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Existují rostlinná intermediální filamenta?

Do plant intermediate filaments exist?

BACHELOR THESIS

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Prohlášení

Prohlašuji, že jsem svou bakalářskou práci vypracovala samostatně a použila jsem pouze podklady (literaturu, projekty, SW atd.) uvedené v přiloženém seznamu.

V Praze dne 29.4.2025 .....

Kristýna Stohanzlová

Poděkování

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Abstrakt

Intermediální filamenta patří mezi cytoskeletální proteiny. Vyskytují pouze u zástupců Metazoa, i když nedávno byly nalezeny homology v superskupině SAR a Amoebozoa. U rostlin se vyskytují analogy intermediálních filament, nuclear matrix constituent proitens (NMCPs). Jedná se o strukturní analogy živočišných laminů. Mimo to se literatuře vyskytují anotace rostlinných intermediálních filament, a daných anotací v současné době stále přibývá. Cílem práce bylo setřídit takto anotované proteiny a rozhodnout, zda jejich název (intermediální filamentum) označuje skutečnost. Výsledky naznačují, že rostliny nemají pravé homology intermediálních filament, nicméně pro potvrzení bude třeba dalšího výzkumu.

Klíčová slova

Intermediální filamenta, nuclear matrix constituent protein (NMCP), jaderná intermediální filamenta (NIF), lamin, homology laminů

Abstract

Intermediate filaments are one of the 4 categories of cytoskeletal proteins. They are restricted to Metazoa, though recently new homologues are found in SAR supergroup and Amoebozoa. In plants, intermediate filaments analogues exist, they are structural components of the plant lamina. These are nuclear matrix constituent proteins (NMCPs). However, other plant intermediate filaments have been recently annotated in literature. Aim of this thesis is to sort out these annotations and declare, whether the use of term "intermediate filament" is justified. The results suggest, that no true intermediate filament homologues occur in plants. To clarify these findings, further research is needed.

Keywords

Intermediate filaments, nuclear matrix constituent protein (NMCP), nuclear intermediate filament (NIF), lamin, lamin homologues

Contents

1 Introduction **1**

2 Metazoan Intermediate Filaments **2**
2.1 Classes of intermediate filaments 5
2.1.1 Type I and type II intermediate filament proteins 6
2.1.2 Type III intermediate filament proteins 6
2.1.3 Type IV intermediate filament proteins 7
2.1.4 Type V intermediate filament proteins 7
2.1.5 Type VI intermediate filament proteins 8

3 Plant Intermediate Filaments **10**
3.1 Plant Homologues of IF Known until Recently 10
3.1.1 Protein Group NMCP/CRWN/LINC (Plant Lamins Analogues) 11

4 Alleged IF Homologues in Plants **18**
4.1 Presumed Plant IF in the Literature 18
4.1.1 Sequence Homologues of Intermediate Filaments 18
4.1.2 PROPSEARCH-found Intermediate Filament Analogues 18
4.1.3 Intermediate Filament Motif Protein 1 20
4.1.4 Plant Keratins 22
4.1.5 Intermediate Filament Antigen Binding Proteins 23
4.1.6 Calcium-dependent Protein Kinase Adapter Protein 1 23
4.1.7 *Oryza Sativa* Intermediate Filament(-like) Protein 25
4.1.8 Neurofilament Light Protein 26
4.2 Plant IF Annotated in Databases 26

5 Conclusion **28**

1 Introduction

Intermediate filaments (IF) are part of cell cytoskeleton along with microfilaments, microtubules and septins (which are nowadays counted as a part of cytoskeleton). These proteins have the ability to form filamentous networks in cells and their properties differ depending on their protein content. Cytoskeletal filaments can serve as a mechanical scaffold of the cell, moreover also as transport structures, signalling molecules, etc.

The omnipresent part of cytoskeletal fibres in all eukaryotic cells are microtubules and microfilaments. Septins also belong to highly conserved protein family, however they are absent in higher plants (Dulal et al. (2020)).

Intermediate filaments are only present in metazoans, but few other homologues are recently emerging in non-metazoans (Odell et al. (2023)). However, sequence homologues have not yet been found in single-cell organisms and neither in plant cells. By criteria other than sequence homology, structure analogues of intermediate filaments (especially lamins) can be found in various eukaryotic cells including plant cells.

Plant IF analogues are known as nuclear matrix constituent proteins (NMCPs) which are lamin analogues. They are able to form filaments and perform other lamin roles in animal cells.

In earlier literature, nuclear intermediate filaments were considered as plant IF homologues and also in recent literature, new proteins named as intermediate filaments are emerging. These proteins together with metazoan IF and NMCPs will be described in following chapters.

2 Metazoan Intermediate Filaments

Intermediate filaments form a group of filamentous proteins that are highly insoluble in a wide range of pH (Renner et al. (1981), Herrmann et al. (2016), and Hargreaves, Goodbody, et al. (1989)). They are known for their diameter size of approximately 10 nm, which is in between the sizes of actin filaments (6 nm) and myosin (15 nm) filaments (and also microtubules of 25 nm diameter) and were previously called 10 nm filaments (Steinert, Jones, et al. (1984), Herrmann et al. (2016), and Hargreaves, Goodbody, et al. (1989)). IFs are able to withstand great mechanical stresses in vitro as well as in vivo (Köster et al. (2015)), and can re-polymerise after solubilisation in urea (Hargreaves, Goodbody, et al. (1989)).

For characterisation, X-ray diffraction and amino acid sequencing were used first. The results of the X-ray diffractions are simple patterns that imply the fibrillar structure of the proteins (Astbury (1939) and Bouameur et al. (2017)). Although the amino acid sequences of different IFs were not the same, the overall structures were similar (Renner et al. (1981)). This was supported by the similarities of the observed diffraction patterns. In addition, electron microscopy of ultrathin sections revealed fibrous structures of 10 nm filaments in muscle tissue. Later, using the 10 nm criteria, these filaments were also observed in other tissues.

Another breakthrough was immunological experiments such as immunoblots and immunofluorescence microscopy. In 1981, Pruss et al. invented monoclonal antibody binding to various IF proteins in the most conserved region, the coiled coil domain (more details are discussed later) (Pruss et al. (1981)). The following research unravelled multiple amino acid sequence similarities of IFs by comparison of vimentin sequences among the animal kingdom (Renner et al. (1981)). The same research team later collated other IFs (e.g. desmin and keratins) and found sequence similarities again.

Not long after, in 1983, Peter M. Steinert et al. described the first full-length amino acid sequence of an epidermal keratin (Steinert, Rice, et al. (1983)).

The IF protein family is divided into five (Dawson et al. (1985)) or six subgroups (Hohmann et al. (2019) and Bouameur et al. (2017)), categorized by sequence homology (Bouameur et al. (2017)). The characterisation of each group will be discussed in Chapter 2.1.

Although the intermediate filaments have different characteristics across the groups, they do share some common qualities. IFs create a sturdy mechanical structure (mechanical buffer) in cells. IFs can be stretched to 300 % of their original length without rupture (Goldman et al. (2008)) and during the cell cycle, they undergo various remodelations (Hohmann et al. (2019) and Meng et al. (1994)). Moreover, IF proteins participate in cell signalling and interact with a wide range of cellular structures, from organelles and vesicles to cytoskeleton parts (actin, microtubules, and even other IFs). Although they interact with other parts of the cytoskeleton through molecular interactions, they do not function as enzymes themselves (Pradeau-Phélut et al. (2024) and Hohmann et al. (2019)). IF proteins can be specifically phosphorylated or elsehow post-translationally edited (Bouameur et al. (2017) and Steinert, Jones, et al. (1984)).

IF proteins typically have a tripartite structure – one central α-helical rod domain surrounded by a non-α-helical domain at each end (Herrmann et al. (2016)). The α-helical rod domain can be further substructured into three α-helical subdomains, helices Ia, Ib and II (Geisler and Weber (1982) and Chou et al. (1978)). The helix II was previously divided into two helices, IIa and IIb, so the rod domain would consist of four α-helices and 3 non-α-helical linkers (more on it later). Two of the helices would be shorter (Ia and IIa) and the other two longer (Ib and IIb) (Geisler, Schünemann, et al. (1992) and Steinert, Jones, et al. (1984)).

The rod domain forms dimers that take the shape of a double-stranded coiled coil, which can then form dimers in a half-staggered antiparallel manner (Geisler and Weber (1982)). Eight tetramers form a cylindrical complex that functions as a monomer for filament formation. This complex is called protofilament or unit length filament (ULF; Herrmann et al. (2016) and Geisler, Schünemann, et al. (1992)). After polymerisation, the filament is shrunk to its final diameter of 10 nm (shown in Figure 1; Hohmann et al. (2019)).

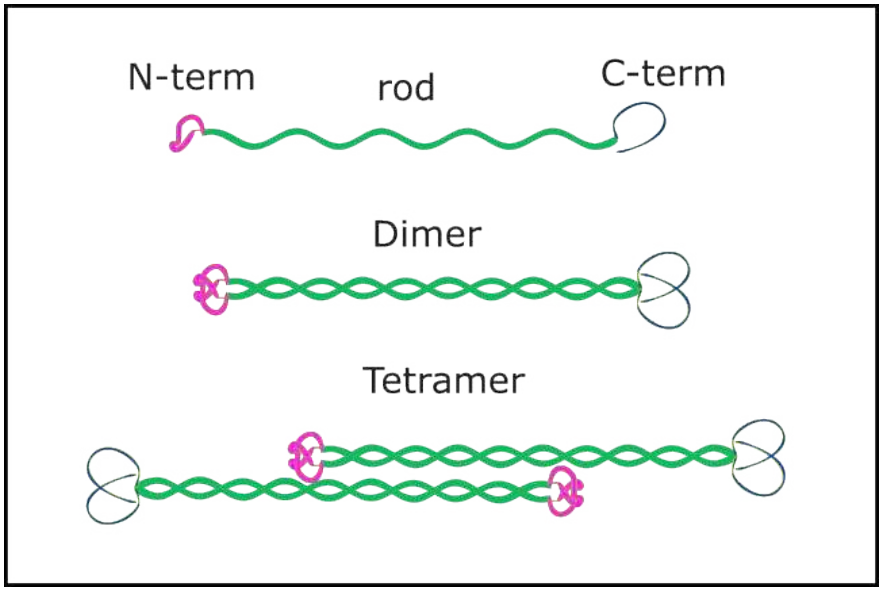

Figure 1: Scheme of dimer and tetramer formation. Modified from Hohmann et al. (2019). Pink part resembles N-terminal domain (N-term), green part coiled-coil rod domain and blue part C-terminal domain (C-term) These tetramers then segregate in parallel way to form unit filaments which do form filaments. This process is not dependent on ATP nor GTP.

The driving force for dimer coiled coil assembly is the hydrophobic and electrostatic interactions between the hydrophobic residues in heptads (Monera et al. (1994)). The heptad is a group of 7 amino acids (similarly, hendecads are repeats of 11 amino acids), the pattern of which is repeated in the protein. In IF proteins, the pattern follows a scheme where the first and the fourth residues are hydrophobic (Meng et al. (1994)).
In dimers, the strands are aligned, so that the same domains are at the same end, and the rod domain is aligned with the other rod domain. In contrast, the tetramer forms in an antiparallel way, so that the N-terminal domains (the head) of one dimer are facing the C-terminal domains (the tails) of the other. The alignment is not exact, but staggered by approximately 20 nm. The end domains are the C-terminal domains of both dimers as can be seen in Figure 1 (Meng et al. (1994) and Geisler, Schünemann, et al. (1992)).
Characteristics of the rod domain have been studied separately from the rest of the protein via crystallography and also by sequence homology. Although the rod region is highly conserved among the IFs, the rod domain cannot form the characteristic 10 nm filament itself, hence the head-tail domains are necessary for filament formation. However, these end domains are divergent in the sequences available so far (Geisler and Weber (1982) and Herrmann et al. (2016)).
Regarding filament assembly in vivo, there were suspected centres of IF organisation (for keratins) that may be located at the nucleus. But no further information is available (Steinert, Jones, et al.

(1984), Eckert et al. (1982b), and Eckert et al. (1982a)).
As mentioned previously, the rod domain is one of the most conserved regions of IF proteins, and is divided into two main α-helices: helix I and II; which could be further subdivided (see Figure 2).

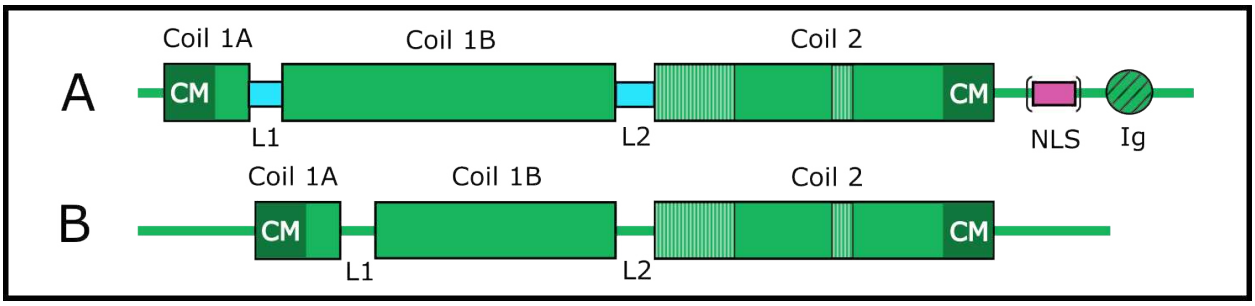

Figure 2: Domain organization lamins and invertebrate cytoplasmic IF proteins (top) and vertebrate cytoplasmic (bottom) IF proteins. Modified from (Herrmann et al. (2016)). Green boxes resemble α-helices (coils) with numbers representing amino acid length of the segments. Stripes indicates hendecad instead of heptads repeats in coil 2. The pink box is for NLS sequence in lamins, but is not for invertebrates cytoplasmic IFs (therefore the brackets). The Ig-fold (striped circle) is conserved among lamins and most invertebrate cytoplasmic IF proteins. CM in boxes stands for consensus sequences LNDR (in coil 1A) and TYRKLLEGEE (Coil 2). L1 and L12 are the linker segments connecting corresponding helixes which are in lamins present in ordered state (blue boxes) and in disordered state in others IF proteins.

The first α-helix of the rod, the helix Ia, contains one of the consensus sequences, the LNDR sequence (Strelkov et al. (2002)). When crystallised, the helix occurs as a monomer and can form dimers only in the complete coil 1. Nevertheless, the helix has a shape that is likely to form a coiled coil; therefore, it does not disturb the final coiled coil rod domain (Meier et al. (2009)).
In contrast, the helix Ib forms a stable parallel coiled coil dimer in crystal and can also form the antiparallel tetramers (Herrmann et al. (2016)).
The structure of the first linker (L1, as in Figure 2) is not flexible, so the peptide acts as a distortion in otherwise continuous coiled coil Ia-Ib (Aziz et al. (2012)).
After the helix Ib, the second linker, L12, follows (see Figure 2), which has the first 4 amino acids conserved among organisms. In type I to type IV IF proteins in vertebrates, the linker sequence is Gln-Ala-Gln-Iso. For lamins and invertebrate IF proteins, it changes to Arg-Arg-Lys-His. These patterns apply with slight changes in homologous amino acids in different species (Herrmann et al. (2004)).
Helix II is more complicated. The helix itself was first divided into helices IIa and IIb, which should be separated by a non-α-helical linker, L2. However, current knowledge supports the idea of an unseparated helix II since the linker L2 does not disturb the coiled coil and even shows an α-helical structure itself (Herrmann et al. (2016)). The first 36 amino acids form hendecad repeats that are distinct from the heptad pattern occurring in the rest of the rod domain. The rest of helix IIb follows the heptad scheme (Herrmann et al. (2016) and Nefedova et al. (2024)). This motif is conserved among all IF proteins and therefore could be crucial for IF assembly (Herrmann et al. (2016)). Last consensus sequence begins three amino acids from the end of helix II. The motif is called TYRKLLEGEE according to its sequence in a one-letter amino acid code (Herrmann et al.

(2016)).
Invertebrate IF proteins are more similar to vertebrate lamin A, with its long Ib helix (42 amino acids longer; Herrmann et al. (2016)). There are documented cases of short IF proteins in invertebrates, in cephalochordates and urochordates, which form the chordate branch of deuterostomia (Karabinos et al. (1998)), and also in echinoderms (Holy (2000)). This indicates that the short version of the IF proteins is shared among all deuterostomia.
Beyond IFs itself, numerous proteins have been described to be related to them. These proteins are IF-associated proteins (IFAPs). Each IF has its own IFAPs that help it to connect with other cellular parts, or to post-translationally edit the IF, etc. The IFAPs as well as the IFs can be found in various types of cells, however some of them are restricted to one cell type only (Steinert, Jones, et al. (1984)).

2.1 Classes of intermediate filaments

In this part, the different classes of IF are discussed. An overview of all classes is included in this chapter in form of a table, see Table 1.
Intermediate filaments form a structurally homologous family of cytoskeletal proteins. As mentioned above, all of these filaments contain an α-helical rod domain with highly conserved sequence. They all occur mainly in proximity (or inside, for the lamins) of the nucleus (Hohmann et al. (2019)).
IF proteins are grouped into 5 (or 6) subgroups named type I-V or based on the sequence homology as SHC1-SHC5. Type I to type IV are cytoplasmic proteins and type V – lamins – are the only nuclear IF proteins. The last class, type VI, is formed by divergent IF of eye lens epithelia, phakinin and filensin (which would otherwise not belong to any class). A single cell can express more than one type of IF proteins that can interact with each other or form separate networks (Steinert, Jones, et al. (1984)).
Type I and **type II** class of IF protein consists of keratins (KtyI and KtyII) which are divided by their acidity – the type I are the acidic keratins and type II are the basic keratins. They form a complex class and are expressed mainly in epithelial cells, where they form heteropolymers (Geisler and Weber (1982), Bouameur et al. (2017), and Hohmann et al. (2019)).
Type III class includes vimentin, desmin (or earlier skeletin), peripherin, glial fibrillary acidic protein (GFAP), and syncoilin (sync1 and sync2). These proteins form homopolymers and are expressed in various tissues (Geisler and Weber (1982), Bouameur et al. (2017), and Hohmann et al. (2019)).
Type IV intermediate filaments include three neurofilament heteropolymers, α-internexin, synemin and nestin. All these proteins are found mainly in cells of the nervous system (Bouameur et al. (2017) and Hohmann et al. (2019)).
Type V are nuclear lamins: lamin A, B, and C. They are considered to be the founding proteins of the IF family (Bouameur et al. (2017)).
Finally, the **type VI** IF proteins, which includes filensin and phakinin. They are expressed in the lens epithelium and form heteropolymers (Bouameur et al. (2017) and Hohmann et al. (2019)).

Table 1: Summary of IF classes, their characteristics and their representative proteins. For detailed description and references see chapter 2.

Class	Description	Members
Type I	acidic proteins of vertebrate epithelial cells	acidic keratins
Type II	basic proteins of vertebrate epithelial cells	basic keratins
Type III	proteins contributing in oxidative--stress responses, expressed in a variety of cells	vimentin, desmin, peripherin, GFAP, syncoilin
Type IV	proteins of neural tissues, important for their growth	NFL, NFM, NFH, α-internexin, synemin, nestin.
Type V	ancestral IF proteins in nuclear lamina, present in all Metazoa	lamins
Type VI	diverging group of IF proteins, present in lens epithelium	filensin, phakanin

2.1.1 Type I and type II intermediate filament proteins

In these types of IF proteins belong **keratins**, one of the first observed IFs. They occur among various vertebrate epithelial cells. No evidence of true cystein-rich keratins was found in invertebrates, even after 70 years (Brown (1950)). In human epithelial tissue, various keratin subunits are found (Steinert, Jones, et al. (1984)), encoded by approximately 50 keratin genes (Moll et al. (2008)).
Keratins provide mechanical stability and integrity of epithelial cells and tissues. Some keratins are also involved in intracellular signalling pathways (Moll et al. (2008)).
Keratins form heterodimers, where one of the monomers is acidic keratin (type I) and the other is basic (type II) (Kayser et al. (2013)). Their molecular weight ranges between 40-70 kDa (Hermann et al. (2016) and Steinert, Jones, et al. (1984)).
In contrast to other IFs, keratins have higher abundance of glycine and serine, mainly in their terminal domains (Steinert, Jones, et al. (1984)).

2.1.2 Type III intermediate filament proteins

This type of IF protein plays an important role in redox signaling in cells. They function as targets and modulators in oxidative stress responses. Conserved residue, C328, is significant for the processes (Pajares et al. (2024)).
In this class, vimentin and desmin form highly connected networks with a small mesh size (Hohmann et al. (2019)).
Desmin is one of the most studied IF proteins. It is a 53kDa protein with two differently phosphorylated variants (Steinert, Jones, et al. (1984)). The N-terminal domain is arginine-rich and consists of about 70 residues and the C-terminal domain of 48 residues (Geisler and Weber (1982)).
Vimentin (decamin) occurs in various cell types. When inhibited, the cell usually collapses towards the nucleus (Steinert, Jones, et al. (1984)).
Syncoilin is a 64kDa protein with atypical structure for the IF protein expressed in the skeletal and

cardiac muscles. It lacks the linker L1 and the periodic distribution of charged residues in the rod domain (Parlakian et al. (2016)). Therefore some resources entitle it as IF-like-protein (Kleyman et al. (2014)).
Glial fibrillary acidic protein (GFAP) is expressed in radial glia, astrocytes, and glioma cells and has many isoforms. Each isoform is different in many ways and it leads to different molecular interactions and mechanical properties of the IF network (Steinert, Jones, et al. (1984), Hohmann et al. (2019), and Reus et al. (2024)).
The last protein in this group is **peripherin** specific to neurons. Its expression alters through the development of neural tissue. It is usually co-expressed with the neurofilaments (type IV) and colocalises with syncoilin and synemin (Parlakian et al. (2016)). The loss of peripherin in mice leads to an approximately 30% loss of small sensory axons (Lariviere et al. (2004)).

2.1.3 Type IV intermediate filament proteins

This type of IFs includes proteins of neural tissue. They are one of the main structural components of the axons and dendrites of neurons (Steinert, Jones, et al. (1984)).
The first to be mentioned are **neurofilament triplet proteins**: neurofilament light (NFL,68 kDa), medium (NFM,160 kDa) and heavy (NFH, 240 kDa) polypeptide (Parlakian et al. (2016)). They influence the development of motor axons. In addition, NFM is necessary for proper radial growth of myelinated axons (Lariviere et al. (2004)).
Another member of class IV IF proteins is **nestin** (240 kDa) expressed in dividing cells in early stages of the central and peripheral nervous systems, and also in other tissues. Nestin is replaced by tissue-specific IF proteins after tissue differentiation (Parlakian et al. (2016)).
α-internexin (66 kDa) is expressed in neurons and adult cerebellar granule cells. It also binds other IFs and, therefore, can function as a connecting piece among IFs (Pachter et al. (1985) and Parlakian et al. (2016)).
The last type IV IF protein is **synemin**, expressed in all muscle types and associated with protein kinase A through the tail domain (Gargano et al. (2024)).

2.1.4 Type V intermediate filament proteins

Type V IF proteins are nuclear proteins, **lamins**. They are considered to be the ancestor of other IF proteins and are abundant in all metazoan cells. Yet, none of the sequenced genomes of plants, fungi, or protozoans encodes a lamin or lamin-like protein (Batsios et al. (2012)). An overview of the evolution of lamin is given in Figure 3 and in more detail in the article Odell et al. (2023).
They differ from other vertebrate IFs by an additional 6 heptads in helix Ib (as mentioned previously), the NLS sequence, and in most cases by a terminal CAAX box in the tail domain (Herrmann et al. (2016) and Karabinos et al. (1998)).
NLS is a localisation signal to the nucleus (Herrmann et al. (2016)), while CAAX is a sequence for post-translational prenylation, which is crucial for anchoring proteins to the nuclear membrane. The CAAX sequence is present in prelamin A, lamin B1, and lamin B2 (Gao et al. (2009)). Both sequences, NLS and CAAX, are localised at the C-terminal along with the immunoglobulin fold (Ig-fold, Sobo et al. (2024)).
There are two structural types of lamins, in vertebrates encoded by three genes. B-type lamins (B1

encoded by gene LMNB1 and B2 by gene LMNB2) are abundant in all somatic cells. However, A-type lamins (lamin A and C, both encoded by gene LMNA) are developmentally regulated, in undifferentiated cells the A-type lamins are almost absent (Nigg (1992) and Shimi et al. (2015)). Furthermore, there is lamin B3 (type B lamin), abundant only in amphibians and fish (Dittmer and Misteli (2011)). All lamins form distinct but interdependent networks in the nuclear lamina, when there is more than one type of lamin expressed Shimi et al. (2015)).
Lamin A and both lamins B are post-translationally prenylated, but lamin A is spliced afterwards, leading to the loss of the prenyl-anchor. Therefore, lamin A can be found solubilised in the nucleoplasm during mitosis. The other A-type lamin, lamin C, completely lacks the CAAX prenylation signal. Lamin C functions similar to lamin A and is possibly derived from lamin A by differential splicing (Nigg (1992)).
Lamin A and C maintain the mechanical properties of the nucleus, their loss leads to susceptibility of the nucleus to mechanical stress. The same thing cannot be said about lamins B1 and B2 (Shimi et al. (2015)).
Lamins are widespread among different metazoan cell types (Gerace and Huber (2012)). They form a filamentous network, nuclear lamina, beneath the inner nuclear membrane. The nuclear lamina, is a stable non-dynamic structure (lamins remain integrated in polymers for hours after integration)(Gerace and Huber (2012)). However, in actively cycling cells, lamins are reversibly phosphorylated during mitosis, leading to temporary disassembly of the network (Gerace and Blobel (1980) and Nigg (1992)).
Lamins are also abundant in nucleoplasm, but the organisation is only partly understood (Hohmann et al. (2019)). In nucleoplasm, A-type lamins (mainly lamin C) are in the form of phosphorylated monomers and can bind to euchromatin. They are important for chromosome organization (Sobo et al. (2024)). Lamins bind to nuclear actin in the nucleoplasm by actin binding domain localised in the immunoglobulin domain on C-terminus (Simon et al. (2010)). They also attach to other components of the nucleoskeleton and to intermembrane linking proteins in the inner nuclear membrane (LINC) (Shimi et al. (2015)).
Beside maintaining nuclear structure, lamins (from nuclear lamina) are responsible for binding heterochromatin by histones and function as silencers. They modulate cell signalling and gene expression, hence control the cell processes as well as cell-differentiation (Sobo et al. (2024), Wong et al. (2022), and Shimi et al. (2015)).
They interact with other parts of cytoskeleton via LINC complexes (linker of nucleoskeleton and cytoskeleton). LINC complexes consist of SUN proteins located in the inner nuclear membrane. SUN proteins interact with KASH domain proteins in the outer nuclear membrane that bind the outer cytoskeleton parts(Stewart-Hutchinson et al. (2008)). In vegetative cells, LINC complexes also represent main microtubule-organizing centre, they link centrosomes to the nucleus (Batsios et al. (2012)).

2.1.5 Type VI intermediate filament proteins

Filensin and **phakinin** are IF proteins found exclusively in fibre cells of the eye lens. They were previously named as CP95/CP94 (filensin) and CP49 (phakinin). To avoid confusion, proteins were renamed filensin or beaded filament structural protein 1 (BFSP1) and phakinin (BFSP2) (Perng et al. (2007)).
The characteristic regions of these proteins are not conserved. In filensin, the TYRKLLEGEE motif is modified to RYHRIIE(I/N)EG and in phakinin it is changed to SYHALLDREE. LNDR

motif is altered to LGER in mammalian filensin, and in phakanin the arginine in LNDR is replaced by cysteine (leading to LNDC sequence) (Perng et al. (2007)). The protein homology is very low compared to other IF proteins. Moreover, filensin and phakinin also differ in their terminal domains among species (Hess et al. (1998)).

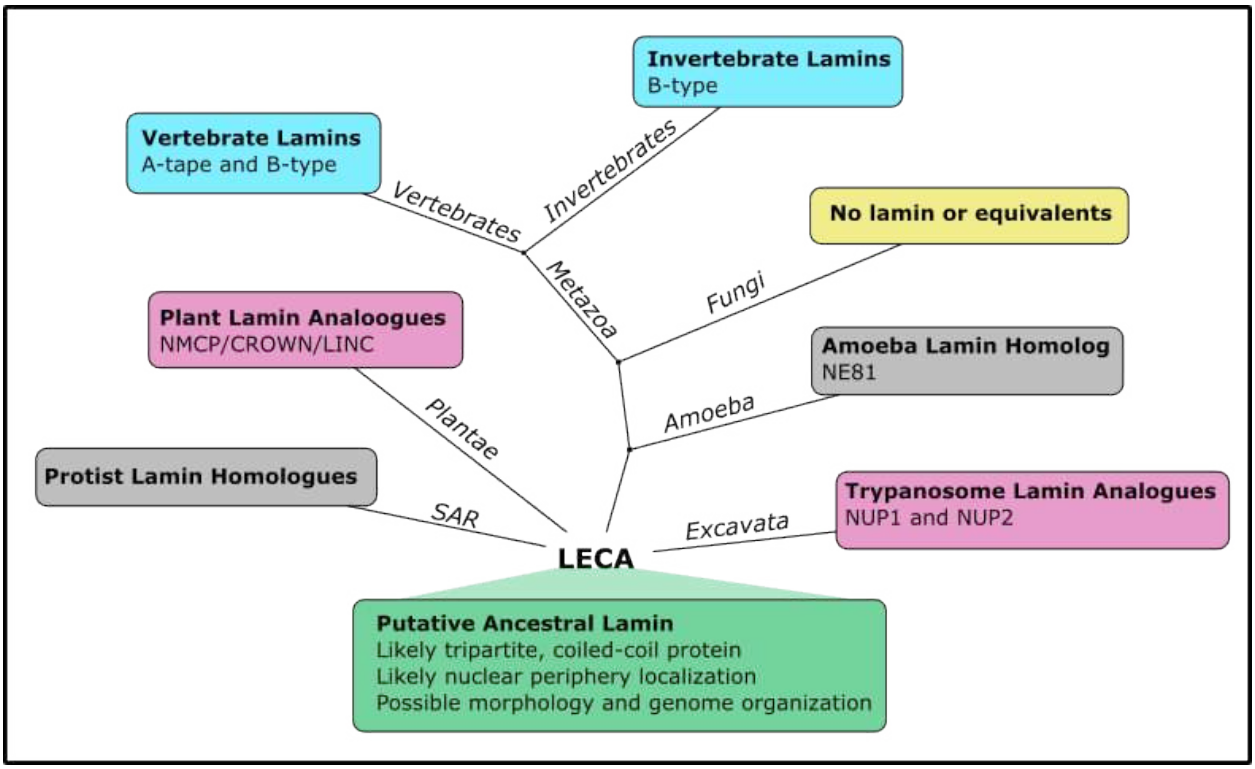

Figure 3: Evolution of lamins and lamin analogues. This figure was modified from Odell et al. (2023). In green box, likely properties of LECA (last eukaryotic common ancestor) are noted. SAR: stramenopiles, alveolates, and rhizaria supergroup. Lengths of branches on the tree are not drawn to scale.

3 Plant Intermediate Filaments

3.1 Plant Homologues of IF Known until Recently

Following the discovery of intermediate filaments in animal cells, many scientists tried to look for similar structures in plant cells. Soon, fibrous structures apparently distinct from both actin and microtubules were found to form around nucleus during cytoskeleton isolation, because the nucleus sustained its shape even after the isolation (Powell et al. (1982)). This structure was further studied using immunological, biochemical, and molecular-biological techniques. In this section, some of the work will be briefly described.

In one of the first articles, Powell et al. (1982) found 7nm filaments localised near the nuclear membrane. They form a sturdy structure that preserves the nucleus during cytoskeleton extraction by lysing adherent protoplasts with detergent under isotonic conditions.

By extracting the cell cytoskeleton, they determined the filament localisation. The filaments were localised near the nucleus and dispersed further away from it. Observations were done in multiple plants (carrot, oil palm, Italian rye, and maize). They did not find out exactly what they were made of, but determined that they were not actomyosin, and proposed that the proteins could be P-proteins (Phloem-specific proteins) (Powell et al. (1982)).

In 1985, Dawson et al. used a special monoclonal antibody to determine the proteins of the filaments (Dawson et al. (1985)). The monoclonal antibody was developed by Pruss et al. (Pruss et al. (1981)), raised against the cross-reacting epitope located within the most conserved regions of the IF proteins and therefore reacting with all 5 classes of IF.

The experiments were performed on homogenates and cells (or protoplasts) of carrot suspension cells and meristematic cells of the onion root tip. They found bundles of 7nm fibres cross-reacting with the antibody localised around the nucleus, which were not microtubules. The bundles were the same as the filaments in the work of Powell et al. (Powell et al. (1982)).

In addition, these filaments colocalised with cortical microtubules, but the staining pattern was different from that of the microtubules. Immunoblotting proved that the proteins reacting with the antibodies used are not microtubules, but some other proteins that are insoluble and resistant to detergents.(Dawson et al. (1985))

Followed by other immunological studies, the presence of the cross-reacting protein was confirmed (Beven et al. (1991), Mínguez et al. (1993), and Blumenthal et al. (2004)). In summary, the new found protein was also detergent resistant and insoluble. Its ability to self-assemble into filaments after denaturation, as a proper IF should, was yet to be proved.

Hargreaves, Goodbody, et al. (1989) demonstrated that the carrot IF antigens can reassemble into filaments in vitro, when renatured after dilution in urea.

In 1992, Frederick et al. tried to detect (and localise) lamins in the nuclear matrix of plant cells. As in the work of other scientists (e.g. Beven et al. (1991)), they tried to isolate proteins that react with the universal antibody as well as with the lamin-specific one (Frederick et al. (1992)). In this way, two proteins with different molecular weights and ratios were identified. One of them is 60 kDa and is more prominent than the other 65 kDa, both present in multiple plant species. On the other hand, they also distinguished proteins that cross-react only with lamin-specific antibodies. Consequently, these proteins were not labelled as IFs (Frederick et al. (1992))

Unlike animal nuclear lamins, plant lamin-like proteins were localised not only in the periphery but also in the nuclear matrix and could penetrate it at least to the nucleolus (Frederick et al. (1992)).

Differences between animal and plant lamins were further studied, however, they will not be mentioned in this work. For further reading this article is recommended: Frederick et al. (1992).
Many studies have focused on examining these proteins in different plant species (J. Wang et al. (1996) and Blumenthal et al. (2004)) and search for other proteins of the nuclear lamina (Wendou et al. (1999)). The aim was not only to characterise the lamin-like proteins, but also to determine whether the structure of the nuclear lamina is conserved among the eukaryotes (J. Wang et al. (1996)). In their findings, they claim that the coiled-coil-based lamina is evolutionarily conserved (Moreno Díaz se la Espina (1995)).
However, these proteins, nucelar intermediate filament (NIF) proteins, were abandoned when Masuda, Xu, et al. (1997) found a protein predicted to have an α-helical domain localised in nuclear periphery with a sequence and a molecular weight different from the lamins. This protein, named nuclear matrix constituent protein (NMCP), was later predicted to be the plant lamin analogue (Ciska, Hikida, et al. (2019)).
The plant NIF proteins, were further explored by a research group in Spain, Blumenthal et al. (2004) and Pérez-Munive et al. (2012), which based on microscopical observations suggested that the proteins (NIFs and NMCPs) might be the same. However, no full-length sequence of NIF proteins was obtained and for some of them, the molecular weights differ significantly – with 60-65 kDa for the NIFs (Ciska, Masuda, et al. (2013)) and much higher (predicted around 140 kDa, in vivo varies from 70 to 200 kDa) of the NMCPs (Masuda, Xu, et al. (1997) and Ciska and Díaz de la Espina (2013)).
Unlike NIFs, NMCPs were further researched and observed in other species via amino acid similarity searches using DcNMCP1 as a query against the other plant proteomes (Dittmer, Stacey, et al. (2007b)). The important characteristics and structures of NMCPs will be discussed in the following chapter.

3.1.1 Protein Group NMCP/CRWN/LINC (Plant Lamins Analogues)

As mentioned before, NMCP stands for the nuclear matrix constituent protein and was firstly observed in *Daucus carota* (Masuda, Xu, et al. (1997)). In *A. thaliana*, the homologues were named after the mutant phenotype with small nuclei as LINC (little nuclei). Due to confusion with LINC proteins (linkers of nucleoskeleton and cytoskeleton), they were renamed CRWN for another mutant phenotype, crowded nucleus (Ciska and Díaz de la Espina (2013)).
NMCPs are considered as plant analogues of metazoan lamin proteins (further described in chapter 2.1.4) because of their similarities in structure and function, including high insolubility in wide range of pH, predicted dimer-formation and in vitro self-assembly into filaments (Ciska and Díaz de la Espina (2013)). However, they do not share a sequence homology with them (Graumann (2021)). The preliminary comparison of NMCPs and lamins can be seen in Table 2.
NMCPs are restricted to Streptophyta clade with origin in Charophyta. There is no evidence of fibrous lamina in Chlorophyta, neither Rhodophyta nor in any single-cell plant (Ciska, Hikida, et al. (2019), Ciska and Díaz de la Espina (2013), Ciska, Masuda, et al. (2013), and Odell et al. (2023)).
The number of genes for NMCPs varies among species. Ancestral plants have only one NMCP gene, which was duplicated in some groups by whole genome duplication (WGD), leading to the creation of NMCP1 and NMCP2 type proteins in Spermatophyta. The WGD of Gunneridae lead to formation of NMCP3 subtype (not yet fully examined) (Ciska, Hikida, et al. (2019)).

Table 2: Comparison of main features of NMCPs and lamins. Table was modified from article of Ciska and Díaz de la Espina (2013), and additional information was added from article J. Wang et al. (1998) and other mention in chapter 3.1.1.

NMCPs	**Lamins**
NMCP1 and NMCP2	A-type and B-type
Central coiled-coil rod domain, head and tail domains	Central coiled-coil rod domain, head and tail domain
Rod domain flanked by cdk1 sites	Rod domain flanked by cdk1 sites
NLS in the tail domain	NLS in the tail domain
Conserved regions inside and in proximity of rod domain, and linkers	Conserved regions in extremes of the rod domain, linkers and C-terminus
Variable mass: 70-200 kDa	Mass of 65-74 kDa
Acidic	Acidic (B-type) and neutral (A-type)
Predicted to dimerize	Dimerize
Self-assemble into filaments	Self-assemble into filaments
Occurrence: Lamina, nucleoplasm	Occurrence: Lamina, nucleoplasm
Function:	Function:
Nuclear shape and size	Nuclear shape and architecture
Chromatin organization	Chromatin organization and positioning
Not involved in light induced nuclear movement	Connecting NSK and CSK
	DNA replication, repair and transcription
	Cell proliferation and differentiation

NMCP1 can be present in 1 to 4 copies while NMCP2 only in 1, or 2 due to recent WGD (Ciska, Hikida, et al. (2019)) This corresponds with the fact that NMCP1 genes are the fastest diverging NMCPs while NMCP2 and the basal NMCPs maintained 'basal NMCP' features (Ciska, Hikida, et al. (2019)). For example in *A. thaliana* and *Z. mays* the NMCPs are (Ciska and Moreno Díaz de la Espina (2014) and Graumann (2021)):

	NMCP1	NMCP2	NMCP3
Arabidopsis thaliana	CRWN1	CRWN4	CRWN2, CRWN3
Zea mays	NCH1	NCH2	–

Further phylogenetic relations of NMCPs can be seen in Figure 4.

Apart from NMCPs and disputable NIFs, other proteins are present in the plant nuclear lamina. Those are NMCP-binding proteins which are important for the proper functioning of NMCPs via their interactions (Ciska and Díaz de la Espina (2013) and Ciska, Hikida, et al. (2019)). These proteins are namely: SUNs, KAKU4, ARP7, NEAPs, MYB3, SINAT and BIM1 (Ciska, Hikida, et al. (2019), Ciska and Díaz de la Espina (2013), and Ciska, Masuda, et al. (2018)).

NMCP1 and NMCP2 are the core element of plant lamina and form independent networks in it (Ciska, Masuda, et al. (2018) and Masuda, Hikida, et al. (2021)). Their structural organisation is conserved and similar to that of lamins: a tripartite structure with a short head domain, a coiled coil rod domain, and a long tail domain (Ciska and Díaz de la Espina (2013)).

The rod domain consists of two coiled coils separated by a linker of about 20 amino acids (and in some cases more short linkers are present). It is similar to that of lamins but twice as long (Ciska,

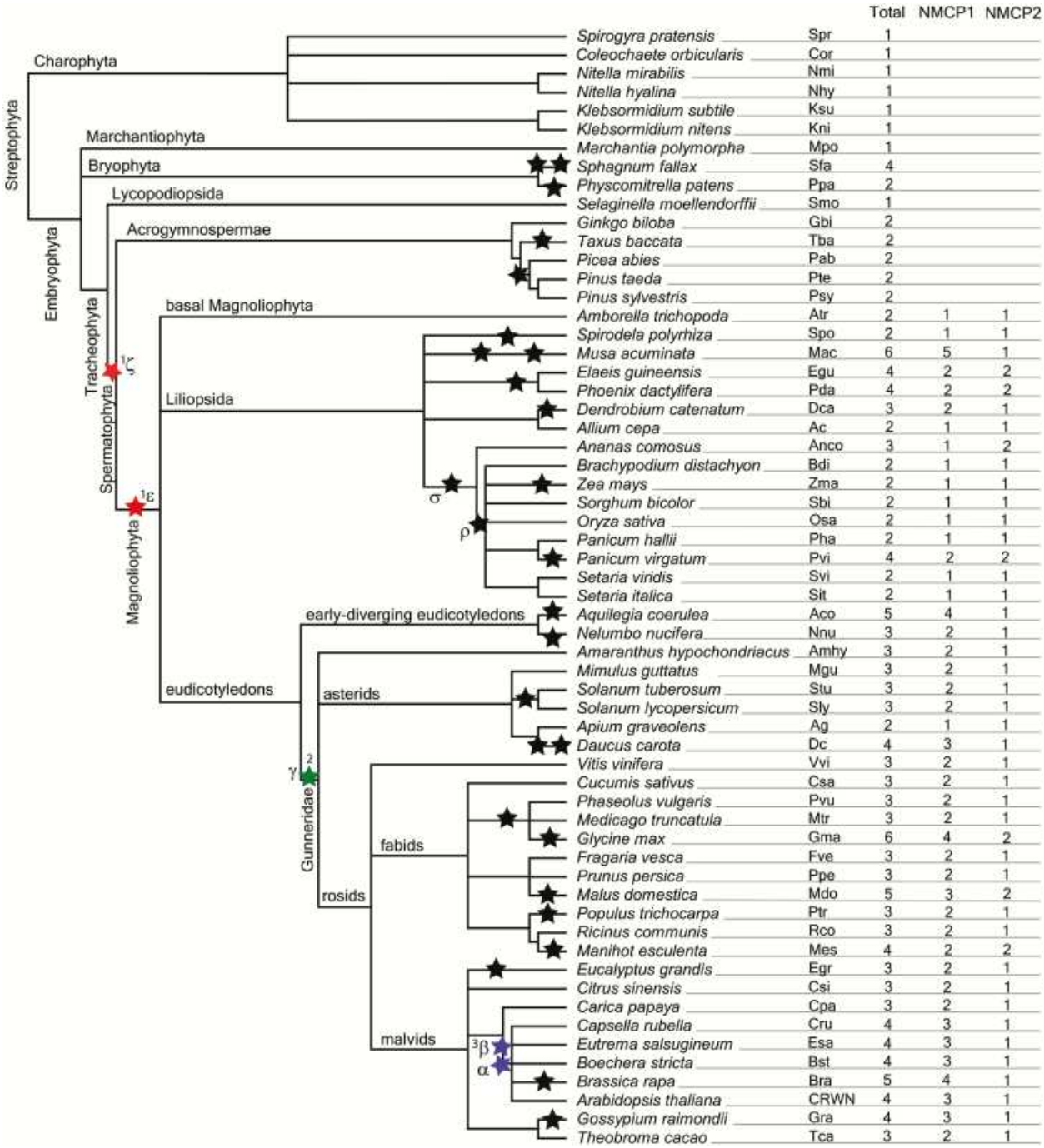

Figure 4: Phylogenetic relationship between species containing NMCP proteins, reported polyploidization events, and numbers of NMCPs. Asterisks, reported WGD; ζ and ε, possible points of appearance of NMCP1 and NMCP2; γ, point of appearance of NMCP3. This figure was borrowed from article (Ciska, Hikida, et al. (2019)).

Masuda, et al. (2013)).
Some tail domains of the basal NMCPs also contain coiled coils and none of the NMCPs contains the characteristic Ig fold of lamins (Ciska, Hikida, et al. (2019))
The length of each domain is less conserved than the overall structure. To illustrate, NMCP1 has a much longer tail domain than NMCP2 (Ciska, Hikida, et al. (2019)), and basal NMCPs vary

in length of all domains. The structures of NMCPs can be seen in Figure 5 and their lengths in Table 3. The length of the rod domain is conserved with the exception of the longer domain of Sporophyta, and the variable domain of basal NMCPs (Ciska, Hikida, et al. (2019)).

In NMCPs, conserved motifs are more abundant and different than the metazoan IF motifs. The

Table 3: Table of total protein length and lengths of the three protein domains in NMCP families from Magnoliophyta. (mean ± SD value) (Ciska, Hikida, et al. (2019))

	Total length	Head	CCD	Tail
Gymnosperm NMCP1-type	1203±72 aa	106±17 aa	673±9 aa	430±72 aa
Gymnosperm NMCP2-type	832±155 aa	51±23 aa	665±30 aa	60–463 aa
Monocot NMCP1-type	1171±75 aa	72±35 aa	674±20 aa	463±91 aa
Monocot NMCP2-type	1034±77 aa	68±18 aa	669±16 aa	297±70 aa
Dicot NMCP1-type	1169±49 aa	64±24 aa	668±16 aa	435±34 aa
Dicot NMCP2-type	1039±60 aa	92±30 aa	657±40 aa	285±60 aa

only similarity located at the C-terminus consists of a stretch of acidic amino acids that may be involved in association with the nuclear envelope (Ciska and Díaz de la Espina (2013) and Ciska, Hikida, et al. (2019))

Majority of the other conserved motifs is in the rod domain (see Figure 5 and Table 4), which are conserved in almost all NMCPs. The putative NLS sequence is conserved only among NMCP1s and few basal NMCPs, yet all NMCPs are located in nuclei and are distributed in the nuclear periphery (Ciska and Díaz de la Espina (2013) and Ciska, Hikida, et al. (2019)). The NLS sequences are KRRRK and RRHK, abundant in seed plants (not in mosses). The sequence RRHK follows the consensus sequence in the tail domain (R/Q/H)YNLR(R/H) which is crucial for proper localisation of NMCP1 in the nuclear periphery (Kimura, Fujino, et al. (2014) and Ciska, Masuda, et al. (2013)). NMCP2s and some basal NMCPs lack these NLS sequences, however, still do occur in the nuclear periphery.

Previously thought NLS sequence was motif I from Table 4 which is conserved in all NMCPs (Ciska, Hikida, et al. (2019) and Kimura, Fujino, et al. (2014)) Another disputable NLS sequence is motif 5 which again present in almost all known NMCPs and seems to be essential for localisation to nuclear periphery. (Ciska, Hikida, et al. (2019))

Apart from the NLS sequence, the minimal consensus phosphorylation motifs for cyclin-dependent kinases have been revealed. One conserved region is located in the head domain (not present in gymnosperm NMCP2 and basal NMCPs). Second site is located in the tail domain after motif 5 and is present in all NMCP1 and NMCP2 and many basal NMCPs (Ciska, Hikida, et al. (2019)). These phosphorylation sites are recognized by multiple protein kinases including cyclin dependent kinase (cdk1) and protein kinase PKC (Masuda, Xu, et al. (1997) and Ciska and Díaz de la Espina (2013)).

Another conserved motif of NMCPs is the one similar to lamins. It is sequence RYNLRR of NMCPs similar to EYNLRSRT of lamins Annette et al. (2012)), which supposedly serves as actin binding site (Ciska, Masuda, et al. (2013)).

Location of NMCPs in cells was studied in interphase cells as well as in mitotic cells. During interphase they are part of the nucleoskeleton in the nuclear periphery and NMCP1s also in the nucleoplasm (Mínguez et al. (1993) and Ciska, Masuda, et al. (2013)). Some of the NMCPs can be located exclusively to nucleoplasm (as it is for CRWN2) or to nuclear periphery (as CRWN1 and CRWN4 are) (Ciska and Díaz de la Espina (2013) and Pradillo et al. (2019)).

After nuclear envelope disintegrates in the beginning of prometaphase, lamina disintegrates as

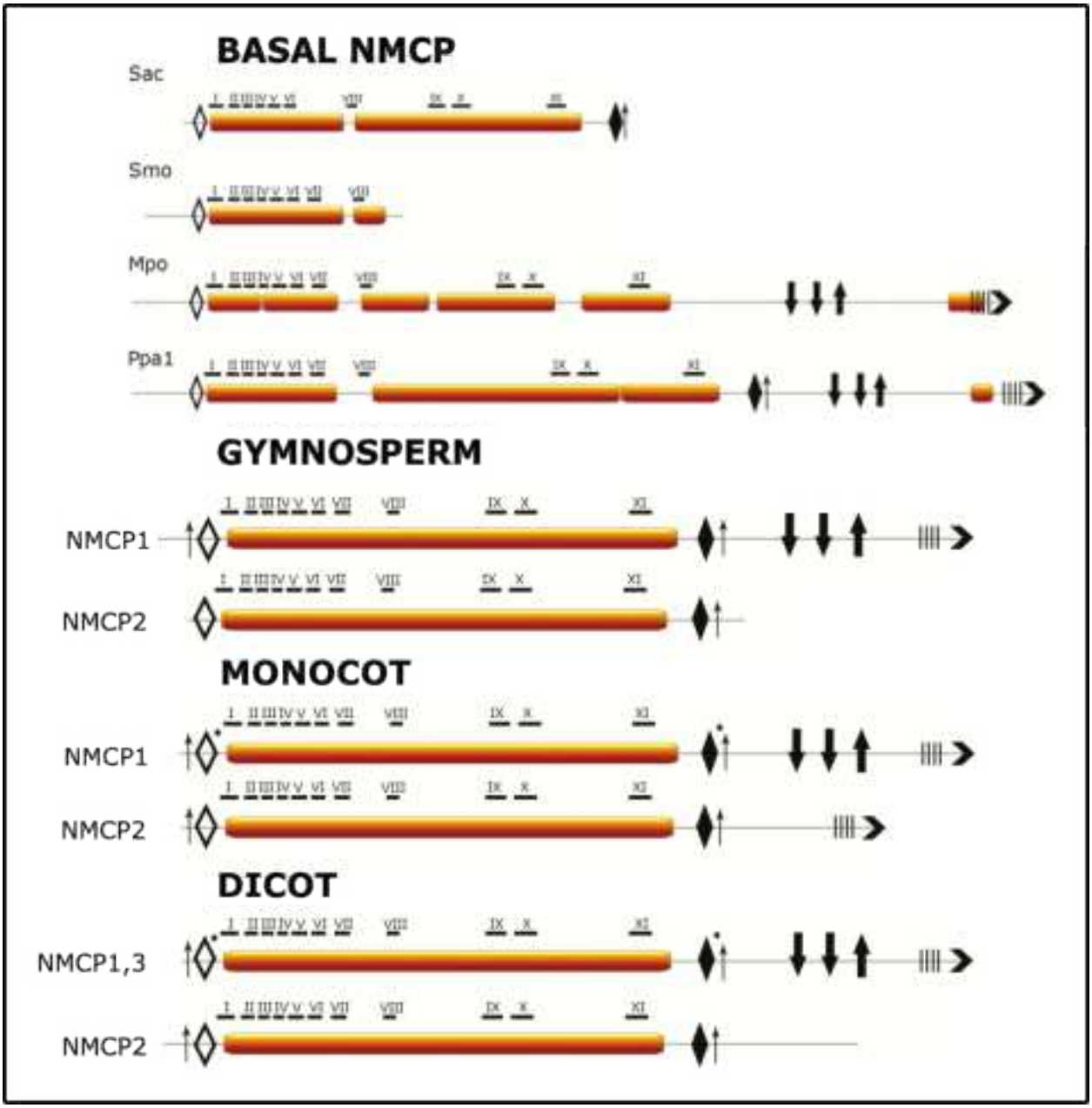

Figure 5: Comparison of NMCP domain layout and the position of conserved motifs in NMCP1 and NMCP2 of Strematophyta and few exemplary basal NMCPs. Dicot NMCP1,3 includes NMCP3. Modified from Ciska, 2019. Central rod domain is resembled by orange box. Highly conserved motifs I–XI (sequences in Table 4). Empty diamonds, motif 11.; filled diamonds, motif 5.; thin upward arrows, phosphorylation sites; thick downward arrows, NLS; thick upward arrows, motif 14.; four vertical bars, stretch of acidic amino acids; chevron arrows at C-terminus, motif 4. Asterisks indicate conserved regions that were present in most but not all NMCPs analysed. Sac, *Selaginella acanthonota*; Smo, *Selaginella moellendorffii*; Mpo, *Marchantia polymorpha*; Ppa, *Physcomitrella patens* (Ciska, Hikida, et al. (2019))

well. NMCP1 and NMCP2 then follow different pathways to later reassemble the nuclear envelope (Ciska and Díaz de la Espina (2013) and Kimura, Kuroda, et al. (2010)).

Table 4: Conserved motifs in NMCPs, modified from Ciska, Hikida, et al. (2019). The regions were ordered from the highest statistical significance (e-value) of the motif to the lowest. Standard amino acid symbols; ambiguous symbols: B for N or D, Z for Q or E; J for L or I; X for any amino acid. Bold letters indicate the most conserved residues. Location in CCD (coiled coil domain) denoted by number of the motifs in Figure 5. F in first column signifies function of respective motif (Ciska and Díaz de la Espina (2013)).

	Pattern	e-value	Location
1.	**E**LYD**YQ**Y**NMGLL**LI**EKKEWT**	1.1e-589	CCD (I)
2.	**ALG**V**EKQCVADLEKAL**KE**MR**	2.2e-428	CCD (III)
3.	E**LKQ**EK**EK**F**EKEWELLDEK**R	1.6e-361	CCD (X)
4.	E**D**EEP**G**EAS**I**GK**KLW**NFL**TT**	8.2e-216	Tail
F	conserved in NMCP1 clusters and in monocot NMCP2 (not dicot, where NMCP3 are). Preceded by a stretch of acidic amino acids present in lamis (may be involved in association with nuclear envelope).		
5.	**SWL**RK**CASKIF**	2.1e-184	Tail
6.	**ERE**E**LLRLQ**SE**LKZEI**DEL**R**	2.1e-262	CCD (IX)
7.	**LKREQAAHL**IALSE**AEKREE**	1.7e-274	CCD (II)
8.	**RKLQEVEARE**DALR**RERLS**F	4.1e-266	CCD (VI)
9.	**WEKKLQEGZERLLEGQRJLN**	1.2e-247	CCD (VII)
10.	**IKKDIEEL**XVQR**EKLKEQRE**	1.2e-257	CCD (XI)
11.	GGL**DEESL**ERK**DRAALJAYI**	1.4e-239	Head
12.	**AE**X**AE**V**K**VTAES**KLAZA**REL	1.8e-220S	CCD (IV)
13.	**LE**A**EAKLHAA**EAL**LAEASRK**	1.5e-194	CCD (V)
14.	**QTPG**EK**RYN**L**RR**STIVN**TVA**	2.5e-184	Tail
F	RYNLRR for proper localisation in nuclear periphery and probably serves as actin biding site		
15.	LD**KKEQELLLLZEKLASRER**	1.9e-158	CCD (VIII)

NMCP1 is then dispersed within the spindle during metaphase and therefore association with mitotic apparatus proteins (e.g. mitotic microtubules) may be possible. In early anaphase to telophase NMCP1 accumulates on segregating chromosomes probably via association with protein AHL1 located on chromosome surface.(Kimura, Kuroda, et al. (2010)). In telophase the NMCP1 then covers the chromatin surface and assists in the nuclear envelope reassembly (Pradillo et al. (2019)).
On the other hand, NMCP2 (and also NMCP3 of *A. thaliana*) follows a different path after dissociation in prometaphase. During metaphase and anaphase it is dispersed in mitotic cytoplasm in nuclear-envelope-derived vesicles and then relocates to chromosome surface during telophase (Ciska and Díaz de la Espina (2013) and Kimura, Kuroda, et al. (2010)).
Similarities with lamins go even further than this. NMCPs control nuclear size and shape and also chromatin organization and gene expression. But the processes are yet not fully understood (Ciska and Díaz de la Espina (2013), Graumann (2021), Ciska, Masuda, et al. (2013), and Tongtong et al. (2017)).
The plant lamina itself is also predicted to undergo structural changes during cell differentiation (Ciska, Masuda, et al. (2018)). The expression of NMCP1 is developmentally regulated (resembles lamin B1) and strongly weakened in differentiated cells (Ciska and Díaz de la Espina (2013)).
Their influence on nuclear size and shape does not affect the amount of DNA content nor chromosome structural domain patterns (Sakamoto (2020), H. Wang et al. (2013), and Dittmer, Stacey,

et al. (2007a)).
The heterochromatin organization and gene expression is based on interaction of NMCPs with NMCP-associated proteins as CRWN1 interacts with nucleoporin (NUP1/136) to tether heterochromatin in the nuclear periphery. CRWN1 also binds to C-terminal domain of PWO1 (plant-specific protein involved in gene repression) and carrot DcNMCP1 interacts with transcription factors, MYB3 and BIM1, and E3 ubiquitin ligase SINAT1 (Mikulski et al. (2019)).
First known downstream repressor of CRWN1 and CRWN3 is ABA insensitive 5 (AIB5) protein. CRWN3 plays an important role in regulating the ABI5 protein level via formation of a degradation body in the nucleus. In conclusion NMCPs may tether the DNA damage sites to the nuclear periphery and form a repair body, which may contribute to the effective repair of DNA damage (Sakamoto (2020)).
CRWN proteins are also involved in pathogen (virulent bacteria and salicylic acid) response. After introduction of the pathogen, CRWN1 is degraded and transcription of *CRWN1* gene is induced. The new CRWN1 via interaction with NTL9 (NAC transcription factor) enhances its binding ability to promotor of *pathogen-related 1* (*PR1*) gene, which is then expressed. This implies the ability of NMCPs to participate in pathogen response (Tongtong et al. (2017)).
Lastly, unlike lamins NMCPs and NMCP associated proteins are not involved in movement of nucleus although new linker, myosin XI-i, attached to nuclear membrane via WIT1 and WIT2 (which interact with SUNs) is required (along with mentioned WIT-proteins) for light-induced nuclear movement. Further research is needed to properly understand these processes (Tamura et al. (2013)).
In conclusion, the NMCPs are lamins analogues with a lot of similarities. Yet no evolutionary connections have been found with metazoan lamins. Since other lamin analogues have been found in different organisms (see Figure 3, Odell et al. (2023)), it could be that metazoan lamins and plant NMCPs are highly modified descendants of IF-gene of LECA, which can suggest the nuclear lamina is evolutionarily conserved in all eukaryotic organisms. As for the plant NIFs sequences, further research is needed to determine their origins as they can be the same proteins as the NMCPs are.

4 Alleged IF Homologues in Plants

True sequence homologues of IF in plants have not been found yet and probably will not be, as was showed in previous chapter. However, in few new articles and in protein databases there are references of plant IF-proteins. The aim of this chapter is to elaborate on these purported proteins and determine whether or not they should be considered as a potential plant IF homologues.

4.1 Presumed Plant IF in the Literature

To begin with, annotations of plant IFs occur in modern studies usually due to a confusion of terms (intermediate filament-like protein, coiled coil protein being confused with IF-proteins) or because of database annotation errors. In following chapters, some of the proteins noted in articles will be described. Names of the chapters correspond with names given to the proteins by authors of respective articles.

4.1.1 Sequence Homologues of Intermediate Filaments

Meagher and Fechheimer looked for *A. thaliana* cytoskeletal sequence homologues with well-characterized animal, fungal, and protist sequences (Meagher et al. (2003)). The proteins were identified using BLAST searches. Their method was developed to "directly approximate alignments that optimize local similarity." (Meagher et al. (2003)). Part of the identified proteins can be seen in table 5.
As for IFs, this included not only putative lamins, but also other cytoplasmic IF proteins. They were represented by individual genes and also as small gene families. The most likely to exist in plants seemed to be the neurofilaments, which had most of the found similarities within the rod domain and in long glutamine rich sequences of the neurofilaments (Meagher et al. (2003)).
However, further details of algorithm used are absent, hence it is possible the observed similarities simply reflect unusual protein sequence composition, such as the glutamine-rich fraction, than actual homology. Furthermore, the reported e-values do not indicate very strict sequence conservation. No experimental data are available for majority of these proteins, hence it is not possible to elaborate further.

4.1.2 PROPSEARCH-found Intermediate Filament Analogues

Gardiner et al. used PROPSEARCH algorithm to determine structural homologues of plant coiled-coil proteins (Gardiner et al. (2011)). PROPSEARCH is designed to detect functional or structural homologues by transforming sequences into vectors based on their properties (molecular weight, acidity, etc.) and for comparison, Euclidian distances between sequences are calculated (Gardiner et al. (2011) and Hobohm et al. (1995)). The authors also verified the true homology using the NCBI Genbank annotations (Gardiner et al. (2011)).
By this approach they achieved about 43 % accuracy of determining protein homologues. This algorithm revealed multiple putative intermediate filaments including filensin, lamin and keratin homologues, in total 23 proteins were detected.

Table 5: Presumable IF-proteins in *A. thaliana* from article Meagher et al. (2003) from which the table was borrowed.

Intermediate filament protein query[a]	Potential family size[b]	Species & accession # of query	# hits & E-value[c] range	a.a./ a.a. query [*]	Annotation in database or reference
Type I IF, Keratin cytoplasmic	0-2	Human Q99456	*2 (3e-4: 0.002)*	208-270/ 494	Kinesin-related, putative protein
Type II IF, Keratin cytoplasmic	0-12	Human P12035	*17 (2e-7: 0.001)*	300-330/ 629	hypothetical protein, myosin-like
Type III IF					
Peripherin	0-1	human XP_006827	*4 (e-4: 0.005)*	180-280 / 471	Myosin-like, putative protein
Vimentin	0-2	*Bovis taurus* AAA53661	*4 (0.001: 0.003)*	300-350 / 466	Kinesin-like
Type IV IF					
Neurofilament, light	0-1	Human XP005159	*1 (0.004)*	310 / 472	Putative protein, similarity to kinesin
Neurofilament, medium	0-6	Human XP_005158	*13 (2e-11: 0.001)*	250-400 / 991	Putative, unknown protein, extensin-like, calmodulin-like
Neurofilament, heavy	0-5	Human XP009907	*7 (5e-11: 0.001)*	320-440/ 879	Unknown, periaxin-like
Periaxin-correctly placed?	4	Human XP_047407	*4 (6e-14: 2e-4)*	1257	periaxin-like, unknown
Type V IF					
Lamin A/C	5	Human XP_044163	*15 (5e-7: 5e-4)*	265-410/ 664	Myosin-like, kinesin-like, unknown protein
Lamin B2	2	Human XP_031921	*2 (4e-5: 9e-4)*	145-220/ 417	unknown protein
Type VI IF, nestin	0-2	Human 1346682	*3 (e-4-.0001)*	300-320 / 1618	Putative myosin, unknown
Total similar to IFs	11-42				

All of them were looked up by EnsemblPlants by given loci (Dyer et al. (2024)). With their accession numbers (AN), these proteins were then looked up in NCBI database for updated names and functions (Biotechnology Information (NCBI) (1988)). All obtained data are concluded in table 6. Only four protein sequences have a probability of containing the putative IF homology since other proteins now belong to different protein groups than IF proteins.

Starting with protein **SCD2 (824047)**, SCD2 is located in plasma membrane and its structure predicted by AlphFold is not similar to structure of IF-proteins. According to UniProt, it participates in cytokinesis and polarized cell expansion and possibly in clathrin-mediated membrane trafficking (Consortium (2024)).

Three similar proteins were found using BLAST (Biotechnology Information (NCBI) (1988)): actin-binding protein Tropomyosin alpha-3 chain of *Mus musculus* (AN: P21107; 57 % similarities, e-value 6,3e-11), another uncharacterised protein of *A. thaliana* (AN: P0CB26) and MAP7 domain-containing protein 1 of *Mus musculus* (AN: A2AJI0). Furthermore, none of these proteins' predicted sequences are similar to IF-protein.

The **uncharacterised protein (AN: 843825)** does not have any annotations of function. Sequence prediction by AlphaFold is strongly diverging from structure of any IF protein known (Consortium (2024)). Furthermore, even BLAST was not able to find any similar protein sequences in protein databases.

On the other hand, **filament-like plant protein 3** (FLPP3) predicted structure (by AlphaFold) is highly similar to structures of IF proteins and even to NMCP proteins. FLPP3 recruits EXO70A1

to xylem cells' cortical microtubules during secondary cell wall deposition (Oda et al. (2015)). According to BLAST search, it is highly similar to multiple animal myosin sequences and other filament-like plant proteins of *A. thaliana* and *Solanum lycopersicum*.
Lastly, **filament-like plant protein 2** (FLPP2) is also involved in EXOCYST recruiting in xylem cells (Oda et al. (2015)). Its structure is highly similar to one of IF-protein sequences. BLAST discovered similarities with various α-helical proteins, including other FLPPs, kinesins, MAP proteins, various coiled-coil proteins and COP1-interactive protein 1 with more than 40 % similarity and low e-values (around 10^{-8}) (Madeira et al. (2024)).
These results show that probably none of the aforementioned proteins is an IF-protein homologue and further research of is needed to confirm their true affiliations.

4.1.3 Intermediate Filament Motif Protein 1

Utsunomiya et al. (2020) studied filament-like plant protein (FLPP) annotated by Gardiner et al. (2011). The protein is encoded by gene At3G05270 and authors named it as an Intermediate Filament Motif Protein 1 (IFMoP1).
According to UniProt (Consortium (2024)), this protein is supposed to have 3 coiled coil domains, two of them separated by linker and the third is separated by more than 100 amino acid residues (not very typical for IFs). It is able to form filament-like structures, and colocalise with microtubules. However the filaments can be formed even after microtubules disruption, signifying its independence (Utsunomiya et al. (2020)).
The article says that low (about 20 %) similarity of IFMoP1 with metazoan IFs is not low in comparison with similarities among IFs themselve (Utsunomiya et al. (2020)), which is disputable. The low similarities of metazoan IFs can be caused by high variability of end domains, but the rod domain is the most conserved part of the IFs. To look for IF consensus sequences in IFMoP1 MSA was run with IFMoP1 and human IFs: keratin type I (AN: J00124), neurofilament light polypeptide (AY156690) and lamin-B2 (BT007441). The consensus motifs were not conserved in IFMoP1, as can be seen in figure 6, therefore this protein should not be named as intermediate filament.

```
AtIFMoP     98  NEAAALKQQLDASTSKVSALEDRNSHLDSALKECVR------      134
Lamin B2    48  EELRELNDRLAHYIDRVRALELENDRLLLKISEKEE-VTTRE      88
Keratin    116  VTMQNLNDRLASYLDKVRALEEANADLEVKIRDWYQRQRPAE      158
NFL         91  AQLQDLNDRFASFIERVHELEQQNKVLEAELLVLRQK--HSE      131
                     *::::    .:*  **  *  *   :    .

AtIFMoP     453  GTKKQLETSQNRLKETERKLTELQTLLHLTKDAKEAAEDGLKAANGK-T  498
Lamin B2    389  AYRKLLEGEEERLKLSPSPSSRVTV-----SRATSS-SSGSLSATGRLG  432
Keratin     413  TYRRLLEGEDAHLSSSQFSSGS--------QS-----SRDVTSSS----  437
NFL         387  AYRKLLEGEETRLSFTSVGSITSGY-----SQ-----SSQVFGRSAYGG  422
                   :: ** .: :*. :              .      .    . .
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Figure 6: Alignment of IFMoP1 and human IFs: keratin type I (AN: J00124), neurofilament light polypeptide (AY156690) and lamin-B2 (BT007441); at the IF consensus sequences **LNDR** and **TYRKLLEGEE**.

Due to the ability to form filaments, EMBOSS-Needle was performed with AtCRWN1 (AN: AK230454) to see, whether the protein could be a derived cytoplasmatic subtype of NMCPs. The

alignment started after the first 3 consensus motifs within the coiled-coil domain and conservation of other consensus motifs was low. Hence it is not probable that this protein would be a descendant of an NMCPs. Therefore it is not easy to predict the classification of the protein further with current knowledge.

Table 6: Putative homologues IF-proteins in *A. thaliana* from article Gardiner et al. (2011). In first column the protein names from articles are written. AN, accession numbers (obtained from EnsemblPlant, Dyer et al. (2024)); HN – homologue name from article Gardiner et al. (2011); Updated name – name from protein entries in NCBI or UniProt database (Biotechnology Information (NCBI) (1988) and Consortium (2024)).

Protein	**AN**	**HN**	**Updated name**
At1g24560.1	839070	filensin	paramyosin
At1g52410.1(2)	841671	filensin	TSK-associating protein 1
At3g16000.1	820845	filensin	MAR binding filament-like protein 1
At3g18480.1	821377	filensin	CCAAT-displacement protein alternatively spliced product (golgin function analogue)
At3g48860.2	824047	filensin	SCD2, coiled-coil protein STOMATAL CYTOKINESIS DEFECTIVE 2
At1g74860	843825	Keratin type I	uncharacterized protein
At2g30880.1	817638	Keratin type I	Pleckstrin homology (PH) domain-containing protein
At5g59210.1(2)	836039	Keratin type I	myosin heavy chain-like protein
At2g37080	818284	Lamin L(I)	ROP interactive partner 3
At3g05270.1	819691	Lamin L(I)	filament-like plant protein 3 (DUF869)
At5g60210.1	836143	Lamin L(I)	ROP interactive partner 5
At3g61570.1	825330	Lamin L(II)	GRIP-related ARF-binding domain-containing protein 1 (golgin function analogue)
At4g17220.1	827434	Lamin L(II)	microtubule-associated proteins 70-5
At3g52115.1	824375	Lamin L(III)	protein gamma response 1
At1g24764.1	839083	Lamin A	microtubule-associated proteins 70-2
At1g68060	843134	Lamin A	microtubule-associated proteins 70-1
At1g21810.1	838784	Lamin B2	filament-like plant protein 2 (DUF869)
At5g13260.1	831167	Lamin C	myosin
At1g12150	837767	Lamin C and C2	weak chloroplast movement under blue light protein (DUF827)
At5g55860	835681	Lamin C and C2	weak chloroplast movement under blue light protein (DUF827)
At1g18190.1	838398	Lamin DmO	golgin Putative 2
At2g26570	817198	NFM	weak chloroplast movement under blue light-like protein (DUF827)
At1g68910	843224	type III IF	WPP domain-interacting protein 2

4.1.4 Plant Keratins

In 1989, Hargreaves, Dawson, et al. (1989) described filament bundles associated with cytoplasmic microtubules and showed its cross-reactions with anti-IF antibodies. These bundles were further studied by different research groups and were determined as plant keratin-like proteins. They were observed as 10 nm filamentous structure cross-reacting with keratin antibodies and showed ability to self-assemble in vitro and were highly insoluble. These bundles were then observed in various species and cell-types (Yang et al. (1992), Chen et al. (1998), and Su et al. (1990)).
In 1999, research group of Zhao isolated these proteins from protoplasts and separated them by SDS-PAGE and got proteins which reacted with antibodies for basic and acidic keratins. They obtained proteins homologue to β-glucosidase and to an unknown protein. Homological analysis of obtained DNAs revealed that it had moderate homology to a variety of α-helical proteins and to rod domain of nestin (Zao et al. (2000)). No further informations were available, therefore it is not possible to specify the proteins further.
Nevertheless, they obtained cDNA fragment for a different protein by RT-PCR ran with primers for keratin-conserved sequence LLEGEE (Zao et al. (1999)). It is available in NCBI under accession number AF078688A (Bostan et al. (2019) and Zao et al. (2000)).
Using Clustal Omega, an MSA (Multiple Sequence Alignment, Madeira et al. (2024)) was run with nucleotide sequence of human keratin I (AN: J00124), lamin 1 of *C. elegans* (AN:X74027) and NMCP1 of *A. cepa* (AN: AB673103) with cDNA obtained by Zao et al. (2000) (AN: AF078688). The putative plant keratin sequence aligns with C-terminals of other IF proteins, excluding the coiled coil section. The percent identity matrix showed the highest similarity with *A. cepa* NMCP1. Therefore another MSA was run with nucleotide sequences of *D. carota* NMCPs (see table 7). The alignment showed highest similarity with NMCP1 and NMCP4 sequences and none with NMCP2. The sequences were aligned at C-terminal domains, including conserved regions shared among dicot NMCP1 (therefore no apparent similarity showed with NMCP2 which is lacking these motifs).

Table 7: Percent identity matrix created with Clustal2.1. MSA was performed with sequences from table below. newIF, AN: AF078688; NMCP1, *D. carota* NMCP1, AN: D64087; NMCP2, *D. carota* NMCP2, AN: AB514509; NMCP3, *D. carota* NMCP3, AN: AB812877; NMCP4, *D. carota* NMCP4, AN: KX828842. Whole alignment length was 4427 residues. In brackets, number of residues in protein pair are listed.

	newIF	**NMCP1**	**NMCP2**	**NMCP3**	**NMCP4**
newIF	100,00%(368)	51,01%(296)	none (0)	43,00%(100)	46,11%(371)
NMCP1	51,01%(296)	100,00%(4033)	45,71%(2,874)	54,65%(3453)	54,05%(3710)
NMCP2	none (0)	45,71%(2,874)	100,00%(2994)	45,82%(2840)	46,83%(2883)
NMCP3	43,00%(100)	54,65%(3453)	45,82%(2840)	100,00%(3718)	72,93%(3436)
NMCP4	46,11%(371)	54,05%(3710)	46,83%(2883)	72,93%(3436)	100,00%(4088)

The obtained cDNA sequence is probably a C-terminal domain of a putative IF (the LLEGEE is localised at the end of coiled coil) and it corresponds highly with the sequence of dicot NMCP1 end domain. Hence, it is possible they isolated new homologue of NMCP of *D. carota* instead of putative keratin. No further research featured this particular protein, therefore it cannot be reliably determined.

Another putative plant keratins were mentioned in article of Cai et al. (2024), stating that "Anther cuticle is composed of keratin and wax, ..." with references which do not include any notes about keratins at all (Cai et al. (2024)). Therefore it is highly probable that this annotation is incorrect and no keratins are in the cuticle of anther.

4.1.5 Intermediate Filament Antigen Binding Proteins

Research featuring one of the Intermediate Filament Antigen Binding Protein (IFABP) was focused on anther-specific gene *LLP13*. This gene encodes possible cytoskeleton-binding glycoprotein which, according to authors, has the potential to be an IF-binding glycoprotein (B. Wang et al. (2014)). The putative protein LLP13 shares significant identity with DUF593 proteins of Oryza and Arabidopsis and also with two IF antigen-binding proteins, rice OsIFABP (AN: NP_001044407.1) and Arabidopsis AtIFABP (AN: NP_564999.2) (B. Wang et al. (2014)).
The protein LLP13 is predicted to bind potential IF in pollen tubes. However, no experiments were performed to show any of this potential binding and this statement was based on bioinformatic analyses and cross-linking with actin filaments only, which does not seem to be completely relevant. Furthermore, it seems that the authors were not fully aware of current IF research in plants, because in their elucidation of plant IFs, they cited articles mostly about putative NIFs and did not mention NMCPs at all (B. Wang et al. (2014)).
The gene ***LLP13*** (AN: EF026011.1) name in GenBank is "*Lilium longiflorum* clone LLP-13 IFA binding protein mRNA" (IFA supposedly for IF antigen) and is annotated by one other article (Hsu et al. (2007)), where the authors state without further references that the protein is IF-antigen binding protein and that the IF may exist in pollen. However no proof was found yet to confirm their statement.
The LLP13 relatives were looked up in GenBank. The **OsIFABP** was removed from GenBank as a result of standard genome annotation processing and is now available under BAF06321.1 (in GenBank) and is also annotated in UniProt database as IFA-binding protein-like. However, only accessible information about the structure of this protein is the presence of coiled-coil domain. Therefore BLAST was run with available sequence and the best matches were myosin-binding proteins from various species including *A. thaliana*. The similarities were close to 50 % with very low e-values (10^{-50}). Therefore it seems, that this protein is most probably a myosin binding protein.
As for **AtIFABP**, it was renamed myosin-binding protein 2 according to its annotation in GenBank and UniProt. Currently, it is a membrane-anchored myosin receptor. This highly corresponds with findings of Wang et al. that the LLP13 is "... apparently decorating actin cytoskeleton ..." (B. Wang et al. (2014)) to which myosins do bind. Thus it is possible, that this protein is not an IFABP but a myosin binding protein.
In conclusion, it seems that as the putative OsIFABP and AtIFABP, the LLP13 is most probably another myosin binding protein. Further research is needed to confirm these results.

4.1.6 Calcium-dependent Protein Kinase Adapter Protein 1

McCAP1 (*M. crystallinum* calcium-dependent protein kinase (CDPK) adapter protein 1) is homologous to a family of proteins in Arabidopsis with unknown function. McCAP1 is predicted

to contain a coiled-coil domain (at C-terminus) and 2 other α-helical domains. McCAP1 and its homologically related proteins are more likely to have a trimeric than a dimeric coiled-coil domain (Patharkar et al. (2006)), which is atypical for IFs. McCAP1 is localised in the nucleus, including the nuclear envelope (in high concentrations also near nucleolus), and in cytoplasmic strands. Expression of McCAP1 is not influenced in stressed plants (Patharkar et al. (2006)).
Using Protein Homology/analogY Recognition Engine (PHYRE) threading algorithm similarity with helix IIb was found in the McCAP1 and in its putative homologues in *A. thaliana* by Patharkar et al. (2006). However, no mention of heptad repeats present in McCAP1 was reported and by aligning protein sequence of McCAP1 (AN: AAK69758.1) with human keratin (AN: J00124) no similarities were found. The conserved sequence TYRKLLEGEE is not aligned with the sequence of McCAP1 when MSA was run with whole protein sequences and percent identity is only 21,6 % (325 aligned residues). Similar alignment request was run with EMBOSS Needle with higher similarity in the coiled-coil region, however overall similarity was only 22,5 % (302 aligned residues) When MSA was run with whole McCAP1 sequence and sequence of only helix IIb of keratin the percent identity rose to 23,62 % (128 aligned residues). When EMBOSS Needle was assigned the same problem, multiple alignments were possible depending on penalty values for gaps in alignment. The similarity grows smaller with higher penalties for gaps. However, the conserved regions of IF-proteins are not conserved in any of the alignments which rules out the possibility of being IF homologue. There still is a possibility of coiled coil domain present in McCAP1, as it was not ruled out by the authors and neither tested in this study.
Six possible plant homologues of McCAP1 were found by BLAST and are written in table 8. The similarities are not negligible and because none of these proteins are IF (or even NMCP) related, McCAP1 is most probably not an IF-protein.

Table 8: BLAST hits ordered by their e-value (Madeira et al. (2024)). In first table proteins and their scores from BLAST are written, in second table their functions are mentioned briefly. Functions and AN were taken from UniProt database (Consortium (2024)).

Protein	AN	Similarity (%)	E-value
OBERON 1 (*A. thaliana*)	DQ059085	41,8	$4,1 \cdot 10^{-13}$
OBERON 2 (*A. thaliana*)	AK317346	43,0	$2,3 \cdot 10^{-12}$
OBERON-like (*N. benthamiana*)	AY271742	40,2	$7,2 \cdot 10^{-8}$
Vernalization insensitive 3 (*A. thaliana*)	AY446960	43,9	$7,7 \cdot 10^{-8}$
VIN3-like (*A. thaliana*)	EF064791	44,4	$1,5 \cdot 10^{-5}$
TITANIA (*O. sativa*)	AK068684	44,4	$2,1 \cdot 10^{-5}$

Protein AN	Function
DQ059085	Probable transcription factor in WUSCHEL-CLAVATA pathway.
AK317346	Probable transcription factor WUSCHEL-CLAVATA pathway.
AY271742	Similar function to OBERON, not fully described.
AY446960	Mediator of initial transcriptional repression of the homeotic gene FLC.
EF064791	Regulator of expression of related floral repressors (FLC and FLM) of vernalization and photoperiod pathways
AK068684	Probable transcription factor regulating metal transporter genes

4.1.7 *Oryza Sativa* Intermediate Filament(-like) Protein

Following articles of Soda et al. (Soda, Sharan, et al. (2016) and Soda, Gupta, et al. (2018)) used for their research a putative IF protein, which they obtained from Rice Genome Annotation Project (RGAP, Hamilton et al. (2024)). They did not studied the possibility of this protein to be an IF protein. In the first article, they named the protein *Oryza sativa* intermediate filament-like protein (OsIFL), but in the second article they removed the alias "like" (OsIF). In next three paragraphs, the incorrect statements are corrected and protein analysis follows afterwards.
In their first article, they claim that presence of 5 α-helices is a characteristic feature of IF-proteins, however it is not, and what they probably meant is that presence of an α-helical domain is a characteristic feature of IF proteins (Soda, Sharan, et al. (2016)).
Their further study on a secondary structure predicted a coiled coil motif at N-terminus which atypical for IF proteins, which contain coiled coil motif only in their central rod domain. Therefore their statement that due to this characteristic the protein is a "true plant IF protein" is also incorrect (Soda, Sharan, et al. (2016)).
Furthermore, their annotations of "recent discoveries" from more than 20 years ago are a bit odd and some of their more current references include putative IFs discussed in this chapter (meaning that no reliable article was cited). They also cite an article Rose et al. (2003) where an IF-motif protein (i. e. coiled coil protein) was discovered, but they never stated that this protein (nuclear matrix protein) is an intermediate filament. Furthermore, in the whole article of Soda, there is no mention of (by then already known) NMCP proteins (Soda, Sharan, et al. (2016)).
The OsIFL was renamed OsIF in their second article and they refer to it as to an intermediate filament. As an evidence, they found 2 similar proteins to OsIF according to database search: *A. thaliana* intermediate filament (AtIF, AN: AAD09217) and *Zea mays* IF (ZmIF, AN: NP_001149106). When searched in UniProt database, the names are now different: AtIF was renamed nuclear envelope-associated protein 1 and ZmIF is now plant IF-like protein with mainly α-helical structure (therefore not likely not an IF protein which has α-helical structure only in central rod domain).
They state the cytoplasmic filaments of OsIF reorient, however in their attached figure, there is no filamentous structure visible even after salt treatment. It is possible, that the putative filaments are polymerized under membrane and in this section none are be visible. No attention was brought to this aspect by the authors and therefore no data are available to clarify it (Soda, Sharan, et al. (2016) and Soda, Gupta, et al. (2018)).
Nevertheless, OsIF is reported to bind to microtubules (Soda, Sharan, et al. (2016)), which could explain protein distribution after salt-induced stress. After inducing stress the OsIF move to cell margins (Soda, Gupta, et al. (2018)) where cortical microtubules are localised.
Similar proteins of OsIF were searched by BLAST and high similarities (around 50 %, close to 0 e-values) were found with nuclear envelope-associated proteins of *A. thaliana* which are coiled coil proteins with α-helical structure localised at nuclear envelope and nucleoplasm. It also shares high similarities with myosins (from human and mice) and other coiled-coil/α-helical proteins.
Based on experimental data of Soda et al. it can be suggested that the OsIF protein may be a microtubule associated protein (which can have coiled coil domain) and by binding to microtubules it makes them more sturdy and salt-resistant, which corresponds to data from both articles.
In rice genome database (Hamilton et al. (2024)) another putative rice intermediate filament is annotated at locus LOC_Os05g04530.1. It showes high similarities to the same proteins as the OsIF does. No further data are available and therefore the true nature of this protein remains unclear,

but it is unlikely that it will be an IF protein.

4.1.8 Neurofilament Light Protein

Lastly, an article of Shymanovich et al. (2022) focused on plant phototropism and gravitropism features a plant neurofilament. The authors claim to observe an effect of NFL (neurofilament light protein), on tropism of *A. thaliana*. In the article only the effects of NFL in plant tropisms were discussed. They cite work of Soda and Utsunomiya of to state that the IFs do exist in plants (Soda, Gupta, et al. (2018) and Utsunomiya et al. (2020)).
According to UniProt, NFL (gene ID AT5G25070) is localised in plastids. BLAST was performed to look for homologues and the most similar ones (more than 40 % similarity, low e-values) are proteins from WEB family, CRWN proteins and COP1-interactive protein 1. The protein structure predicted by AlphaFold is different from the structure of any intermediate protein or the *A. thaliana* proteins found by BLAST. Therefore the NFL protein is most probably not an intermediate filament. To confirm, further research is needed.

4.2 Plant IF Annotated in Databases

To find putative plant intermediate filaments in databases the names of all IF-proteins mentioned in chapter 2.1 was searched in three databases including EnsemblPlant (Dyer et al. (2024)), UniProt (Consortium (2024)) and NCBI (Biotechnology Information (NCBI) (1988)). In UniProt, the searches were restricted to *Streptophyta* clade and in NCBI to plant species.
The search (see table 9) gave mostly uncharacterised, unreviewed or predicted proteins with none annotations and only with predicted structure (not in all cases). Due to the huge number of obtained results it was not possible to study each protein separately. Structures of some of these proteins which were checked had α-helical structure, however none of them is similar to any of the IFs'.
Following proteins, noted in the thesis references, are studied in this thesis. Two of the proteins were removed (superseded by a new assembly of the genom), these are POF22112.1 and POE82372.1. AG1369807.1 could not be found by NCBI nor by UniProt. The rest of proteins are still in the database and described in the following paragraphs.
XP_020571099.1 is annotated vimentin isoform X3 of *Phalaenopsis equestris*. This record is predicted by automated computational analysis and annotated using gene prediction method: Gnomon, supported by EST evidence. No structure is available in UniProt. EMBOSS Needle was run with human vimentin (AN: X56134). The identity of the sequences is around 15 %, the similarity around 30 %. Neither of IFs consensus sequences is conserved, therefore this protein is not an IF-protein and should not be named as vimentin.
KAG7030997.1 is neurofilament heavy polypeptide of *Cucurbita argyrosperma* subsp. *Argyrosperma*. Again, no sequence is available in UniProt. EMBOSS Needle was run with human neurofilament heavy polypeptide (AN: AF203032). The putative NFH polypeptide was aligned with the beginning of C-terminal domain of human NFH polypeptide. This could mean that the putative protein is similar to end domain, which in IFs is not α-helical, and therefore it cannot be an IF-protein.
Last protein, KAB2635032.1, is supposed to be keratin of *Pyrus ussuriensis x Pyrus communis*.

This protein is available in UniProt database, the sequence was derived by EMBL/GenBank/DDBJ whole genome shotgun entry which is preliminary data (Consortium (2024)). The protein structure is not similar to any IF structures. EMBOSS Needle was run with human keratin (BT007186). The putative keratin similarity with human keratin is very low and aligment in the human keratin N-terminal domain part with overlap to helix 1a. This suggests presence of very short α-helix at the putative keratin C-terminus. This is however still not enough to classify it as an IF, therefore this protein also should not be annotated as keratin.
This suggests that by automatic annotation of organism genomes, the protein names/functions are not determined properly causing a confusion in new protein research. No further study of similar proteins will be done in this work.

Table 9: Obtained number of proteins (hits) from databases (UniProt, NCBI and EnsemblPlant). The first column corresponds to searched term, other columns include number of hits for each searches in respective databases. Numbers in brackets mean number of reviewed entries. These were usually non-IF proteins with the searched word written somewhere in the protein description.

	UniProt	**NCBI**	**EnsemblPlant**
keratin	1322	1056	73
desmin	0	1	0
vimentin	40	14	7
syncoilin	1	19	2
glial fibrillary acidic protein	62	1	7
peripherin	6652 (19)	209	48
neurofilament	2430	1064	589
nestin	12	11	0
internexin	2	2	1
synemin	0	4	0
lamin	1610 (7)	355	43
intermediate filament	196	85	12

5 Conclusion

Nuclear intermediate filaments are supposed to be ancestral nucleoskeletal part of eukaryotic cells. It has been proven, that all metazoan cells express nuclear intermediate filaments. e.g. lamins. The question is, whether there is an equivalent of lamins in yeasts and protists, which would suggest there was a common eukaryotic ancestor who did express IFs. That would mean the IFs are necessary for nucleus functioning and other IFs were derived later from lamins by evolution to fulfil various roles in cells. It would also suggest that animal lamins and plant NMCPs belong to one protein family that diverged during evolution.

What can be suggested is a sequence homology study for lamins exclusively to prove this idea. There could be other conserved regions than the ones conserved in animal IFs, as there are non-canonic IFs (type VI) which have the consensus sequences modified. To support this idea further, the basal lamins also have modification in otherwise conserved sequences and as mentioned by Batsios et al. (2012), the lamins in lower eukaryotes do not share sequence homologies even among themselves. The sequences vary among the Dictyostelia species, and yet they are considered as lamin homologues (Odell et al. (2023)).

Further question is, whether these proteins should be considered lamin homologues (due to doubtful homology) or lamin orthologues, which would signify same evolutional origin. Then the NMCPs could potentially fulfil other lamin-roles in plant cell, e.g. be a centriole substitution of plant cells, still remains to be determined. Lot of further research is needed to prove any of these theories.

For now, the NMCPs should be considered as lamin analogues thanks to their similar domain organization, function and localization. As no full sequence was obtained for any of NIF members and they highly resemble the NMCPs, it is possible these two protein groups may actually be the same one. To prove this theory, sequences of NIFs need to be acquired, to compare their sequences, and more colocalisation studies need to be done.

Other proteins annotated as plant intermediate filaments usually do not even fulfil the basic IF requirements as having central coiled-coil domain and non α-helical end domains. To be classified as an intermediate filament, there is also a need of IF consensus sequences, which no known plant protein contains yet. It seems that many scientist tend to use IF as a synonym to coiled-coil proteins. However this substitution is in many ways not right and should not be used.

Similar problem goes with automatic annotations in databases of unknown predicted proteins, which are apparently named based on the most similar sequence to them (even if it is low similarity). These proteins should not be given names of already existing proteins if they do not have strong sequence homology.

Simple solution is to give these plant putative intermediate filaments a new name by which all of them can be addressed to further elaborate on their functions and origin without the prejudice of incorrectly given name. One of these name can be something like filament-like plant proteins (FLPs, which is already used) or coiled-coil domain proteins (CCDs).

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