venn diagrams of important genes that were altered 5 and 20 min after novobiocin was added. The time points are each represented by a circle with the time given above the circle. B: We can see hierarchical gene clusters that were altered after 5 and 20 min after novobiocin was added ($p<0.05$). Genes are represented by horizontal stripes and treatment protocols are illustrated as columns. Log2-fold alterations of gene ratios were colour-coded as is illustrated in the bar. Adapted from (Sioud, Boudabous, et al., 2009).

Furthermore, Schröder *et al*. (2013) compared the impact of two various DNA gyrase inhibitors on the *S. aureus* SOS response. These inhibitors were ciprofloxacin and novobiocin (Schröder, Goerke, et al., 2013). Ciprofloxacin, a quinolone, interacts with the subunit A of DNA gyrase and results in double-stranded breaks in DNA. This induces the SOS response. The double-stranded breaks are then turned into ssDNA and this causes ssDNA-RecA nucleoprotein filaments to form. Autocleavage of the repressor of LexA is induced by the previously activated complex ssDNA-RecA. This enables the transcription of genes, which participate in the repair of DNA and of the *recA* and *lexA* genes themselves (Kelley, 2006; Michel Bénédicte, 2005). Schröder *et al.* (2013) then showed that aminocoumarins inhibit the SOS response induced by ciprofloxacin. This was caused by aminocoumarins, through the inhibition of the expression of *recA* at the level of transcription. Supposedly the inhibition was caused by changes in supercoiling and was not dependent on LexA (Schröder, Goerke, et al., 2013). Later, Schröder *et al.* (2014) analysed how novobiocin, a DNA relaxing agent, impacts the gene expression in *S. aureus*. When they used a mutant that was resistant to novobiocin, it became clear that the change in expression of *recA* was caused by the inhibition of DNA gyrase. They discovered that the levels of expression of specific sets of genes were upregulated and other were downregulated after the treatment with

novobiocin. The expression of *recF-gyrB-gyrA* genes, the *rib* operon and the *ure* operon increased, while the expression of *arlRS, recA, lukA, hlgC* decreased (Schröder, Bernhardt, et al., 2014). Earlier on, it had been found that the two-component ArlRS system decreased the amount of supercoiling in *S. aureus*. It is therefore possible, that if *arlRS* is downregulated it could partially compensate for the relaxation caused by novobiocin (Fournier & Klier, 2004).

Schröder *et al*. (2014) also discovered that there is a tight link between the inhibition of the expression of *recA* and the inhibition of the subunit B of DNA gyrase. When DNA gyrase was inhibited by aminocoumarins it caused the alteration of gene expression. The sensitivity to supercoiling was not dependent on the Arl system and neither was it dependent on the chromosomal location. Using global analysis and gene mapping of genes sensitive to supercoiling did not show that they are clustered in the genome. The dislocation of three various promoters indicated that the responsiveness of a gene is probably not dependent on the chromosomal location but is given by the promoter region and its molecular properties. Lastly, in the case of *S. aureus*, the promoter before *recF-gyrB-gyrA* supposedly measures directly the supercoiling status and when DNA is relaxed it leads to the upregulation of DNA gyrase (Schröder, Bernhardt, et al., 2014).

5 Summary

This Thesis is an overview on bacterial DNA topology and how environmental change impacts it and subsequently gene expression. Transcription affects DNA topology through the formation of positive supercoils in front of the transcription complex during elongation and negative supercoils behind it. This is resolved by various enzymes, which affect DNA topology. The enzymes topoisomerase I, topoisomerase IV and DNA gyrase play a major role in the alteration of DNA topology in bacteria and in the response to environmental change.

Usually gene expression of certain genes is regulated not only by the promoter sequence but also by the chromosomal localisation. However, according to the study on the effect of antibiotics on *Staphylococcus aureus* this might not always be the case, as the responsiveness of a gene in *S. aureus* is probably determined by molecular properties of the promoter region and not by the chromosomal localisation (Schröder, Bernhardt, et al., 2014). In this area further studies are necessary. Some antibiotics, for example quinolones or coumarins, have an inhibitory effect on DNA gyrase, which then in turn regulates gene expression of various genes. Topo IV also plays a role in the regulation of gene expression and is affected by antibiotics. It might be interesting to do further experiments focused on the different effects that Topo IV may have on the topology and gene expression in other bacteria than *E. coli*.

Furthermore, gene expression may be influenced for example by temperature change, oxidative stress, extreme pH or by antibiotics and other drugs. High temperature causes a transient relaxation, while low temperature causes an increase in negative supercoiling. This results in a change in the response from the promoters of the topoisomerase I *topA* gene. Important roles are played by the heat shock protein DnaK and the cold shock protein CspA.

Oxidative stress induces plasmid relaxation. Hydrogen peroxide also affects the transcription from the promoters of the *topA* gene; however, the response differs in exponential phase and stationary phase probably because of the FIS protein that binds to DNA. The defence mechanism to hydrogen peroxide is controlled by two regulons: OxyR in exponential phase and RpoS in the other phases and transitions. The superoxide radical has only one regulon, SoxRS.

Low pH results in a decrease in negative supercoiling and high pH in its increase. The gene *topA* also plays a role in the pH-regulated expression and affects the *aniG* locus. When enteric bacteria are exposed to non-lethal pH they respond by synthesising proteins, which then allow them to become more resistant when exposed to lethal acidic pH.

In conclusion, this study shows the vast diversity of physiological situations in which DNA topology plays a role. This review will be of use in studies of the effects of DNA topology on gene expression in *Bacillus subtilis* in the laboratory I work in.

6 References

(*Secondary citation)

Adachi, T., Mizuuchi, M., Robinson, E. A., Appella, E., O'Dea, M. H., Gellert, M., & Mizuuchi, K. (1987). DNA sequence of the *E. coli gyrB* gene: application of a new sequencing strategy. *Nucleic Acids Research*, *15*(2), 771–784.

Aguilar, P. S., Cronan, J. E., & De Mendoza, D. (1998). A *Bacillus subtilis* gene induced by cold shock encodes a membrane phospholipid desaturase. *Journal of Bacteriology*, *180*(8), 2194–2200.

Ananthaswamy, H. N., & Eisenstark, A. (1977). Repair of hydrogen peroxide-induced single-strand breaks in *Escherichia coli* deoxyribonucleic acid. *Journal of Bacteriology*, *130*(1), 187–191.

*Atkinson, B. G., & Walden, D. B. (1985). *Changes in Eukaryotic Gene Expression in Response to Environmental Stress* (5th ed.). Academic Press.

Atlung, T., & Hansen, F. G. (1999). Low-temperature-induced DnaA protein synthesis does not change initiation mass in *Escherichia coli* K-12. *Journal of Bacteriology*, *181*(18), 5557–5562.

Azam, T. A., Iwata, A., Nishimura, A., Ueda, S., & Ishihama, A. (1999). Growth phase-dependent variation in protein composition of the Escherichia coli nucleoid. *Journal of Bacteriology*, *181*(20), 6361–6370.

Baba, T., Kuwahara-Arai, K., Uchiyama, I., Takeuchi, F., Ito, T., & Hiramatsu, K. (2009). Complete genome sequence of *Macrococcus caseolyticus* strain JCSCS5402, reflecting the ancestral genome of the human-pathogenic staphylococci. *Journal of Bacteriology*, *191*(4), 1180–1190.

Bae, W., Phadtare, S., Severinov, K., & Inouye, M. (1999). Characterization of *Escherichia coli cspE*, whose product negatively regulates transcription of *cspA*, the gene for the major cold shock protein. *Molecular Microbiology*, *31*(5), 1429–1441.

Bae, W., Xia, B., Inouye, M., & Severinov, K. (2000). *Escherichia coli* CspA-family RNA chaperones are transcription antiterminators. *Proceedings of the National Academy of Sciences*, *97*(14), 7784–7789.

Bailly, C., Waring, M. J., & Travers, A. A. (1995). Effects of base substitutions on the binding of a DNA-bending protein. *Journal of Molecular Biology*, *253*(1), 1–7.

Ball, C. A., Osuna, R., Ferguson, K. C., & Johnson, R. C. (1992). Dramatic changes in Fis levels upon nutrient upshift in *Escherichia coli*. *Journal of Bacteriology*, *174*(24), 8043–8056.

Bang, I. S., Audia, J. P., Park, Y. K., & Foster, J. W. (2002). Autoinduction of the *ompR* response regulator by acid shock and control of the *Salmonella enterica* acid tolerance response. *Molecular Microbiology*, *44*(5), 1235–1250.

Barne, K. A., Bown, J. A., Busby, S. J. W., & Minchin, S. D. (1997). Region 2.5 of the *Escherichia coli* RNA polymerase σ 70 subunit is responsible for the recognition of the "extended-10" motif at promoters. *The EMBO Journal*, *16*(13), 4034–4040.

*Bates, A. D., & Maxwell, A. (2005). *DNA topology*. Oxford University Press.

*Blaser, M. J., & Newman, L. S. (1982). A Review of Human Salmonellosis: I. Infective Dose. *Reviews of Infectious Diseases*, *4*(6), 1096–1106.

*Boyd, R. F. (1995). *Basic medical microbiology* (5th ed.). Little, Brown and Company.

Brandi, A., Pon, C. L., & Gualerzi, C. O. (1994). Interaction of the main cold shock protein CS7.4 (CspA) of *Escherichia coli* with the promoter region of hns. *Biochimie*, *76*(10–11), 1090–1098.

*Brown, M., Falla, T., Foster, J. W., Galinski, E. A., Gilkes, N. R., Hall, H. K., Hancock, R. E. W., Inoue, Y., Kimura, A., Smith, R. J., Tomme, P., & Warren, R. A. J. (1995). Molecular Responses of Microbes to Environmental pH Stress. In R. K. Poole (Ed.), *Advances in Microbial Physiology* (Vol. 37, pp. 229–272). Academic Press.

*Browning, D. F., & Busby, S. J. W. (2004). The regulation of bacterial transcription initiation. *Nature Reviews Microbiology*, *2*(1), 57–65.

*Champoux, J. J. (2001). DNA Topoisomerases: Structure, Function, and Mechanism. *Annual Review of Biochemistry*, *70*(1), 369–413.

Cohen-Or, I., Shenhar, Y., Biran, D., & Ron, E. Z. (2010). CspC regulates rpoS transcript levels and complements hfq deletions. *Research in Microbiology*, *161*(8), 694–700.

Cramer, P., Bushnell, D. A., & Kornberg, R. D. (2001). Structural basis of transcription: RNA

polymerase II at 2.8 ångstrom resolution. *Science*, *292*(5523), 1863–1876.
Crick, F. (1970). Central dogma of molecular biology. *Nature*, *227*(5258), 561–563.
Crisona, N. J., Strick, T. R., Bensimon, D., Croquette, V., & Cozzarelli, N. R. (2000). Preferential relaxation of positively supercoiled DNA by *E. coli* topoisomerase IV in single-molecule and ensemble measurements. *Genes and Development*, *14*(22), 2881–2892.
Czapski, T. R., & Trun, N. (2014). Expression of *csp* genes in *E. Coli* K-12 in defined rich and defined minimal media during normal growth, and after cold-shock. *Gene*, *547*(1), 91–97.
Dame, R. T. (2005). The role of nucleoid-associated proteins in the organization and compaction of bacterial chromatin. *Molecular Microbiology*, *56*(4), 858–870.
Deibler, R. W., Rahmati, S., & Zechiedrich, E. L. (2001). Topoisomerase IV, alone, unknots DNA in *E. coli*. *Genes and Development*, *15*(6), 748–761.
Delius, H., & Worcel, A. (1974). Electron microscopic studies on the folded chromosome of *Escherichia coli*. *Cold Spring Harbor Symposia on Quantitative Biology*, *38*, 53–58.
*Deweese, J. E., Osheroff, M. A., Osheroff, N., Mol, B., & Author, B. E. (2008). DNA Topology and Topoisomerases: Teaching a "Knotty" Subject. *Biochem Mol Biol Educ*, *37*(1), 2–10.
Dong, Q., Hyde, D., Herra, C., Kean, C., Murphy, P., O'Morain, C. A., & Buckley, M. (2001). Identification of genes regulated by prolonged acid exposure in *Helicobacter pylori*. *FEMS Microbiology Letters*, *196*(2), 245–249.
Dorman, C. J. (1995). DNA topology and the global control of bacterial gene expression: Implications for the regulation of virulence gene expression. *Microbiology*, *141*(6), 1271–1280.
Dorman, C. J. (2008). Regulation of Transcription in Bacteria by DNA Supercoiling. *Bacterial Physiology*, 155–178.
Dorman, C. J., Bhriain, N. N., & Higgins, C. F. (1990). DNA supercoiling and environmental regulation of virulence gene expression in *Shigella flexneri*. *Nature*, *344*, 789–792.
Drlica, K. (1984). Biology of Bacterial Deoxyribonucleic Acid Topoisomerases. *Microbiological Reviews*, *48*(4), 273–289.
Drlica, K., & Franco, R. J. (1988). Inhibitors of DNA Topoisomerases. *Biochemistry*, *27*(7), 2253–2259.
Eisenstark, A., Calcutt, M. J., Becker-Hapak, M., & Ivanova, A. (1996). Role of *Escherichia coli* rpoS and associated genes in defense against oxidative damage. *Free Radical Biology and Medicine*, *21*(7), 975–993.
Etchegaray, J.-P., Jones, P. G., & Inouye, M. (1996). Differential thermoregulation of two highly homologous cold-shock genes, *cspA* and *cspB* , of *Escherichia coli*. *Genes to Cells*, *1*(2), 171–178.
Ferrero, L., Cameron, B., Manse, B., Lagneaux, D., Crouzet, J., Famechon, A., & Blanche, F. (1994). Cloning and primary structure of *Staphylococcus aureus* DNA topoisomerase IV: a primary target of fluoroquinolones. *Molecular Microbiology*, *13*(4), 641–653.
Forterre, P., Gribaldo, S., Gadelle, D., & Serre, M. C. (2007). Origin and evolution of DNA topoisomerases. *Biochimie*, *89*(4), 427–446.
Foster, J. W., & Aliabadi, Z. (1989). pH-regulated gene expression in *Salmonella*: genetic analysis of *aniG* and cloning of the *earA* regulator. *Molecular Microbiology*, *3*(11), 1605–1615.
Foster, J. W., & Hall, H. K. (1990). Adaptive acidification tolerance response of Salmonella typhimurium. *Journal of Bacteriology*, *172*(2), 771–778.
Fournier, B., & Klier, A. (2004). Protein A gene expression is regulated by DNA supercoiling which is modified by the ArlS-ArlR two-component system of Staphylococcus aureus. *Microbiology*, *150*(11), 3807–3819.
Franklin, R. E., & Gosling, R. G. (1953). Molecular configuration in sodium thymonucleate. *Nature*, *171*(4356), 740–741.
Gellert, M. (1981). DNA topoisomerases. *Annual Review of Biochemistry*, *50*, 879–910.
Gellert, M, Fisher, L. M., & O'Dea, M. H. (1979). DNA gyrase: purification and catalytic properties of a fragment of gyrase B protein. *Proceedings of the National Academy of Sciences*, *76*(12), 6289–6293.
Gellert, M, O'Dea, M. H., Itoh, T., & Tomizawa, J. (1976). Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. *Proceedings of the National Academy of Sciences*, *73*(12), 4474–4478.

Gellert, Martin, Mizuuchi, K., O'dea, M. H., & Nasht, H. A. (1976). DNA gyrase: An enzyme that introduces superhelical turns into DNA. *Proceedings of the National Academy of Sciences*, *73*(11), 3872–3876.
Ghilarov, D. A., & Shkundina, I. S. (2012). DNA topoisomerases and their functions in a cell. *Molecular Biology*, *46*(1), 47–57.
Giuliodori, A. M., Di Pietro, F., Marzi, S., Masquida, B., Wagner, R., Romby, P., Gualerzi, C. O., & Pon, C. L. (2010). The *cspA* mRNA Is a Thermosensor that Modulates Translation of the Cold-Shock Protein CspA. *Molecular Cell*, *37*(1), 21–33.
Gmuender, H., Kuratli, K., Di Padova, K., Gray, C. P., Keck, W., & Evers, S. (2001). Gene expression changes triggered by exposure of *Haemophilus influenzae* to Novobiocin or ciprofloxacin: Combined transcription and translation analysis. *Genome Research*, *11*(1), 28–42.
Goldstein, J., Pollitt, N. S., & Inouye, M. (1990). Major cold shock protein of *Escherichia coli*. *Proceedings of the National Academy of Sciences*, *87*(1), 283–287.
González-Flecha, B., & Demple, B. (1997). Transcriptional regulation of the *Escherichia coli oxyR* gene as a function of cell growth. *Journal of Bacteriology*, *179*(19), 6181–6186.
Grau, R., Gardiol, D., Glikin, G. C., & Mendoza, D. (1994). DNA supercoiling and thermal regulation of unsaturated fatty acid synthesis in *Bacillus subtilis*. *Molecular Microbiology*, *11*(5), 933–941.
Graumann, P. L. (2001). SMC proteins in bacteria: condensation motors for chromosome segregation? *Biochimie*, *83*(1), 53–59.
Graumann, P., & Marahiel, M. A. (1996). Some like it cold: Response of microorganisms to cold shock. *Archives of Microbiology*, *166*(5), 293–300.
Graumann, P., Wendrich, T. M., Weber, M. H. W., Schröder, K., & Marahiel, M. A. (1997). A family of cold shock proteins in *Bacillus subtilis* is essential for cellular growth and for efficient protein synthesis at optimal and low temperatures. *Molecular Microbiology*, *25*(4), 741–756.
Halliwell, B. (1978). Biochemical mechanisms accounting for the toxic action of oxygen on living organisms: The key role of superoxide dismutase. *Cell Biology International Reports*, *2*(2), 113–128.
Harvey, S. C., Luo, J., & Lavery, R. (1988). DNA stem-loop structures in oligopurine-oligopyrimidine triplexes. *Nucleic Acids Research*, *16*(24), 11795–11809.
Hébraud, M., & Potier, P. (1999). Cold Shock Response and Low Temperature Adaptation in Psychrotrophic Bacteria. *Article in Journal of Molecular Microbiology and Biotechnology*, *1*(2), 211–219.
Hein, P. P., & Landick, R. (2010). The bridge helix coordinates movements of modules in RNA polymerase. *BMC Biology*, *8*(141), 1–4.
Helmann, J. D., & DeHaseth, P. L. (1999). Protein-nucleic acid interactions during open complex formation investigated by systematic alteration of the protein and DNA binding partners. *Biochemistry*, *38*(19), 5959–5967.
Higgins, N. P., Peebles, C. L., Sugino, A., & Cozzarelli, N. R. (1978). Purification of subunits of *Escherichia coli* DNA gyrase and reconstitution of enzymatic activity. *Proceedings of the National Academy of Sciences*, *75*(4), 1773–1777.
*Hinton, D. M. (2016). Prokaryotic Transcription. In R. A. Bradshaw & P. D. Stahl (Eds.), *Encyclopedia of Cell Biology* (1st ed., Vol. 1, pp. 468–480). Elsevier Inc.
Hirata, A., Klein, B. J., & Murakami, K. S. (2008). The X-ray Crystal Structure of RNA Polymerase from Archaea. *Nature*, *451*(7180), 851–854.
Horiuchi, H., Takagi, M., & Yano, K. (1984). Relaxation of Supercoiled Plasmid DNA by Oxidative Stresses in *Escherichia coli*. *Journal of Bacteriology*, *160*(3), 1017–1021.
Hormeño, S., Ibarra, B., Carrascosa, J. L., Valpuesta, J. M., Moreno-Herrero, F., & Arias-Gonzalez, J. R. (2011). Mechanical properties of high-G·C content DNA with A-type base-stacking. *Biophysical Journal*, *100*(8), 1996–2005.
Htun, H. A. N., & Dahlberg, J. E. (1988). Single strands, triple strands, and kinks in H-DNA. *Science*, *241*(4874), 1791–1796.
Jaworski, A., Higgins, N. P., Wells, R. D., & Zacharias, W. (1991). Topoisomerase mutants and physiological conditions control supercoiling and Z-DNA formation in vivo. *The Journal of Biological Chemistry*, *266*(4), 2576–2581.
Jiang, W., Hou, Y., & Inouye, M. (1997). CspA, the major cold-shock protein of *Escherichia coli*, is

an RNA chaperone. *The Journal of Biological Chemistry*, *272*(1), 196–202.
Jones, P. G., VanBogelen, R. A., & Neidhardt, F. C. (1987). Induction of proteins in response to low temperature in *Eschericia coli*. *Journal of Bacteriology*, *169*(5), 2092–2095.
Jones, Pamela G., & Inouye, M. (1994). The cold-shock response - a hot topic. *Molecular Microbiology*, *11*(5), 811–818.
Jones, Pamela G., & Inouye, M. (1996). RbfA, a 30S ribosomal binding factor, is a cold-shock protein whose absence triggers the cold-shock response. *Molecular Microbiology*, *21*(6), 1207–1218.
Jones, Pamela G, Krah, R., Tafuri, S. R., & Wolffe, A. P. (1992). DNA Gyrase, CS7.4, and the Cold Shock Response in *Escherichia coli*. *Journal of Bacteriology*, *174*(18), 5798–5802.
Jovanovich, S. B., & Lebowitz, J. (1987). Estimation of the effect of coumermycin A1 on *Salmonella typhimurium* promoters by using random operon fusions. *Journal of Bacteriology*, *169*(10), 4431–4443.
Kandror, O., & Goldberg, A. L. (1997). Trigger factor is induced upon cold shock and enhances viability of *Escherichia coli* at low temperatures. *Proceedings of the National Academy of Sciences*, *94*(10), 4978–4981.
Karem, K., & Foster, J. W. (1993). The influence of DNA topology on the environmental regulation of a pH-regulated locus in *Salmonella typhimurium*. *Molecular Microbiology*, *10*(1), 75–86.
Kataoka, K., Mizushima, T., Ogata, Y., Miki, T., & Sekimizu, K. (1996). Heat shock-induced DNA relaxation *in vitro* by DNA gyrase of *Escherichia coli* in the presence of ATP. *Journal of Biological Chemistry*, *271*(40), 24806–24810.
Kato, J. ichi, Nishimura, Y., Imamura, R., Niki, H., Hiraga, S., & Suzuki, H. (1990). New topoisomerase essential for chromosome segregation in *E. coli*. *Cell*, *63*(2), 393–404.
Kelley, W. L. (2006). Lex marks the spot: the virulent side of SOS and a closer look at the LexA regulon. *Molecular Microbiology*, *62*(5), 1228–1238.
*Keniry, M. A. (2000). Quadruplex structures in nucleic acids. *Biopolymers*, *56*(3), 123–146.
*Keto-Timonen R., Hietala N., Palonen E., H. A., & Lindström M., K. H. (2016). Cold Shock Proteins: A Minireview with Special Emphasis on Csp-family of Enteropathogenic *Yersinia*. *Frontiers in Microbiology*, *7*(1151).
Kim, Y., Wang, X., Zhang, X.-S., Grigoriu, S., Page, R., Peti, W., & Wood, T. K. (2010). *Escherichia coli* toxin/antitoxin pair MqsR/MqsA regulate toxin CspD. *Environmental Microbiology*, *12*(5), 1105–1121.
Kim, Y., & Wood, T. K. (2010). Toxins Hha and CspD and small RNA regulator Hfq are involved in persister cell formation through MqsR in *Escherichia coli*. *Biochemical and Biophysical Research Communications*, *391*(1), 209–213.
Klevan, L., & Wang, J. C. (1980). Deoxyribonucleic acid gyrase-deoxyribonucleic acid complex containing 140 base pairs of deoxyribonucleic acid and an alpha 2 beta 2 protein core. *Biochemistry*, *19*(23), 5229–5234.
Kreuzer, K. N., & Cozzarelli, N. R. (1980). Formation and resolution of DNA catenanes by DNA gyrase. *Cell*, *20*(1), 245–254.
Landick, R. (2005). NTP-entry routes in multi-subunit RNA polymerases. *TRENDS in Biochemical Sciences*, *30*(12).
Lelivelt, M. J., & Kawula, T. H. (1995). Hsc66, an Hsp70 homolog in *Escherichia coli*, is induced by cold shock but not by heat shock. *Journal of Bacteriology*, *177*(17), 4900–4907.
Lesley, S. A., Jovanovich, S. B., Tse-Dinh, Y.-C., & Burgess', R. R. (1990). Identification of a Heat Shock Promoter in the *topA* Gene of *Escherichia coli*. *Journal of Bacteriology*, *172*(12), 6871–6874.
Levin, D. E., Hollstein, M., Christman, M. F., Schwiers, E. A., & Ames, B. N. (1982). A new *Salmonella* tester strain (TA102) with A.T base pairs at the site of mutation detects oxidative mutagens. *Proceedings of the National Academy of Sciences*, *79*(23), 7445–7449.
*Levine, C., Hiasa, H., & Marians, K. J. (1998). DNA gyrase and topoisomerase IV: Biochemical activities, physiological roles during chromosome replication, and drug sensitivities. *Biochimica et Biophysica Acta*, *1400*(1–3), 29–43.
Lilley, D. M. J., Chen, D., & Bowater, R. P. (1996). DNA supercoiling and transcription: topological coupling of promoters. *Quarterly Reviews of Biophysics*, *29*(3), 203–225.
Liu, L. F., & Wang, J. C. (1987). Supercoiling of the DNA template during transcription. *Proceedings*

of the National Academy of Sciences, *84*(20), 7024–7027.
Liu, Leroy F., Liu, C. C., & Alberts, B. M. (1980). Type II DNA topoisomerases: Enzymes that can unknot a topologically knotted DNA molecule via a reversible double-strand break. *Cell*, *19*(3), 697–707.
Lockshon, D., & Morrs, D. R. (1983). Positively supercoiled plasmid DNA is produced by treatment of *Escherichia coli* with DNA gyrase inhibitors. *Nucleic Acids Research*, *11*(10), 2999–3017.
*López-García, P., & Forterre, P. (2000). DNA topology and the thermal stress response, a tale from mesophiles and hyperthermophiles. *BioEssays*, *22*(8), 738–746.
López, V., Martinez-Robles, M.-L., Herná Ndez, P., Krimer, D. B., & Schvartzman, J. B. (2012). Topo IV is the topoisomerase that knots and unknots sister duplexes during DNA replication. *Nucleic Acids Research*, *40*(8), 3563–3573.
Lozinski, T., Adrych-Rozek, K., Markiewicz, W. T., & Wierzchowski, K. (1991). Effect of DNA bending in various regions of a consensus-like *Escherichia coli* promoter on its strength *in vivo* and structure of the open complex *in vitro*. *Nucleic Acids Research*, *19*(11), 2947–2953.
*Luijsterburg, M. S., White, M. F., Van Driel, R., & Th. Dame, R. (2008). The major architects of chromatin: Architectural proteins in bacteria, archaea and eukaryotes. *Critical Reviews in Biochemistry and Molecular Biology*, *43*(6), 393–418.
Lyamichev, V. I., Mirkin, S. M., Frank-Kamenetskii, M. D., & Mirkin, S. M. (1985). A ph-dependent structural transition in the homopurine-homopyrimidine tract in superhelical dna. *Journal of Biomolecular Structure and Dynamics*, *3*(2), 327–339.
Malik, M., Bensaid, A., Rouviere-Yaniv, J., & Drlica, K. (1996). Histone-like protein HU and bacterial DNA topology: Suppression of an HU deficiency by gyrase mutations. *Journal of Molecular Biology*, *256*(1), 66–76.
Marians, K. J. (1987). DNA gyrase-catalyzed decatenation of multiply linked DNA dimers. *The Journal of Biological Chemistry*, *262*(21), 10362–10368.
Massé, E., & Drolet, M. (1999). *Escherichia coli* DNA topoisomerase I inhibits R-loop formation by relaxing transcription-induced negative supercoiling. *Journal of Biological Chemistry*, *274*(23), 16659–16664.
*Maxwell, A., Bush, N. G., & Evans-Roberts, K. (2015). DNA Topoisomerases. *EcoSal Plus*, *6*(2).
Maxwell, A., & Gellert, M. (1986). Mechanistic aspects of DNA topoisomerases. *Advances in Protein Chemistry*, *38*, 69–107.
Menzel, R., & Gellert, M. (1983). Regulation of the genes for *E. coli* DNA gyrase: Homeostatic control of DNA supercoiling. *Cell*, *34*(1), 105–113.
Menzel, R., & Gellert, M. (1987). Fusions of the *Escherichia coli gyrA* and *gyrB* Control Regions to the Galactokinase Gene Are Inducible by Coumermycin Treatment. *Journal of Bacteriology*, *169*(3), 1272–1278.
Michel Bénédicte. (2005). After 30 Years of Study, the Bacterial SOS Response Still Surprises Us. *PlLoS Biology*, *3*(7), 1174–1176.
Minakhin, L., Bhagat, S., Brunning, A., Campbell, E. A., Darst, S. A., Ebright, R. H., & Severinov, K. (2000). Bacterial RNA polymerase subunit and eukaryotic RNA polymerase subunit RPB6 are sequence, structural, and functional homologs and promote RNA polymerase assembly. *PNAS*, *98*(3), 892–897.
*Mitra, P., Ghosh, G., Hafeezunnisa, M., & Sen, R. (2017). Rho Protein: Roles and Mechanisms. *Annual Review of Microbiology*, *71*(1), 687–709.
Mizushima, T., Kataoka, K., Ogata, Y., Inoue, R., & Sekimizu, K. (1997). Increase in negative supercoiling of plasmid DNA in *Escherichia coli* exposed to cold shock. *Molecular Microbiology*, *23*(2), 381–386.
Mizushima, T., Natori, S., & Sekimizu, K. (1993). Relaxation of supercoiled DNA associated with induction of heat shock proteins in *Escherichia coli*. *MGG Molecular & General Genetics*, *238*, 1–5.
Mizuuchi, K., Fisher, L. M., O'Dea, M. H., & Gellert, M. (1980). DNA gyrase action involves the introduction of transient double-strand breaks into DNA. *Proceedings of the National Academy of Sciences*, *77*(4), 1847–1851.
Murakami, K. S., Masuda, S., Campbell, E. A., Muzzin, O., & Darst, S. A. (2002). Structural basis of transcription initiation: An RNA polymerase holoenzyme-DNA complex. *Science*, *296*(5571),

1285–1290.
Murphy, L. D., & Zimmerman, S. B. (1997). Isolation and characterization of spermidine nucleoids from *Escherichia coli*. *Journal of Structural Biology*, *119*(3), 321–335.
Nakashima, K., Kanamaru, K., Mizuno, T., & Horikoshi, K. (1996). A novel member of the cspA family of genes that is induced by cold shock in *Escherichia coli*. *Journal of Bacteriology*, *178*(10), 2994–2997.
Ogata, Y., Inoue, R. I., Mizushima, T., Kano, Y., Miki, T., & Sekimizu, K. (1997). Heat shock-induced excessive relaxation of DNA in *Escherichia coli* mutants lacking the histone-like protein HU. *Biochimica et Biophysica Acta*, *1353*(3), 298–306.
Ogata, Y., Mizushima, T., Kataoka, K., Kita, K., Miki, T., & Sekimizu, K. (1996). DnaK heat shock protein of *Escherichia coli* maintains the negative supercoiling of DNA against thermal stress. *Journal of Biological Chemistry*, *271*(46), 29407–29414.
Ogata, Y., Mizushima, T., Kataoka, K., Miki, T., & Sekimizu, K. (1994). Identification of DNA topoisomerases involved in immediate and transient DNA relaxation induced by heat shock in *Escherichia coli*. *MGG Molecular & General Genetics*, *244*(5), 451–455.
Palangi, F., Samuel, S. M., Thompson, I. R., Triggle, C. R., & Emara, M. M. (2017). Effects of oxidative and thermal stresses on stress granule formation in human induced pluripotent stem cells. *PLoS ONE*, *12*(7), 1–18.
Paul, B. J., Berkmen, M. B., & Gourse, R. L. (2005). DksA potentiates direct activation of amino acid promoters by ppGpp. *Proceedings of the National Academy of Sciences*, *102*(22), 7823–7828.
Peng, H., & Marians, K. J. (1995). The interaction of *Escherichia coli* topoisomerase IV with DNA. *Journal of Biological Chemistry*, *270*(42), 25286–25290.
Peng, H, & Marians, K. J. (1993). *Escherichia coli* topoisomerase IV. *The Journal of Biological Chemistry*, *268*(32), 24481–24490.
Peng, Hong, & Marians, K. J. (1993). Decatenation activity of topoisomerase IV during *oriC* and pBR322 DNA replication *in vitro*. *Proceedings of the National Academy of Sciences*, *90*(18), 8571–8575.
*Peters, J. M., Vangeloff, A. D., & Landick, R. (2011). Bacterial Transcription Terminators: The RNA 3′-End Chronicles. *J. Mol. Biol.*, *412*(5), 793–813.
Phadtare, S., & Inouye, M. (2001). Role of CspC and CspE in regulation of expression of RpoS and UspA, the stress response proteins in *Escherichia coli*. *Journal of Bacteriology*, *183*(4), 1205–1214.
Phadtare, Sangita. (2004). Bacterial Cold-Shock Response . *Current Issues Mol. Bio.*, *6*, 125–136.
Phadtare, Sangita, Inouye, M., & Severinov, K. (2002). The nucleic acid melting activity of *Escherichia* coli CspE is critical for transcription antitermination and cold acclimation of cells. *Journal of Biological Chemistry*, *277*(9), 7239–7245.
Pruss, G. J., & Drlica, K. (1986). Topoisomerase I mutants: The gene on pBR322 that encodes resistance to tetracycline affects plasmid DNA supercoiling. *Proceedings of the National Academy of Sciences*, *83*(23), 8952–8956.
Pruss, Gail J. (1985). DNA topoisomerase I mutants. Increased heterogeneity in linking number and other replicon-dependent changes in DNA supercoiling. *Journal of Molecular Biology*, *185*(1), 51–63.
Pruss, Gail J., & Drlica, K. (1989). DNA supercoiling and prokaryotic transcription. *Cell*, *56*(4), 521–523.
Pruss, Gail J., Manes, S. H., & Drlica, K. (1982). Escherichia coli DNA topoisomerase I mutants: Increased supercoiling is corrected by mutations near gyrase genes. *Cell*, *31*(1), 35–42.
Qi, H., Menzel, R., & Tse-Dinh, Y. C. (1997). Regulation of *Escherichia coli* topA gene transcription: Involvement of a σ(S)-Dependent promoter. *Journal of Molecular Biology*, *267*(3), 481–489.
Qi, L., Menzel, R., & Tse-Dinh, Y. C. (1996). Effect of the deletion of the σ32-dependent promoter (P1) of the *Escherichia coli* topoisomerase I gene on thermotolerance. *Molecular Microbiology*, *21*(4), 703–711.
Rath, D., & Jawali, N. (2006). Loss of expression of *cspC*, a cold shock family gene, confers a gain of fitness in *Escherichia coli* K-12 strains. *Journal of Bacteriology*, *188*(19), 6780–6785.
Ray, M. K., Kumar, G. S., Janiyani, K., Kannan, K., Jagtap, P., Basu, M. K., & Shivaji, S. (1998). Adaptation to low temperature and regulation of gene expression in antarctic psychrotrophic

bacteria. *Journal of Biosciences*, *23*(4), 423–435.
*Roberts, J., & Park, J. S. (2004). Mfd, the bacterial transcription repair coupling factor: Translocation, repair and termination. *Current Opinion in Microbiology*, *7*(2), 120–125.
*Roberts, J. W. (2019). Mechanisms of Bacterial Transcription Termination. *Journal of Molecular Biology*, *431*(20), 4030–4039.
Rohde, J. R., Fox, J. M., & Minnich, S. A. (1994). Thermoregulation in *Yersinia enterocolitica* is coincident with changes in DNA supercoiling. *Molecular Microbiology*, *12*(2), 187–199.
*Rui, S., & Tse-Dinh, Y. C. (2003). Topoisomerase function during bacterial responses to environmental challenge. *Frontiers in Bioscience*, *8*, 256–263.
Salakhetdinova, O. Y., Pshenichnov, M. R., Nesterova, L. Y., Dracheva, N. A., & Tkachenko, A. G. (2000). Changes in the topological state of dna during *Escherichia coli* adaptation to oxidative stress under glucose starvation and after the transition to growth. *Microbiology*, *69*(1), 58–62.
Schneider, R., Travers, A., Kutateladze, T., & Muskhelishvili, G. (1999). A DNA architectural protein couples cellular physiology and DNA topology in Escherichia coli. *Molecular Microbiology*, *34*(5), 953–964.
Schröder, W., Bernhardt, J., Marincola, G., Klein-Hitpass, L., Herbig, A., Krupp, G., Nieselt, K., & Wolz, C. (2014). Altering gene expression by aminocoumarins: The role of DNA supercoiling in *Staphylococcus aureus*. *BMC Genomics*, *15*(291).
Schröder, W., Goerke, C., & Wolz, C. (2013). Opposing effects of aminocoumarins and fluoroquinolones on the SOS response and adaptability in Staphylococcus aureus. *Journal of Antimicrobial Chemotherapy*, *68*, 529–538.
*Schvartzman, J. B., & Stasiak, A. (2004). A topological view of the replicon. *EMBO Reports*, *5*(3), 256–261.
Shenhar, Y., Biran, D., & Ron, E. Z. (2012). Resistance to environmental stress requires the RNA chaperones CspC and CspE. *Environmental Microbiology Reports*, *4*(5), 532–539.
Shi, X., Waasdorp, B. C., & Bennett, G. N. (1993). Modulation of Acid-Induced Amino Acid Decarboxylase Gene Expression by *hns* in *Escherichia coli*. *Journal of Bacteriology*, *175*(4), 1182–1186.
Šiková, M., Wiedermannová, J., Převorovský, M., Barvík, I., Sudzinová, P., Kofroňová, O., Benada, O., Šanderová, H., Condon, C., & Krásný, L. (2020). The torpedo effect in Bacillus subtilis : RN ase J1 resolves stalled transcription complexes. *The EMBO Journal*, *39*(3).
Sioud, M., Boudabous, A., & Cekaite, L. (2009). Transcriptional responses of *Bacillus subtillis* and *thuringiensis* to antibiotics and anti tumour-drugs. *International Journal of Molecular Medicine*, *23*(1), 33–39.
Sissi, C., & Palumbo, M. (2010). In front of and behind the replication fork: bacterial type IIA topoisomerases. *Cellular and Molecular Life Sciences*, *67*(12), 2001–2024.
Snoep, J. L., van der Weijden, C. C., Andersen, H. W., Westerhoff, H. V., & Jensen, P. R. (2002). DNA supercoiling in *Escherichia coli* is under tight and subtle homeostatic control, involving gene-expression and metabolic regulation of both topoisomerase I and DNA gyrase. *European Journal of Biochemistry*, *269*(6), 1662–1669.
Soutourina, O. A., Krin, E., Laurent-Winter, C., Hommais, F., Danchin, A., & Bertin, P. N. (2002). Regulation of bacterial motility in response to low pH in *Escherichia coli*: The role of H-NS protein. *Microbiology*, *148*(5), 1543–1551.
Steck, T. R., Franco, R. J., Wang, J.-Y., & Drlica, K. (1993). Topoisomerase mutations affect the relative abundance of many *Escherichia coli* proteins. *Molecular Microbiology*, *10*(3), 473–481.
Sugino, A, Higgins, N. P., Brown, P. O., Peebles, C. L., & Cozzarelli, N. R. (1978). Energy coupling in DNA gyrase and the mechanism of action of novobiocin. *Proceedings of the National Academy of Sciences*, *75*(10), 4838–4842.
Sugino, Akio, Higgins, N. P., & Cozzarelli, N. R. (1980). DNA gyrase subunit stoichiometry and the covalent attachment of subunit A to DNA during DNA cleavage. *Nucleic Acids Research*, *8*(17), 3865–3874.
Sundquist, W. I., & Klug, A. (1989). Telomeric DNA dimerizes by formation of guanine tetrads between hairpin loops. *Nature*, *342*(6251), 825–829.
Svetlov, V., & Nudler, E. (2020). Towards the unified principles of transcription termination. *The EMBO Journal*, *39*(3).

Swanberg, S. L., & Wang, J. C. (1987). Cloning and sequencing of the *Escherichia coli gyrA* gene coding for the a subunit of DNA gyrase. *Journal of Molecular Biology*, *197*(4), 729–736.
Takami, H., Takaki, Y., & Uchiyama, I. (2002). Genome sequence of *Oceanobacillus iheyensis* isolated from the Iheya Ridge and its unexpected adaptive capabilities to extreme environments. *Nucleic Acids Research*, *30*(18), 3927–3935.
Tapias, A., López, G., & Ayora, S. (2000). *Bacillus subtilis* LrpC is a sequence-independent DNA-binding and DNA-bending protein which bridges DNA. *Nucleic Acids Research*, *28*(2), 552–559.
Thiara, A. S., & Cundliffe, E. (1989). Interplay of novobiocin-resistant and -sensitive DNA gyrase activities in self-protection of the novobiocin producer, *Streptomyces sphaeroides*. *Gene*, *81*(1), 65–72.
Tkachenko, A. G., Salakhetdinova, O. I., & Pshenichnov, M. R. (1997). Exchange of putrescine and potassium between cells and media as a factor in the adaptation of *Escherichia coli* to hyperosmotic shock. *Mikrobiologiia*, *66*(3), 329–334.
Tse-Dinh, Y C, & Beran, R. K. (1988). Multiple promoters for transcription of the Escherichia coli DNA topoisomerase I gene and their regulation by DNA supercoiling. *Journal of Molecular Biology*, *202*(4), 735–742.
Tse-Dinh, & Yuk-Ching. (1985). Regulation of the *Escherichia coli* DNA topoisomerase I gene by DNA supercoiling. *Nucleic Acids Research*, *13*(13), 4751–4763.
Tse-Dinh, Yuk Ching. (2000). Increased sensitivity to oxidative challenges associated with *topA* deletion in *Escherichia coli*. *Journal of Bacteriology*, *182*(3), 829–832.
Ullsperger, C., & Cozzarelli, N. R. (1996). Contrasting enzymatic activities of topoisomerase IV and DNA gyrase from *Escherichia coli*. *Journal of Biological Chemistry*, *271*(49), 31549–31555.
Unniraman, S., Chatterji, M., & Nagaraja, V. (2002). DNA Gyrase Genes in *Mycobacterium tuberculosis*: a Single Operon Driven by Multiple Promoters. *Journal of Bacteriology*, *184*(19), 5449–5456.
Unniraman, S., & Nagaraja, V. (1999). Regulation of DNA gyrase operon in *Mycobacterium smegmatis*: a distinct mechanism of relaxation stimulated transcription. *Genes to Cells*, *4*(12), 697–706.
Uppal, S., Rao Akkipeddi, V. S. N., & Jawali, N. (2008). Posttranscriptional regulation of *cspE* in *Escherichia coli* : involvement of the short 5-untranslated region. *FEMS Microbiology Letters*, *279*(1), 83–91.
*Uptain, S. M., Kane, C. M., & Chamberlin, M. J. (1997). Basic mechanisms of transcript elongation and its regulation. *Annual Review of Biochemistry*, *66*(1), 117–172.
Varshavsky, A. J., Nedospasov, S. A., Bakayev, V. V, Bakayeva, T. G., & Georgiev, G. P. (1977). Histone-like proteins in the purified *Escherichia coli* deoxyribonucleoprotein. *Nucleic Acids Research*, *4*(8), 2725–2745.
*Vos, S. M., Tretter, E. M., Schmidt, B. H., & Berger, J. M. (2011). All tangled up: how cells direct, manage and exploit topoisomerase function. *Nature Reviews Molecular Cell Biology*, *12*(12), 827–841.
Wang, J.-Y., & Syvanen, M. (1992). DNA twist as a transcriptional sensor for environmental changes. *Molecular Microbiology*, *6*(14), 1861–1866.
Wang, J C. (1985). DNA Topoisomerases. *Annual Review of Biochemistry*, *54*(1), 665–697.
Wang, James C. (1971). Interaction between DNA and an *Escherichia coli* protein ω. *Journal of Molecular Biology*, *55*(3), 523–533.
*Wang, James C. (2009). *Untangling the double helix : DNA entanglement and the action of the DNA topoisomerases* (1st ed.). Cold Spring Harbor Laboratory Press.
Wang, James C., & Lynch, A. S. (1993). Transcription and DNA supercoiling. *Current Opinion in Genetics and Development*, *3*(5), 764–768.
Wang, N., Yamanaka, K., & Inouye, M. (1999). CspI, the ninth member of the CspA family of *Escherichia coli*, is induced upon cold shock. *Journal of Bacteriology*, *181*(5), 1603–1609.
Watson, J. D., & Crick, F. H. (1953a). The structure of DNA. *Cold Spring Harbor Symposia on Quantitative Biology*, *18*, 123–131.
Watson, J. D., & Crick, F. H. C. (1953b). Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. *Nature*, *171*(4356), 737–738.
Weinstein-Fischer, D., Elgrably-weiss, M., & Altuvia, S. (2000). *Escherichia coli* response to

hydrogen peroxide: a role for DNA supercoiling, Topoisomerase I and Fis. *Molecular Microbiology*, *35*(6), 1413–1420.
Xia, B., Ke, H., & Inouye, M. (2001). Acquirement of cold sensitivity by quadruple deletion of the *cspA* family and its suppression by PNPase S1 domain in *Escherichia coli*. *Molecular Microbiology*, *40*(1), 179–188.
Yamanaka, K., Fang, L., & Inouye, M. (1998). The CspA family in *Escherichia coli* : multiple gene duplication for stress adaptation. *Molecular Microbiology*, *27*(2), 247–255.
Yamanaka, K., & Inouye, M. (1997). Growth-phase-dependent expression of cspD, encoding a member of the CspA family in *Escherichia coli*. *Journal of Bacteriology*, *179*(16), 5126–5130.
Yamanaka, K., Zheng, W., Crooke, E., Wang, Y.-H., & Inouye, M. (2001). CspD, a novel DNA replication inhibitor induced during the stationary phase in *Escherichia coli*. *Molecular Microbiology*, *39*(6), 1572–1584.
Yan, Y., Ding, Y., Leng, F., Dunlap, D., & Finzi, L. (2018). Protein-mediated loops in supercoiled DNA create large topological domains. *Nucleic Acids Research*, *46*(9), 4424.
Zechiedrich, E. L., & Cozzarelli, N. R. (1995). Roles of topoisomerase IV and DNA gyrase in DNA unlinking during replication in *Escherichia coli*. *Genes and Development*, *9*(22), 2859–2869.
Zechiedrich, E. Lynn, Khodursky, A. B., Bachellier, S., Schneider, R., Chen, D., Lilley, D. M. J., & Cozzarelli, N. R. (2000). Roles of topoisomerases in maintaining steady-state DNA supercoiling in *Escherichia coli*. *Journal of Biological Chemistry*, *275*(11), 8103–8113.
Zhang, G., Campbell, E. A., Minakhin, L., Richter, C., Severinov, K., & Darst, S. A. (1999). Crystal Structure of *Thermus aquaticus* Core RNA Polymerase at 3.3 A ˚ Resolution. *Cell*, *98*, 811–824.