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Review

Scaffolds, levers, rods and springs: diverse cellular functions of long coiled-coil proteins

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Abstract. Long alpha-helical coiled-coil proteins are involved in a variety of organizational and regulatory processes in eukaryotic cells. They provide cables and networks in the cyto- and nucleoskeleton, molecular scaffolds that organize membrane systems, motors, levers, rotating arms and possibly springs. A growing number of human diseases are found to be caused by mutations in long coiled-coil proteins. This review summarizes our current understanding of the multifaceted group of long coiled-coil proteins in the cytoskeleton, nucleus, Golgi and cell division apparatus. The biophysical features of coiled-coil domains provide first clues toward their contribution to the diverse protein functions and promise potential future applications in the area of nanotechnology. Combining the power of fully sequenced genomes and structure prediction algorithms, it is now possible to comprehensively summarize and compare the complete inventory of coiled-coil proteins of different organisms.

Key words. Adapter; ARABI-COIL; coiled-coil protein; genome analysis, golgin; lamin; MultiCoil; scaffold.

Introduction

Coiled-coil domains are protein-protein interaction motifs which consist of two or more alpha helices that twist around one another to form a supercoil [1]. It was the three-strand protein alpha-keratin in which the coiled-coil structural motif was first discovered [2]. Interestingly, one of the early X-ray diagrams depicting the characteristic alpha pattern of the coiled-coil was taken of a lock of Mozart's hair [3]. Sequences with the capacity to form left-handed alpha-helical coiled-coils are characterized by a heptad repeat pattern in which residues in the first and fourth position are hydrophobic, and residues in the fifth and seventh position are predominantly charged or polar. (For a recent excellent review on structural features of the heptad repeat, see [4]). The stability of the coiled-coil is derived from a characteristic packing of the hydrophobic side chains into a hydrophobic core ('knobs into holes' [2, 5]), an extreme case of the more general 'ridges into grooves' model of helix packing in globular proteins [6]. By computational methods, the pattern of hydrophobic and polar residues can be used to predict coiled-coil domains in amino acid sequences [7, 8].

Coiled-coil proteins can be grouped into two general classes. Short coiled-coil domains of six or seven heptad repeats, also called leucine zippers, are frequently found as homo- and heterodimerization domains in transcription factors (e.g. [9]). Similar domains have been found in macromolecular ion channel signaling complexes [10, 11] or other signal transduction components [12–15] where they mediate specific protein-protein interactions. In contrast, long coiled-coil domains of several hundred amino acids are found in a variety of proteins. In these, the coiled-coil domains usually form 'rod'-like tertiary structures [1] and may be combined with characteristic functional domains (see fig. 1 for examples). These long coiled-

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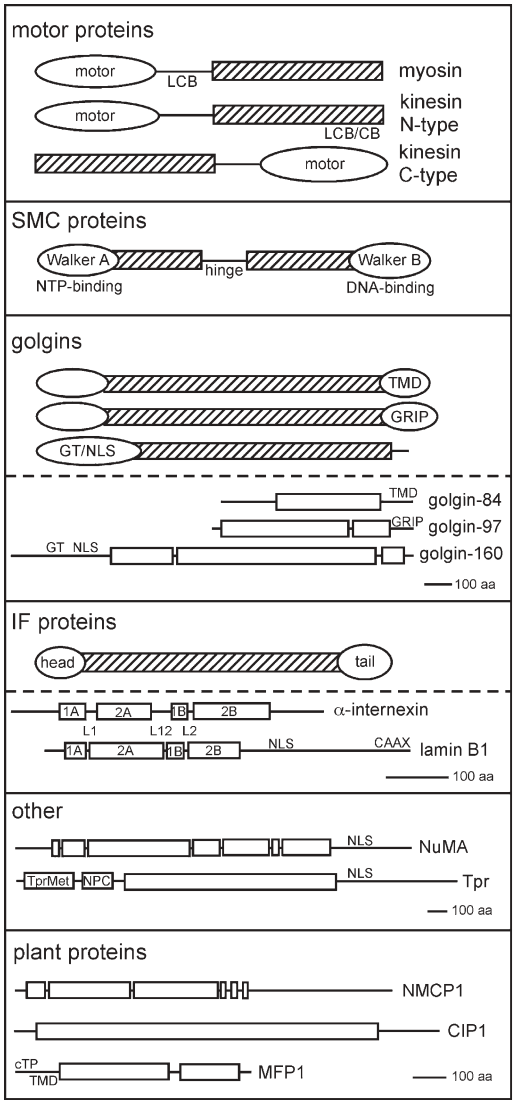

Figure 1. Schematic representation of selected coiled-coil proteins. Several classes of coiled-coil proteins are characterized by non-coiled-coil functional domains. For example, myosins and kinesins can be identified through their respective motor domains, and structural maintenance of chromosomes (SMC) proteins have highly conserved head and tail domains (Walker A and Walker B). Golgins can be recognized through the presence of Golgi-targeting domains, and intermediate filament (IF) proteins are characterized by a conserved coiled-coil domain structure. Examples for the three classes of golgins as well as cytoplasmic (internexin) and nuclear (lamin) intermediate filament proteins are shown below their schematic representations. Other coiled-coil proteins, such as NuMA or Tpr, cannot as easily be classified beyond the fact that they contain long stretches of coiled-coil. The known plant coiled-coil proteins NMCP1, CIP1 and MFP1 do not fit any of the characterized coiled-coil protein classes from animal and yeast systems. Hatched boxes in the schematic representations and open boxes in the individual protein representations indicate coiled-coil rod domains. Ellipses indicate globular domains. CB, cargo binding domain; cTP, chloroplast-targeting peptide; GRIP, golgin-97/RanBP2α/Imh1p/p230 domain; GT, Golgi-targeting domain; LCB, light-chain binding domain; NLS, nuclear localization signal; NPC, nuclear pore complex-targeting domain; TMD, transmembrane domain; TprMet, domain of Tpr fused to Met tyrosine kinase in the TprMet oncoprotein.

coil proteins are involved in diverse cellular functions. The intermediate-filament proteins of the cytoskeleton represent a well-characterized group of coiled-coil proteins. A subgroup of these are the nuclear lamins, which are involved in attaching chromatin to the nuclear envelope and have recently been implicated in inherited human diseases [16, 17]. Besides structural components of the cytoskeleton, the motor proteins such as myosin and kinesin also contain coiled-coil motifs. Other coiled-coil proteins, including the SMC (structural maintenance of chromosomes) proteins, are involved in the organization of chromatin. Membrane-bound coiled-coil proteins such as the golgins form a scaffold for membrane structures within the cell.

In the past few years, the number of investigated long coiled-coil proteins has rapidly grown. Besides the 'classic' cytoskeletal proteins, others function in contexts as different as the nuclear pore, the Golgi, kinetochores and centrosomes. Some have been identified as oncogenes or tumor-suppressor genes, based on their oligomerization function. At the other end of the spectrum, our knowledge about the biophysical features of coiled-coil proteins has grown as well. Molecules ranging from the myosin heavy chain to rationally designed synthetic coiled-coils are being tested for their applicability as units of nanomachines. In their biological context, long coiled-coil proteins emerge as a versatile toolbox of the cell, containing scaffolds, levers, rotating arms and possibly springs.

How to identify all large coiled-coil proteins of an organism? In the post-genomics era, structure-prediction algorithms can be applied to whole proteomes. These approaches show that roughly 10% of all proteins contain coiled-coil domains. Databases presenting all coiled-coil proteins of an organism linked to functional information and (eventually) to other organisms are beginning to provide a framework toward understanding whether there is a unifying principle to the diverse functions of all long coiled-coil proteins. This review compares and contrasts the recent developments toward understanding the biological function, biophysical characteristics and genomic representation of these still underappreciated gadgets of the cell.

Diverse biological functions of long coiled-coil domains

Long coiled-coil proteins as components of the cytoskeleton

One of the three main classes of cytoskeletal proteins, the intermediate-filament proteins, represents a well-characterized group of coiled-coil proteins with an elongated coiled-coil rod flanked by non-alpha-helical head and tail domains (fig. 1 [18]). Besides microtubules and actin filaments, the intermediate filaments (IFs) constitute the third filament network of metazoan cells. Their precise molecular assembly is still less well understood than that

of actin filaments and microtubules. The assembly of IF arrays occurs by oligomerization of their rod domains. In vitro, tetramers associate laterally to form unit-length filaments (ULFs), which anneal longitudinally to form loosely packed filaments that finally undergo internal reorganization to form mature filaments (reviewed in [18]). Besides the structural proteins of the cytoskeleton, the motor proteins [19] interacting with actin and tubulin filaments also contain coiled-coil motifs. Myosin, the actin motor protein, has an extended coiled-coil tail domain necessary for the assembly of the thick muscle filaments [3]. Kinesin and dynein, two microtubule motor proteins, also contain coiled-coil domains (fig. 1 [20, 21]).

Nuclear coiled-coil proteins

The lamins, a subgroup of the intermediate-filament proteins in animals, are involved in a number of functions, including chromatin organization, nuclear envelope assembly, DNA synthesis, transcription and apoptosis. Mutations in A-type lamins or associated proteins have been linked to at least eight different inherited human diseases affecting skeletal and cardiac muscle and/or the loss and redistribution of white fat [17] and premature aging [22–24]. The function of nuclear lamins was recently expertly reviewed (e.g. [17, 25–28]). This review therefore focuses on long coiled-coil proteins other than nuclear lamins.

Besides the lamins, other proteins with long coiled-coil domains are involved in the organization of the nuclear envelope and nuclear architecture. NuMA (nuclear mitotic apparatus protein), also known as centrophilin, is a nuclear, intermediate filament-like protein with a large central coiled-coil domain (fig. 1 [29–31]). It is present in the nuclear matrix during interphase and at the spindle poles during mitosis [29, 31]. NuMA, as well as the centrosomal scaffold protein pericentrin, are recruited to the spindle poles via dynein-driven transport [32]. NuMA induces the formation of a three-dimensional lattice inside the interphase nucleus in HeLa cells when overexpressed [33] and self-assembles into multiarm oligomers in vitro [34], indicating that it may play a role in the architecture of the nuclear matrix. NuMA is anchored to a lamin scaffold inside the nucleus in an RNA-dependent manner [35]. During mitosis, it is involved in centrosome function and is essential for the organization and stabilization of the spindle poles [36]. NuMA is a component of the microtubule aster promoting activity (APA), and promotes spindle growth, a process regulated by Ran and importin beta [37]. Interestingly, the fusion of the NuMA coiled-coil domain with the retinoic acid receptor in the NuMA-RAR(alpha) oncoprotein confers dimerization ability and binding to the NuMA network inside the nucleus, resulting in transforming activity [38].

The SMC proteins are a class of chromosome scaffold proteins involved in sister chromatid cohesion (cohesin), chromosome condensation (condensin) and DNA repair. They are composed of five characteristic domains: two head domains, a central hinge domain and two long coiled-coil stretches between the hinge and the head domains which have been shown to form antiparallel dimers (fig. 1 [39, 40]). SMC proteins are encoded in all eukaryotic genomes and have also been identified in Gram-negative bacteria and archaea [41].

Tpr (for translocated promoter region [42]) and its closest structural relatives in *Drosophila* (Bx34 [43]), and yeast (Mlp1 and Mlp2 [44]), are large coiled-coil proteins located on the nucleoplasmic side of the nuclear pore complex (NPC) [43–46]. A nuclear localization signal has been identified in the C-terminal non-coiled-coil domain of human Tpr that is responsible for the nuclear targeting of the protein (fig. 1). The coiled-coil domain has been shown to mediate attachment to the NPC once the protein is inside the nucleus [46]. *Drosophila* Tpr/Bx34 cofractionates exclusively with the insoluble nuclear matrix [43]. The coiled-coil domain of *Xenopus* Tpr is structurally organized into a parallel helix dimer of rodlike shape [47]. In yeast, Tpr/Mlp proteins are involved in the regulation of telomere length [48].

The proposed role of Tpr in forming filaments extending from the nuclear pore into the nuclear interior [49–51] has recently become controversial. Antibody and GFP localizations show that Tpr is present in the nuclear basket and in discrete foci inside the nucleus (similar to other nucleoporins), but not in intranuclear filaments extending from the pore complex [52, 53]. In mammalian cells, attachment of Tpr to the NPC occurs via interaction with the nucleoporin Nup153 in late telophase and is not necessary for NPC assembly [54]. Increasing evidence strengthens the proposed role of Tpr in messenger RNA export [55, 56]. Analogous to the NuMA-RAR(alpha) oncoprotein, the fusion of the N-terminal part of the Tpr coiled-coil domain with the receptor tyrosine kinase Met in the Tpr-Met oncoprotein confers dimerization ability that results in a cytosolic, constitutively active kinase and is essential for the transforming activity of the oncoprotein [57, 58].

Another group of nuclear envelope proteins are the nesprins, large spectrin-related proteins of the alpha-actinin superfamily containing an N-terminal actin-binding domain, a central spectrin-repeat rod domain and a C-terminal transmembrane domain. Splicing variants of the nesprin family are associated with both the outer and inner nuclear envelope and might be involved in anchoring the nucleus to the actin skeleton [59, 60]. In contrast to the lamins and Tpr, which localize to the inner surface of the nuclear envelope, NUANCE (nucleus and actin connecting element), also known as nesprin-2, localizes to the outer nuclear membrane via a transmembrane domain at its C-terminus [61]. Nesprin-1alpha is a nuclear variant of nesprin-1 interacting with lamin and emerin [62], whereas nesprin-1beta is localized at the Golgi [59, 60, 63].

Coiled-coil proteins in spindle-pole and centrosome formation

The yeast coiled-coil protein Mps2 (monopolar spindle 2) is attached to the nuclear envelope via a C-terminal transmembrane domain and shows a second localization at the spindle pole body (SPB) [64]. It is required for the insertion of the SPB into the nuclear envelope and has been shown to interact through its coiled-coil domain with a number of other coiled-coil proteins localized at the SPB and kinetochores [65, 66]. Several additional kinetochore proteins with large coiled-coil domains have been identified in budding and fission yeast, such as Slk19 [67] and Sim4 [68], the absence of which causes a short spindle phenotype [68, 69].

Several coiled-coil proteins appear to be involved in connecting the inner and outer plaque of the yeast SPB with the central SPB core embedded in the nuclear envelope. Cnm67p (chaotic nuclear migration) bridges the outer plaque with the central plaque with its coiled-coil domain acting as a spacer element [70]. Spc72p is a coiled-coil protein present in the outer plaque and bridge of the spindle pole body [71, 72]. Spc110p (also known as Nuf1p) has been shown to span the distance between the inner plaque and the central plaque in similar fashion to Cnm67p [73]. Spc110p homologues have been identified in *Aspergillus nidulans* and fission yeast (Pcp1p, pole target of calmodulin in *Schizosaccharomyces pombe* [74]), and in humans (Kendrin/pericentrin-B [74, 75]).

Kendrin, a larger isoform of the gamma-tubulin- and dynein-binding protein pericentrin [76, 77], forms a complex with another large coiled-coil protein, known as CG-NAP (centrosomal and Golgi N-kinase anchoring protein [78]) or AKAP450 (A-kinase anchoring protein 450 kDa [79]). This complex is located at the center of microtubule asters nucleated from centrosomes, strengthening its proposed role in providing sites for microtubule formation [80]. Spc110p and Spc72p and their animal homologues as well as GC-NAP/AKAP450 have been shown to bind gamma tubulin, suggesting a role in microtubule nucleation [80–82]. They act as targets for the binding of calmodulin, which is thought to be involved in microtubule-mediated stabilization of the pericentriolar matrix [83]. CG-NAP/AKAP450 also provides a scaffold for the attachment of centrosomal isoforms of protein kinase C [84, 85], while displacement of the protein from the centrosome leads to delocalization of protein kinase A type I(alpha) [86].

In mammalian cells, the large coiled-coil protein ninein has been identified as another component of the pericentriolar matrix of the centrosome [87]. Ninein associates with the minus end of microtubules and might play an important role in the stabilizing, positioning and anchorage of microtubules [88]. The presence of ninein is essential for the centrosome to function as a microtubule organization center [89, 90].

C-Nap1 (centrosomal Nek2-associated protein 1) is a large coiled-coil protein found associated specifically with the proximal ends of the mother and daughter centrioles in interphase [91]. It is thought to be involved in intercentriolar linkage, and its dissociation from the centrosome at the onset of mitosis is stimulated by cell-cycle-dependent phosphorylation [92, 93].

The TACC (transforming acidic coiled-coil) proteins constitute a recently discovered family of centrosomal proteins implicated in vertebrate and *Drosophila* microtubule stabilization, acentrosomal spindle assembly, translational regulation, development and cancer progression [94, 95]. TACC1 has been shown to interact with a NuMA-binding protein, and it has been suggested that TACC proteins act as scaffolding/bridging proteins in multiple complexes important for centrosomal function [96]. Alterations in TACC protein level could result in changes of the centrosomal matrix, leading to spindle defects and increasing genetic instability, thus explaining the tumor-promoting potential of the TACC genes in humans [97]. In general, dysregulation of centrosomal proteins, including the large coiled-coil components such as pericentrin, can trigger cancer development in mammals [98, 99].

Coiled-coil proteins associated with the spindle apparatus

Astrin, a constitutively expressed mammalian microtubule-associated nonmotor protein, relocates from the cytoplasm to the spindle poles during mitosis [100]. Its long coiled-coil domain forms a parallel dimer with a 'lollipop'-shaped structure, which can aggregate into oligomers resembling astral structures. Cells lacking astrin are unable to complete mitosis and undergo apoptosis [101]. It has been suggested that astrin oligomers may function in microtubule bundling and/or providing a scaffold for linking regulatory and structural components to spindle microtubules.

In nematodes, the coiled-coil proteins PUMA1 [102] and LIN-5 [103] have been found to localize to the spindle apparatus in a cell cycle- and microtubule-dependent manner. Both proteins contain a large central coiled-coil domain, in the case of PUMA1 stretching over ~1750 amino acids, the longest single coiled-coil domain known to date. PUMA1 might be part of a 'centromeric matrix', whereas LIN-5 is thought to play a role in localizing or regulating a motor-protein complex and/or connecting the spindle apparatus with the cell cortex.

Kinetochore/centromere-associated coiled-coil proteins

A number of coiled-coil proteins are associated with the kinetochore/centromere in vertebrates. CENP-F, a

350-kDa protein also known as AH antigen [104] or mitosin [105], consists of two 1600-amino-acid-long coiled-coil domains connected with a central flexible core [106]. It changes its subcellular localization significantly during the cell cycle and has been employed as a temporal marker for G2/M phase transition [107]. CENP-F is targeted to the nucleus in early G2 phase, concentrates around the nuclear rim at G2/M phase transition via farnesylation [108], and relocates to the kinetochore/centromere regions and the spindle apparatus during M phase before being degraded after mitosis [104, 105]. Another vertebrate kinetochore protein, CENP-H, is a coiled-coil protein required for the centromere targeting of CENP-C [109–111]. The human coiled-coil kinetochore-associated protein Hzwint-1 binds the essential centromere protein ZW10 and may play a role in associating ZW10 to the kinetochore specifically in prometaphase [112].

Coiled-coil proteins shared between the centromere and the Golgi

Myomegalin, a centrosome/Golgi-associated long coiled-coil protein in humans, interacts with PDE (cyclic nucleotide phosphodiesterase), indicating its involvement in the cyclic AMP (cAMP) signal transduction pathway. It has been proposed to provide an anchor for PDE at the centrosome/Golgi [113]. Interestingly, PKA (cAMP-dependent protein kinase A), another enzyme in the cAMP signal transduction pathway, has also been shown to be anchored to the same locations via the aforementioned large coiled-coil protein CG-NAP/AKAP450 [79, 114], suggesting a role of these coiled-coil proteins in cAMP signal compartmentalization.

Golgi-associated coiled-coil proteins

The golgin family comprises a group of coiled-coil peripheral or integral membrane proteins associated with the Golgi apparatus. They have been shown to function in a variety of membrane-membrane and membrane-cytoskeleton tethering events at the Golgi and are regulated by small GTPases of the Rab and Arl families [115]. A subgroup of mammalian golgins with homologues in yeast and invertebrates is characterized by the presence of the Golgi-targeting GRIP domain in their non-coiled-coil C-termini [116, 117]. This domain binds preferably to the ARF-like GTPases Arl1 and Arl3 [118, 119]. Another subgroup of golgins contains transmembrane domains at their C-termini for anchorage at the Golgi membrane [120–123]. It has been suggested that golgins are forming a Golgi matrix, which serves as the structural scaffold for the enzyme-containing membranes of the Golgi apparatus and may provide the means of partitioning the Golgi during mitosis [124, 125].

Lva (Lava Lamp) is a microfilament/microtubule-associated protein of the Golgi apparatus in *Drosophila* with a golgin-like coiled-coil domain structure. It may form an Lva/spectrin scaffold at the Golgi and facilitate the interaction of Golgi bodies with microtubules in membrane-trafficking events essential for cytokinesis [126].

Coiled-coil proteins involved in developmental and behavioral processes

Mutations in large coiled-coil proteins can lead to specific developmental or even behavioral phenotypes in *Drosophila*. The *mud* (*mushroom body defect*) gene encodes a protein with a long coiled-coil core domain and a C-terminal transmembrane domain. It is expressed mainly during embryogenesis and plays a role in the control of neuroblast proliferation [127]. Mutations in the *qtc* (*quick-to-court*) gene cause increased sexual activity in male flies. The gene was found to encode a coiled-coil protein expressed specifically in olfactory organs, the central nervous system and the male reproductive tract [128].

Plant coiled-coil proteins

In contrast to animals and yeast, only a small number of long coiled-coil proteins have been studied in plants. Seventeen candidates for myosins and 61 putative kinesins have been identified in the completely sequenced *Arabidopsis* genome based upon homologies in the motor domains [129, 130]. A number of kinesin-like proteins have been characterized in plants, most of which appear to play a role in cytokinesis [131–135]. Homologues of the mammalian SMC proteins have been identified in *Arabidopsis,* and mutant analyses confirm their involvement in DNA repair and seed development [136–138].

A small number of plant proteins with long coiled-coil domains has been described for which there are no recognizable nonplant homologues. The carrot coiled-coil protein NMCP1 is located at the nuclear rim and has been shown to migrate to the spindle poles in dividing carrot suspension culture cells, a pattern similar to some of the aforementioned animal coiled-coil proteins involved in spindle organization [139]. CIP1 (COP1-interactive protein 1) is a cytoskeleton-associated coiled-coil protein and a binding partner of the photomorphogenesis suppressor COP1 [140]. It has been suggested that it is involved in retaining COP1 in the cytoplasm and thereby regulating its nucleocytoplasmic partitioning. A family of long coiled-coil proteins designated FPPs (filament-like plant proteins) appearing to be unique to the plant kingdom has been identified in *Arabidopsis* [141]. The cellular function of these plant proteins is presently not known.

Interestingly, chloroplasts have their own set of coiled-coil proteins. MFP1 is a DNA-binding protein with an ex-

tended coiled-coil domain that associates with the thylakoids in plant chloroplasts [142, 143]. It binds in vivo to the chloroplast nucleoids and has therefore been suggested to provide a connection between the thylakoid membrane and the nucleoids in mature chloroplasts. CpPTP (*Craterostigma plantagineum* plastid targeted protein) shows a similar domain structure to MFP1 and exhibits DNA-binding properties in its coiled-coil domain [144]. It is targeted to the plastids and expressed in response to drought stress and therefore has been proposed to play a structural role in protecting chloroplast DNA from dehydration.

PF2 is a large coiled-coil protein found in a screen for motility mutants in the algae *Chlamydomonas* [145]. It is required for the assembly of the dynein regulatory complex (DRC) and has been suggested to form a molecular scaffold for the attachment of other components of the DRC.

Overall, searches for plant homologues of animal and yeast coiled-coil proteins have been rather unsuccessful. Despite immunohistochemical evidence for the existence of lamin-like and NuMA-like proteins in plant nuclei [146–150], no lamin or NuMA homologues have been found in the *Arabidopsis* genome. In BLAST searches of the whole *Arabidopsis* genome for all animal and yeast proteins discussed here, we found significant homologies only for SMC proteins and myosins, with E-values typically below e^{-100}, and kinesins with E-values in the e^{-50} to e^{-100} range. This clearly demonstrates the need to use methods other than sequence comparison for the identification of plant long coiled-coil proteins potentially involved in the diverse cellular functions discussed above (see below). Figure 2 summarizes the subcellular localization during interphase and mitosis of those animal and plant long coiled-coil proteins for which localization data are presently available.

Toward understanding structure-function relationships of coiled-coil proteins

What is it that these functionally diverse proteins have in common? In other words, what is the biological function of a coiled-coil rod domain in the context of the diverse proteins discussed here? While there is no clear answer to this question at present, the biophysical features of the coiled-coil domain may allow formulating some hypotheses to be tested with individual proteins or protein classes.

Dimerization

The most obvious feature of the coiled-coil domain is its ability to form dimers (or oligomers). This allows for the combination of identical or different head and tail domains and – through higher-order interactions – for the

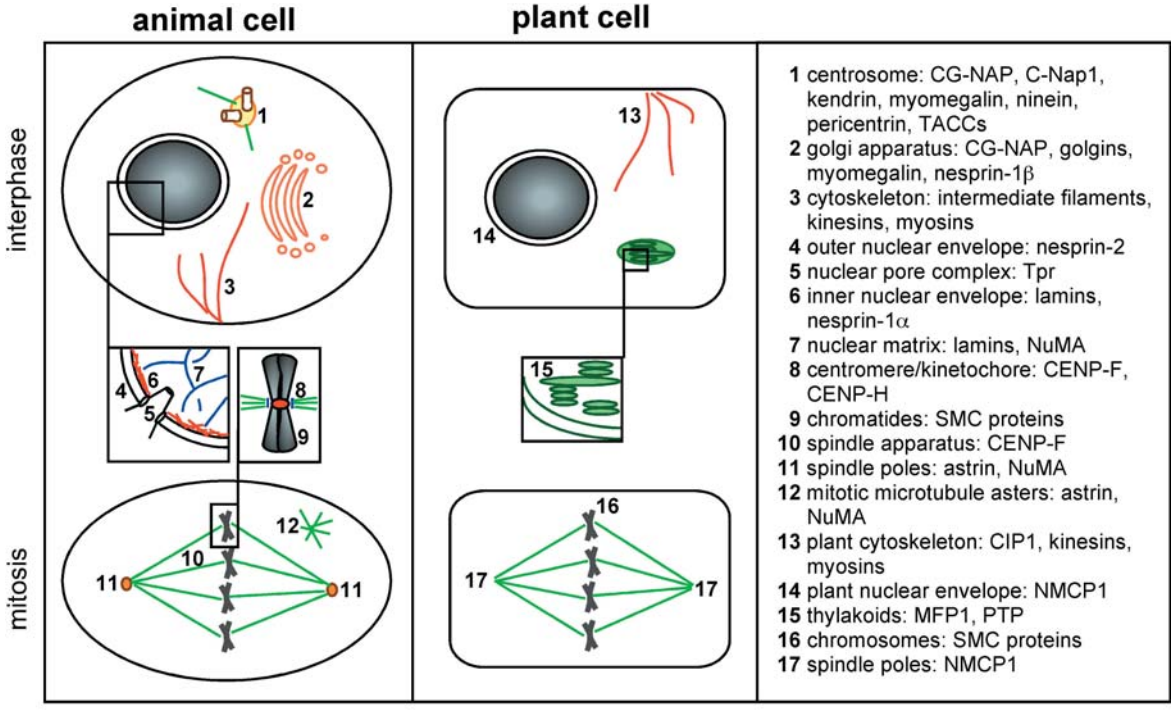

Figure 2. Association of coiled-coil proteins with subcellular structures during interphase and mitosis. Only proteins that have been cloned and characterized regarding subcellular localization are indicated. The numbers in the right column refer to the numbers in the left and middle columns and indicate proteins found in the different subcellular locations. For details regarding protein functions, see text.

formation of filaments. IF proteins can be classified depending on their dimerization preferences. Keratins, for example, are obligatory heterodimers, whereas other IF proteins, such as vimentin, assemble into homodimers [18].

While dimerization might be static in most long coiled-coil proteins, there is a small number of known proteins which undergo regulated coiled-coil dimerization. Interestingly, they sense environmental conditions, such as pH, temperature and solute availability, indicating that their regulation makes direct use of physical features of the coiled-coil motif.

The TlpA protein of *Salmonella* encodes a repressor protein that makes use of the coiled-coil motif to sense temperature changes. At temperatures below 37 °C, TlpA forms a dimer that can bind DNA and act as a repressor. Temperatures above 37 °C cause the coiled-coil dimerization domain to unfold. The random-coiled monomers are unable to remain bound to DNA, lifting the repression of the promoter [151, 152]. The thermal folding/unfolding of the coiled-coil domain is rapidly reversible and makes this protein an interesting candidate for a molecular thermosensor in nanotechnology [153].

The stability of the coiled-coil dimer is highly dependent on the distribution of charged residues along the helix axis. 'Unfavorable' charges leading to electrostatic repulsion will make the dimer less stable. A direct consequence of this is an influence of pH on coiled-coil stability. In an acidic environment, for example, acidic residues in unfavorable positions will be uncharged and therefore less disruptive than at neutral pH. Phosphorylation/dephosphorylation of a coiled-coil dimer can add another level of regulation to its stability [154, 155].

An example for pH regulation of a coiled-coil dimer is the apoptosis-associated protein Par-4. The C-terminal region of Par-4 is required for apoptotic activity, self-association and association with all presently known interaction partners, and contains a coiled-coil domain [156–160]. The protein forms a coiled-coil dimer only at an acidic pH and low temperature. Through site-directed mutagenesis predicting the effects of unfavorable charged residues at higher pH, Par-4 was altered from a pH-dependent, labile dimer into a pH-independent, stabile dimer [161]. It will be very exciting to see the effect of these point mutations on the in vivo function of the protein.

Another environmentally influenced coiled-coil formation was recently discovered in a nematode homologue of an LEA protein. LEA (late embryogenesis abundant) proteins are involved in desiccation tolerance in plant seeds and in desiccation-tolerant plants (reviewed in [162]). Group 3 LEA proteins contain extended amphipathic alpha helices predicted to form coiled-coils. However, Goyal et al. [163] showed that AavLEA1, the nematode LEA protein, is predominantly monomeric in solution and shows the characteristics of an unfolded, random-coil protein. In contrast, Fourier transform infrared (FT-IR) spectroscopy showed that AavLEA1 adopts the characteristics of an alpha-helical, coiled-coil protein after dehydration. The function of this structural change is presently unknown. However, given the role of LEA proteins in desiccation tolerance, it is tempting to speculate that desiccation might induce this structural change in the protein in vivo. This could allow the protein to either become active in a signaling event, or – as the authors suggest – form a filamentous network that might stabilize the desiccation-stressed amorphous cytoplasm in analogy to steel-reinforced concrete.

The coiled-coil as a rod

Besides dimerization of an adjacent functional domain, the most obvious feature of the coiled-coil dimer is the formation of a rod domain. The rod domain in IF proteins and lamins not only dimerizes but also contributes to the assembly of filaments. Filament formation by IF-type coiled-coil proteins was recently reviewed [18] and will therefore not be discussed here. Instead, this section focuses on rod formation of non-IF-type long coiled-coil proteins.

What does it take to make a molecular rod? Not all rod-like proteins consist of long, uninterrupted coiled-coils. Two examples of other domains that form rods are found in spectrin and titin. Spectrin, and the related proteins alpha-actinin, dystrophin and nesprin, are rod-shaped actin-binding proteins anchored to membranes. The rod-like structure of spectrin-like proteins comes from a series of spectrin repeats, regular 106-amino-acid-long triple-helical coiled-coil bundles [164]. Titin is a giant elastic protein responsible for the passive force generated by the stretched striated-muscle sarcomere. It consists of tandemly arranged immunoglobin-like domains, which together form a rod-like structure [165].

Providing adequate spacing between head and tail (and internal) domains is the most obvious function of a rod domain. Acting as a spacer has been proposed for the golgins, which might thereby be involved in early stages of vesicle tethering [166]. Indeed, several long coiled-coil golgins have been shown to interact with each other and with membrane-associated proteins on the Golgi and COPI vesicles, making them potential long-distance bridging molecules for vesicle tethering [166]. Another group of coiled-coil proteins acting as spacers are those associated with the outer and inner plaque of the spindle pole body. It has been shown impressively that shortening the coiled-coil domain of Cnm67p shortens the distance between the outer plaque and the central plaque [70], while shortening the coiled-coil of Spc110p shortens the space between the central plaque and the ends of the microtubules [73].

Elasticity and mechanical force transduction

Coiled-coils appear in a number of proteins having mechanical roles. A classic example is hair elasticity, in part provided by features of the coiled-coil in alpha-keratin [167]. The elastic properties of the myosin coiled-coil have been studied using single-molecule force spectroscopy [168]. The protein undergoes massive structural transitions, extending to about 2.5 times its original length. Interestingly, this transition is fully reversible on a short time scale, showing that the myosin coiled-coil is a truly elastic protein. This is in contrast to the elastic features of titin, which has a much slower recovery period, making it more suitable for its putative 'shock-absorber' function in muscle. It is currently unknown whether this feature of the coiled-coil domain has a function in vivo. However, it makes coiled-coil proteins attractive candidates for the design of molecular springs, which are gaining attention in nanotechnology (e.g. [169]).

All known cytoskeletal motor proteins contain coiled-coil domains. It was suggested earlier that unwinding of the extended coiled-coil in the myosin heavy chain is involved in motor function ([170] and refs therein). However, more recent evidence shows that the rod domain does not uncoil in solution [171]. More likely, its function is that of a flexible connecting arm. The amino-terminal subfragment of the rod swings out from the thick filament at a hinge in the coiled-coil, allowing the myosin heads to create tension by interacting with actin [172].

A connecting function can also be assumed for the stalk domain of kinesin, which connects the motor domain and the cargo-binding domain and – like the stalk of dynein – might provide space between these domains. In contrast, the shorter, coiled-coil neck domain of kinesin has a mechanical function in the unidirectional motion along actin fibers. The neck amplifies small conformational changes to act as a lever for swinging forward the second head, thus allowing for the 80-Å step size of kinesin (reviewed in [173]).

Except for space keeping (in the case of the spindle pole body proteins) or filament and network formation (e.g. nuclear lamins), it is presently not known precisely which of these features (or possibly others) are involved in the function of the diverse coiled-coil proteins discussed above. One area worth investigating is specific protein-protein interactions between coiled-coil proteins and with other, non-coiled-coil proteins. Homo- and heterodimerization can provide the means to combine and position different functional head and tail domains. Binding of other proteins to both the coiled-coil domains and the attached non-coiled-coil portions of a protein allows for the assembly of multi-subunit complexes in specific locations of the cell. In the post-genomic era, these questions can now be addressed for the first time in high-throughput approaches involving most or all of the long coiled-coil proteins of an organism. At the other end of the spectrum, increased investigations into the in vitro properties of long coiled-coil proteins could provide important clues about differences and similarities of their biophysical features, which – in combination with localization and protein-protein interaction data – could help to map their cellular functions.

Whole genome approaches: toward a map of coiled-coil protein function

To identify long coiled-coil proteins in new model organisms and to acquire a more complete picture of coiled-coil proteins in an organism, whole genome information in combination with structure prediction algorithms can now be applied. To predict coiled-coil structures based on amino acid sequence, several programs with differing performance rates are available. COILS and NEWCOILS [174], based on Parry's algorithm [175], have become the standard for coiled-coil prediction and are widely referenced in the literature. However, COILS generates a high number of false positives by predicting non-coiled-coil alpha-helical regions as coiled-coil structures [7, 176]. In tests on the PDB database of solved protein structures, two out of three sequences predicted by COILS did not contain coiled-coils [177]. Thus, this program would generate a high number of false hits if used for a genome-wide screen. The PAIRCOIL program takes pairwise residue correlations within the heptad repeat into account and performs significantly better than COILS in avoiding false positives. However, PAIRCOIL often fails to predict antiparallel or multistranded coiled-coils [176]. MultiCoil, based on data of two-stranded as well as three-stranded coiled-coils, is capable of predicting both types of structures while achieving a similar low rate of false predictions as PAIRCOIL [8].

By using the MultiCoil algorithm on a total yeast genome translation, ~300 two-stranded and 250 three-stranded coiled-coils have been identified [178]. More than half of these open reading frames have no assigned function. An investigation of a number of structural motifs in several whole genomes showed independently that the human, *Drosophila*, *Caenorhabditis elegans* and yeast translated genomes contain ~10% coiled-coil proteins [179]. Applying the yeast two-hybrid assay to determining protein-protein interactions specifically between the coiled-coil domains, Newman et al. [178] found 213 unique interactions between 162 putative coiled-coil sequences. Only one interaction for approximately every 100 pairwise combinations tested was positive, implying a large degree of specificity for heterodimeric coiled-coil interactions. However, with almost 10% of the yeast open reading frames coding for proteins with coiled-coil motifs, a large number of putative interactions remain to be discovered. In the future, it is likely that a combination of experimen-

tal and computational approaches will aid in a whole-genome map of yeast coiled-coil interactions [180].

With the goal of identifying plant long coiled-coil proteins, the MultiCoil algorithm has been applied to the *Arabidopsis* genome to identify all *Arabidopsis* proteins containing long stretches of coiled-coil domains [181]. The resulting data has been used to build the searchable protein database ARABI-COIL (http://www.coiled-coil.org/arabidopsis), which integrates processed data from the original MultiCoil output with features from a battery of additional sequence and structure analysis programs. In this study, no protein-protein interactions have been determined; instead, proteins have been sorted by predicted subcellular localization, presence of predicted transmembrane domains, and putative functions predominantly based on conserved sequences in the non-coiled-coil portions of the proteins.

One result of this analysis is that long coiled-coil proteins are predicted in all compartments of the plant cell. Each compartment contains some members predicted to be membrane attached, which might be attractive candidates for anchoring molecules in plants. A number of predicted nuclear long coiled-coil domains were found, which can now be investigated for putative functions in forming a plant nuclear skeleton. Combining this computational approach with the power of the genome-saturating, sequenced knockout mutations available for *Arabidopsis* [182], addressing whole-organism phenotypes of large numbers of knockout mutants of plant long coiled-coil proteins will now be feasible. The localization predictions will help to develop priorities for this investigation as well as for targeted or high-throughput analyses of protein-protein interactions. Following the establishment of the computational pipeline that delivered ARABI-COIL, this type of approach is now applicable to other full genomes as well, which can help to initiate more targeted, experimental investigations of the numerous coiled-coil proteins having unknown functions in eukaryotic cells.

Summary and outlook

Long coiled-coil proteins emerge as a versatile molecular toolbox of the eukaryotic cell. They provide cables and networks within the cyto- and nucleoskeleton as well as scaffolds to stabilize and organize membrane systems such as the nuclear envelope and the Golgi apparatus. In combination with flexible head and tail domains and hinges within the coiled-coil regions, they act as motors, levers, rotating arms and springs to move molecules, vesicles or large macromolecular complexes within the cell. Coiled-coil domains specifically and reversibly interact with binding partners, thus providing the means of recruiting regulatory components in a spatially and temporarily controlled manner.

The availability of structure prediction tools and their use on fully sequenced genomes allows for data mining of the complete set of coiled-coil proteins encoded within a genome. The comprehensive analysis of these proteins and comparison between different species and kingdoms will provide further insight into the evolution of coiled-coil proteins and the genomes encoding them. Several coiled-coil proteins in mammalian cells, including lamins and centrosomal proteins, are gaining increasing interest in the medical field as mutations in their genes are implicated in hereditary diseases and cancer. Aside from these biological functions, the study of large coiled-coil proteins also has the potential to lead to the development of interesting applications of molecular tools in nanotechnology.

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