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Review

The Dim protein family: from structure to splicing

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Abstract. The spliceosome is a dynamic macromolecular machine that catalyzes pre-mRNA splicing through a mechanism controlled by several accessory proteins, including the Dim proteins. The Dim protein family is composed of two classes, Dim1 and Dim2, which share a common thioredoxin-like fold. They were originally identified for their role in cell cycle progression and have been found to interact with Prp6, an essential component of the spliceosome, which forms the bridge of U4/U6.U5-tri-snRNP. In spite of their biological and structural similarities, Dim1 and Dim2 proteins differ in many aspects. Dim1 bears distinctive structural motifs responsible for its interaction with other spliceosome components. Dim2 forms homodimers and contains specific domains required for its interactions with partners. This originality suggests that although both proteins are involved in pre-mRNA splicing, they are likely to be involved in different biological pathways. In the present article we review the structure and function of the Dim proteins.

Keywords. Splicing, Dim1, Dim2, protein structure, thioredoxin-fold, spliceosome.

Introduction to the splicing machinery

In higher eukaryotes the primary transcripts, pre-mRNAs, of almost all protein-coding genes contain non-coding sequences of genes called introns, which must be entirely and precisely removed to yield mature and functional mRNAs. RNA splicing constitutes the process by which introns are removed and exons are joined, a pre-requisite for the expression of most eukaryotic genes. RNA splicing occurs through two sequential trans-esterification reactions that are carried out by a multi-component complex known as the spliceosome. As pre-mRNA can lead to different types of mRNA molecules and therefore to different proteins, mRNA splicing also plays a major role in the production of complexity and protein diversity in eukaryotes. This mechanism explains the disparity between the number of genes and the myriad of proteins that are believed to be necessary for complex organisms like humans. The spliceosome is a macromolecular machine, composed of more than 300 distinct proteins and five RNAs [1–3], that catalyzes pre-mRNA splicing. The spliceosome orchestrates the sequential binding and release of numerous proteins by formation and disruption of RNA helices as well as RNA-protein and protein-protein interactions, many of which require hydrolysis of ATP [4–7]. The small nuclear ribonucleoproteins (snRNPs) are highly conserved RNA/protein complexes of the spliceosome. Five snRNPs (U1, U2, U5 and U4/U6) are involved in splicing processes, and they all contain an snRNA (U1, U2, U5 or U4/U6) and a set of proteins that are either specific to one particle or common to all of them [7–10].

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According to the current model of the spliceosome mechanism, the different snRNPs interact with the spliceosome in an orderly fashion (Fig. 1). The U1 snRNP initially recognizes the pre-mRNA substrate by base-pairing between U1 snRNA at the conserved 5' splice site exon-intron junction forming the E complex [12–17]. In the second step, the U2 snRNP base-pairs with the branch site of the intron to form complex A or the pre-splicing complex in an ATP-dependent mechanism [18–22]. The tri-snRNP complex containing the U5 and the base-paired U4/U6 then stably joins the spliceosome to form complex B [11, 23, 24]. The most decisive step during the spliceosome maturation process is the conversion of complex B into the catalytically activated spliceosome complex B*, which catalyzes both steps of splicing. This conversion requires numerous rearrangements, in particular conformational changes of the tri-snRNP RNAs [25–29]. Within the U4/U6 snRNPs, the U4 and U6 snRNAs share sequence complementarities, forming a phylogenetically highly conserved interaction domain consisting of two intermolecular helices termed stem I and stem II [30–33]. Both of these intermolecular helices are disrupted as the spliceosome becomes catalytically active; the region of U6 constituting stem II folds back onto itself to form a new intramolecular stem-loop, and the region of U6 residing in stem I base-pairs with U2 snRNA [4, 34–36], while U1 snRNP dissociates from the 5' splice site to allow U6 snRNA and other components to come into juxtaposition with the 5' splice site. In contrast to U6, U4 snRNP is released from the spliceosome. The first catalytic reaction of trans-esterification is ATP-controlled and triggered by the release of U4. Formation of the lariat at the branch site and the 5' splice site generates complex C, and this is rapidly followed by a second splicing reaction. The U5 snRNP has been shown to base-pair with sequences in both the 5' and 3' exons and to position the ends of the two exons for the second step of splicing: the 3' hydroxyl of the 5' exon attacks the phosphate at the 3' splice site, yielding ligated mRNA and a lariat intron. After the second step has been completed, the ligated exons and a lariat intron are released. Finally, the spliceosome components dissociate and are recycled for further rounds of splicing [1, 2, 24].

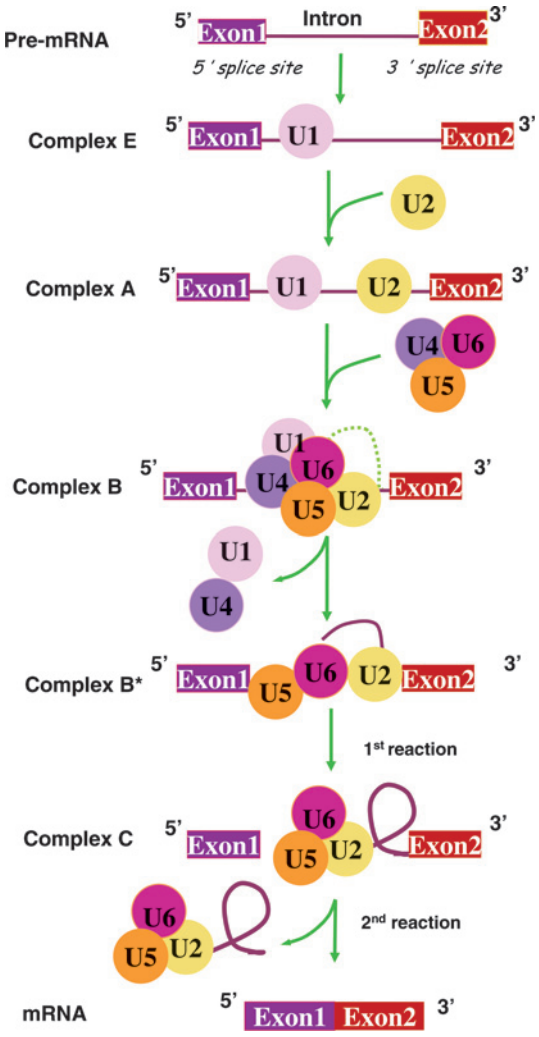

Figure 1. Mechanism of pre-mRNA splicing and snRNP remodelling. The different snRNPs interact with the spliceosome in an orderly fashion: The U1-snRNP initially recognizes the pre-mRNA substrate forming the E complex; then the U2 snRNP base-pairs with the branch site of the intron to form complex A; finally, the tri-snRNP complex U5/U4/U6 associates with the spliceosome to form complex B. The most decisive step during the spliceosome maturation process is the conversion of complex B into the catalytically activated spliceosome complex B*, which catalyzes both steps of splicing. After assembly of the spliceosome, RNA splicing involves two trans-esterification reactions. In the first catalytic reaction, the branch site of the intron that is located close to the 3' splice site attacks the 5' splice site, forming the lariat and generating complex C. In the second splicing reaction, the 3' hydroxyl of the 5' exon attacks the phosphate at the 3' splice site, cleaving the RNA molecule, the two exon sequences are ligated, and the lariat intron is released.

The U5 snRNPs

The major step of the catalytic activation of the spliceosome corresponds to the conversion of the inactive complex B into a fully catalytically activated spliceosome complex B* (Fig. 1). Activation of the spliceosome involves major structural changes, including the displacement of U1 from the 5' splice site, unwinding of the U4/U6 base-pairing interaction, and subsequent base pairing of U6 with the 5' splice site and the U2 snRNA [4, 7]. These rearrangements trigger dissociation of the U1 and U4 snRNPs and the related formation of the catalytic core of the spliceosome.

More than other snRNPs, the U5 snRNP undergoes major changes during the splicing process; the U5 snRNP particle has to be reformed before formation of the U4/U6.U5 tri-snRNP, and its protein composition varies during the splicing process [24–29]. Determination of the protein composition of the U5 snRNP by mass spectroscopy has revealed that this particle exhibits a rich diversity of proteins, including major actor proteins involved in conformational rearrangements of the spliceosome. Proteins of the DEAD/H box family of ATPases of RNA/RNP unwindases, such as Prp28/U5–100K or Brr2/U5–200K, appear to be one of the driving forces that mediate RNA rearrangements at this stage. In yeast, Prp28/U5–100K is involved in displacing U1 from the 5' splice site [22, 29]. Brr2/U5–200K and the GTPase Snu114 facilitate U4/U6 unwinding prior to spliceosome activation [21, 30, 37–39]. The protein Prp8 has an essential role in the catalytic core of the spliceosome by stabilizing all elements of the pre-mRNA at the 5' and 3' splice site and by modulating the activity of the U5 proteins involved in these rearrangements [39, 40–45].

Likewise, the Prp6/U5–102K protein, which contains a tetra-tricopeptide repeatdomain (TPR), has recently been shown to interact with hBrr2, hSnu114, and hPrp8 proteins *via* HAT (Half A TPR)/ TPR repeats [45]. The TPR domain represents a platform for binding partners in multiple protein-protein interactions during the assembly of the macromolecular machinery [46, 47]. Moreover, Prp6 constitutes the most important bridge between human U5 snRNPs and U4/U6, since it recruits a network of proteins involved in the U4/U6 snRNPs and interacts simultaneously with the tri-snRNP-specific hSnu66 and the U4/U6-specific hPrp31 and hPrp3 [45]. Both of these proteins are destabilized during the catalytic activation of complex B and are released from the spliceosome [24]. The N-terminal domain of Prp6 protein has recently been shown to interact with the thioredoxin-like Dim1/U5–15K protein [45], which is also destabilized in the activated spliceosome B* [29].

Biological characterization of Dim1/U5–15K protein

Dim1 (defective entry into mitosis) is a small protein of 15 kDa that was initially identified in *S. pombe* as being essential for cell cycle progression [48]. Dim1p is extraordinarily well conserved throughout the eukaryotic kingdom and is present in numerous species including mammals, plants, yeasts, nematodes, slime molds and alveolate, with about 79 % sequence identity throughout the entire length of 142 amino acids [49, 50] (Fig. 2). The biological functions of Dim1 in both pre-mRNA splicing and the control of cell cycle progression are conserved among different species. Its role in cell cycle regulation, which can be direct or indirect (depending on the species), seems to be a consequence of a fault in pre-mRNA splicing of proteins responsible for accurate cell cycle progression.

In *S. pombe*. Dim1p was initially identified as an essential player in the control of cell cycle progression, required for both entry and progression through M phase [51]. Cells containing a *dim1* null mutation are arrested at the G2/M transition with an elongated shape. *dim1–35* temperature-sensitive mutants exhibit loss of viability in mitosis, a defect in sister chromatid separation, and high sensitivity to microtubule-destabilizing agents; this mutant shows a cut ('cell untimely torn') phenotype similar to that of the anaphase promoting complex component *cut4* and *cut9* mutants. The role of Dim1p protein in cell cycle progression was further supported by isolation of a mutation in the LID1 (lethal in *dim1–35*) gene from a synthetic lethal screen with *dim1–35* aimed at identifying proteins that interact with Dim1p. *Lid* mutant cells have been reported to be unable to undergo anaphase at restrictive temperatures and to exhibit short metaphase spindles that do not elongate as well as chromosomes that do not separate. The association between Lid1p and Cut9p and Nuc2p as well as the dependence of Cut9p in the 20S complex on Lid1p function have been demonstrated *in vivo* by co-immunoprecipitation. Thus, Lid1p is a component of the *S. pombe* APC/C, and its function is required for ubiquitination of the APC/C target Cut2p; Dim1p is required to maintain steady-state levels of Lid1p and the APC/C [48]. Recently Dim1p was co-purified with the U4/U6.U5 tri-snRNP component Prp1 (Prp6 in *S. cerevisiae*) [52] and was shown to be required for efficient splicing of Lid1 pre-mRNA. The *dim1–35* mutation selectively reduces Lid1p protein production not only because pre-mRNA splicing is affected but also because Dim1p participates in the nuclear export of Lid1 mRNA to the cytoplasm [52]. Like *dim1–35* mutation, mutation of Prp1 has been shown to affect pre-mRNA splicing, poly(A)+ RNA nuclear transport, and cell cycle control [53–57] suggesting that both proteins might be critical in the transition steps between pre-mRNA splicing and transport of the mature transcript from the nucleus to the cytoplasm [52].

In *S. cerevisiae*. Deletion of the DIM1 homolog DIB1 is lethal. Moreover, Dib1p was found to be stoichiometrically associated with the U4/U6.U5 tri-snRNP [58] and to interact with Prp6 (equivalent of

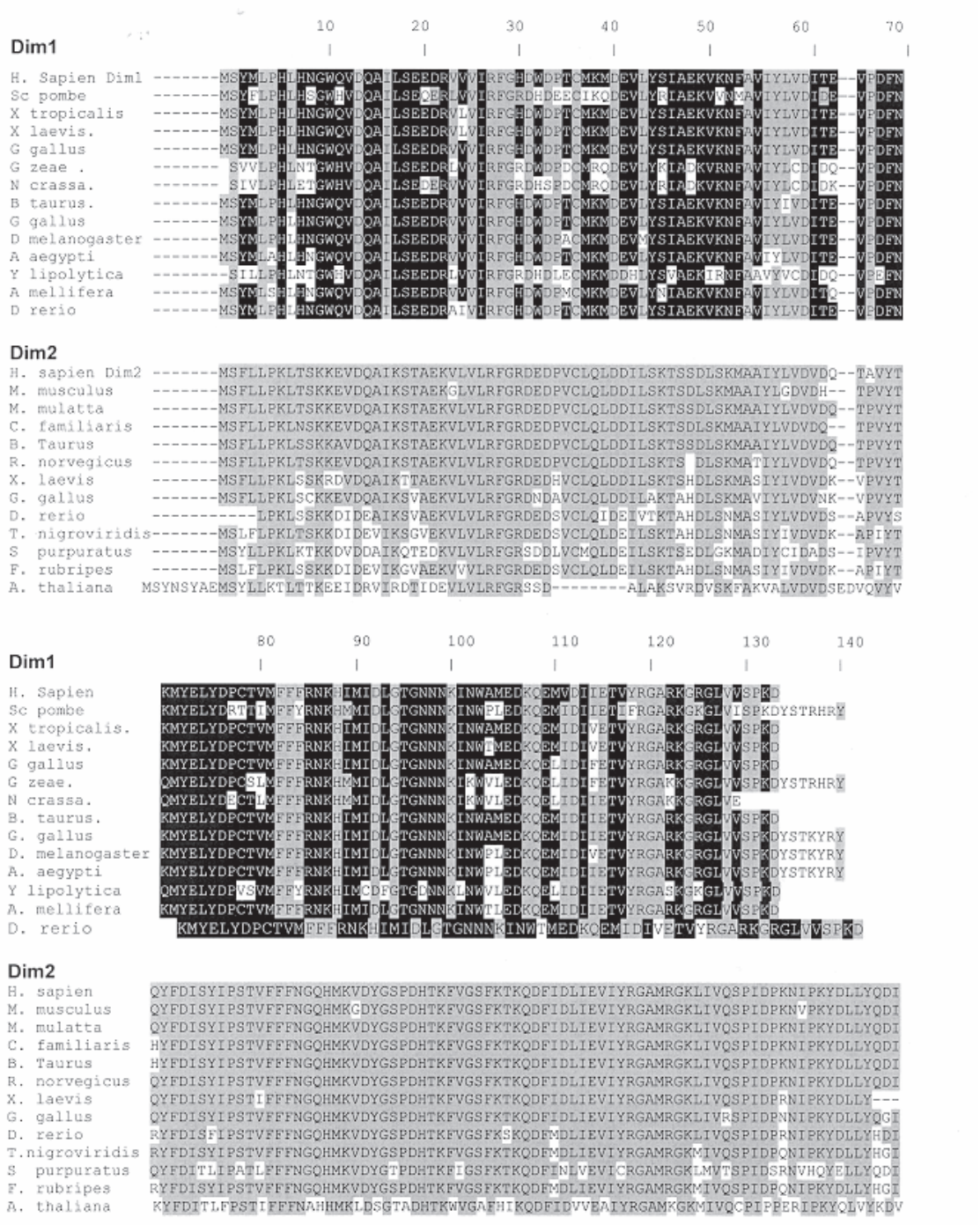

Figure 2. Sequence homology of the Dim protein family. Amino acid sequence alignment of Dim1 and Dim2 proteins. Conserved residues in both Dim1 and Dim2 or only in Dim1 are highlighted in grey and black, respectively.

Prp1 in *S. pombe*) [59–60], suggesting that Dib1 might function in pre-mRNA splicing and that its role in cell cycle progression could be indirect; a role in mRNA splicing is supported by the finding that Dib1 depletion in yeast leads to failure to splice some small RNAs [50].

In *C. elegans.* Knockdown of the DIM1 homolog (DML-1) by RNA interference leads to embryonic lethality during gastrulation following failure in the expression of early zygotic transcripts. These results suggest a role for DML1 at a stage defined as critical for synthesis and pre-mRNA splicing of zygotic mRNAs [61]. In particular, the severity of the defects

resulting from DML-1 ablation is strongly reminiscent of phenotypes observed upon loss of RNA polymerase II [62] or the SL1 trans-spliced mRNA leader [63].

In Human. Dim1 is located on chromosome 18q23 and plays an active role in the spliceosome. In a two-hybrid screen, it was shown to interact with components of pre-mRNA splicing machinery such hnRNP-F, hnRNP-H, and the RNA-binding protein Npw38/PQBP-1 [61]. hnRNP-F and hnRNP-H proteins were initially defined as possessing an RNA recognition motif (RRM)-like sequence that confers the ability to interact directly with poly(rG) and to control their function in tissue-specific enhancement of pre-mRNA. The RNA-binding protein Npw38/PQBP-1 contains a specific RNA-binding activity for poly(rG) and co-localizes with the SRm160/SRm300 splicing co-activators when co-expressed with hDim1 [64]. Under some circumstances Npw38/PQBP-1 regulates RNA polymerase II-dependent transcription [65]. PQBP-1 was identified as a binding protein of the polyglutamine tract, which is present in various transcription-related factors as well as causative genes involved in neurodegenerative disorders such as the triplet repeat disease [66]. In triplet repeat disease, expansion of the polyglutamine tract leads to neuronal cell death; it was therefore proposed that the interaction between Dim1 and PQBP-1, which is activated by triplet repeat disease genes, disturbs RNA splicing and leads to neuronal cell death. Likewise, the causative gene for spinal muscular atrophy, SMN, is involved in spliceosome assembly [68], suggesting that RNA splicing is involved in the pathogenic cascade of neurodegeneration in a variety of disorders.

hDim1 also interacts with the U5/52K protein, which is recruited to the spliceosome during B complex formation [68, 69]. The 52K protein binds hDim1 *via* a known protein-protein interaction motif called the "GYF" domain, which is located within the C-terminal 86 residues of the protein [70]. This motif has been characterized as a polyproline-targeting molecule [71] and was identified as interacting with oligo-proline stretches in the cytoplasmic part of the human T cell surface protein CD2 [70, 71]. Interestingly, hDim1 is devoid of potential proline-rich binding motifs [50], and this is the first case in which a "GYF" domain binds a partner in a proline-independent manner. In addition, it has been shown that the 52K protein interacts with hPrp6/U5–102K protein *via* its N-terminal domain [69]. These two interactions may occur simultaneously, as different domains of 52K are involved, and may be responsible for its integration into the U5 particle. The N-terminal fragment of Prp6 predicted to contain a coiled-coil domain is involved in hDim1 interaction [45, 72]. Simultaneously Prp6, *via* its "HAT" and "TPR" motifs, may interact with the U5 snRNPs hBrr2, hSnu114, and hPrp8 and with U4/U6 hPrp31 and hPrp3, assuming the role of a bridge between the human U5 snRNPs and U4/U6 snRNPs.

hDim1 protein has also been reported to be involved in cell cycle progression. hDim1 interacts with the p55 isoform of HEF1 generated by caspase-dependent cleavage of wild-type HEF1 [73, 74]. The p55 protein is produced specifically at mitosis and associates with the mitotic spindle, which suggests that as in *S. pombe*, hDim1p may either be a spindle component or regulates G2/M transition.

The biological function of Dim1 seems to be conserved among species and is essential in *S. pombe, S. cerevisiae* and *C. elegans*. In contrast, in mammalian cells there is as yet no evidence that Dim1 is an essential protein; knockout studies are required to elucidate its biological role in mammals. Dim1 constitutes an essential protein at the crossroads between splicing and the cell cycle. The role of Dim1 in this intricate complex of the spliceosome is not yet known. There is no evidence of catalytic activity of Dim1 or of its direct interaction with RNA. From a structural point of view, Dim1 is clearly an adapter protein connecting different components of the U5 snRNP. Its role in cell cycle regulation seems to be a consequence of a fault in pre-mRNA splicing of proteins responsible for accurate cell cycle progression.

Biological characterization of Dim2 protein

The Dim2 protein family was originally identified in sequence homology- and motif-based screens to probe for new members of the evolutionarily conserved Dim1 family [49]. Zhang and colleagues isolated three sequences of a hypothetical protein (in human, mouse, and aspen) that were closely related to Dim1, forming a separate Dim1-like family designated the 'Dim2' group [48]. More recently, the full-length sequence of hDim2 (Gene bank: FLJ20511) was identified *in silico*, and Dim2 cDNA was isolated by reverse transcription of placental RNA [75]. hDim2 is a small protein (17 kDa) that is highly conserved in higher eukaryotes including rat (*Rattus norvegicus*), mouse (*M. musculus*), amphibia (*X. laevis*), fish (*D. rerio*), and plants (*A. thaliana*), with a sequence homology of 89.2 %, 83.6 %, 81.4 %, 80.4 %, 77.3 %, and 74.8 %, respectively (Fig. 2). In contrast to its homologue Dim1, Dim2 does not have an ortholog in the genomes of *S. pombe*, *S cerevisiae, or C. elegans*, suggesting that Dim2 may be associated with functions specific to higher eukaryotes.

Human Dim2 is located on chromosome 16q22, and although its function remains poorly defined in comparison to that of Dim1, Dim2 protein seems to be involved in several cellular pathways. Like hDim1, Dim2 has been reported to be involved in pre-mRNA splicing and is required for cell cycle progression, mainly at the S/G2 transition [76]. Dim2 has been shown to be directly associated with pre-mRNA splicing, and its depletion from nuclear cell extracts totally abolishes *in vitro* pre-mRNA splicing, which can be rescued by addition of GST-Dim2 [76]. Sun and coworkers have demonstrated that Dim2 (Dim1-like protein; DLP), like Dim1, co-localizes to the cell nucleus and interacts with the N-terminal region of Prp6/U5–102K protein [77]. A number of proteomic analyses of sub-complexes or of the entire spliceosome have been reported, and so far more than 300 proteins and five RNAs have been identified as components of the spliceosome [1–3]. However, in contrast to Dim1, in none of the proteomic investigations was Dim2 shown to be a component of the spliceosome. Given the dynamic nature of the splicing machinery, the possibility that Dim2 interacts at some stage with the spliceosome, although not as a core splicing protein, cannot be excluded. One hypothesis could be that Dim2 is involved in several cellular pathways and is occasionally associated with the spliceosome machinery. Dim2 has been reported to be present either in the nucleus or in the cytoplasm depending on the cell cycle stage. The ability of Dim2 to shuttle between the nucleus and the cytoplasm is in perfect agreement with a role for this protein in more functions than pre-mRNA splicing [Simeoni and Divita, unpublished results].

Dim proteins share a common structural fold

The three-dimensional structures of both Dim1 and Dim2 have been determined by either X-ray crystallography [50, 75] or nuclear magnetic resonance (NMR) [77]. Dim1 and Dim2 share a common thioredoxin-like fold characterized by a four-stranded β-sheet comprising pairs of parallel and antiparallel strands flanked by three α-helices with a C-terminal extension (Fig. 3a, b).

Structure of Dim1 proteins. The three-dimensional structure of hDim1 was solved by crystallography at high resolution (1.5 Å) [50] (PDB: 1QGV) and by nuclear magnetic resonance (NMR) [74]. Dim1 contains a four-stranded β-sheet containing pairs of parallel (β1–β2) and antiparallel (β3–β4) strands flanked by three α-helices, characteristic of a thioredoxin fold with a C-terminal extension of 14 amino acids (Fig. 3b). Although Dim1 proteins share a common fold with thioredoxin, they do not exhibit any oxidoreductase activity and can be considered more like "topological cousins" rather than functionally close relatives of the thioredoxin protein family. Several important structural requirements for thioredoxin activity are not conserved in Dim1. The canonical Cys-X-X-Cys motif forming a functional disulfide bond typical of the thioredoxin family is replaced in Dim1 by Asp-X-X-Cys (amino acids 35–38). Dim1 contains a second cysteine residue (Cys^{79}) that is not evolutionarily conserved, suggesting that this disulfide bond is not essential for Dim1 function. Moreover, based on structural constraints, the formation of a Cys^{38}-Cys^{79} disulfide bond has been shown to be energetically unfavorable [77].

The extended C-terminus of helix α3 and the loop α3–β5 contain four basic residues (Arg^{121}, Arg^{124}, Lys^{125}, Arg^{127}), which together with Arg^{86} and Lys^{88} of the adjacent loop β3–β4 form a positively charged patch that is evolutionarily conserved in the Dim1 protein (Fig. 2) and is reminiscent of an RNA-binding surface. Although Dim1 has not been shown to interact directly with RNA, this basic patch represents a functionally important region of the U5–15kD protein [50]. Berry and Gould have demonstrated that mutation of the central glycine residue at position 126 of the *S. pombe* U5–15K ortholog in the basic domain of Dim1 to an aspartate yields the temperature-sensitive cell cycle-defective mutant *dim1–35* [48, 51]. Mutation of Gly^{126} to Asp introduces a negatively charged residue into the basic patch of the protein that may alter contacts of Dim1 protein with other components of the U5-snRNP or the spliceosome. However, further studies will be required to determine whether Dim1 interacts with RNA together with other spliceosome proteins.

Likewise, the C-terminal region of Dim1 constitutes an essential domain for its activity. The truncated form of hDim1 lacking the C-terminal region (1–128) not only fails to rescue a *dim1–35* mutant, it additionally acts as a dominant negative, associated with cell cycle arrest in G2, a phenotype similar to the *dim1*-null mutant. The impact of the C-terminal domain of Dim1 lies at the structural level *via* mediation of the interaction of Dim1 protein with key partners or substrates and by stabilization the core thioredoxin fold, which is particularly important for appropriate Dim1 function [49, 77].

Structure of Dim2 proteins. The structure of hDim2 was solved by X-ray crystallography at a resolution of 2.5 Å [74] (PDB: 1XBS). As observed for hDim1, Dim2 adopts a thioredoxin-like fold with a C-terminal extension, and its structure contains a

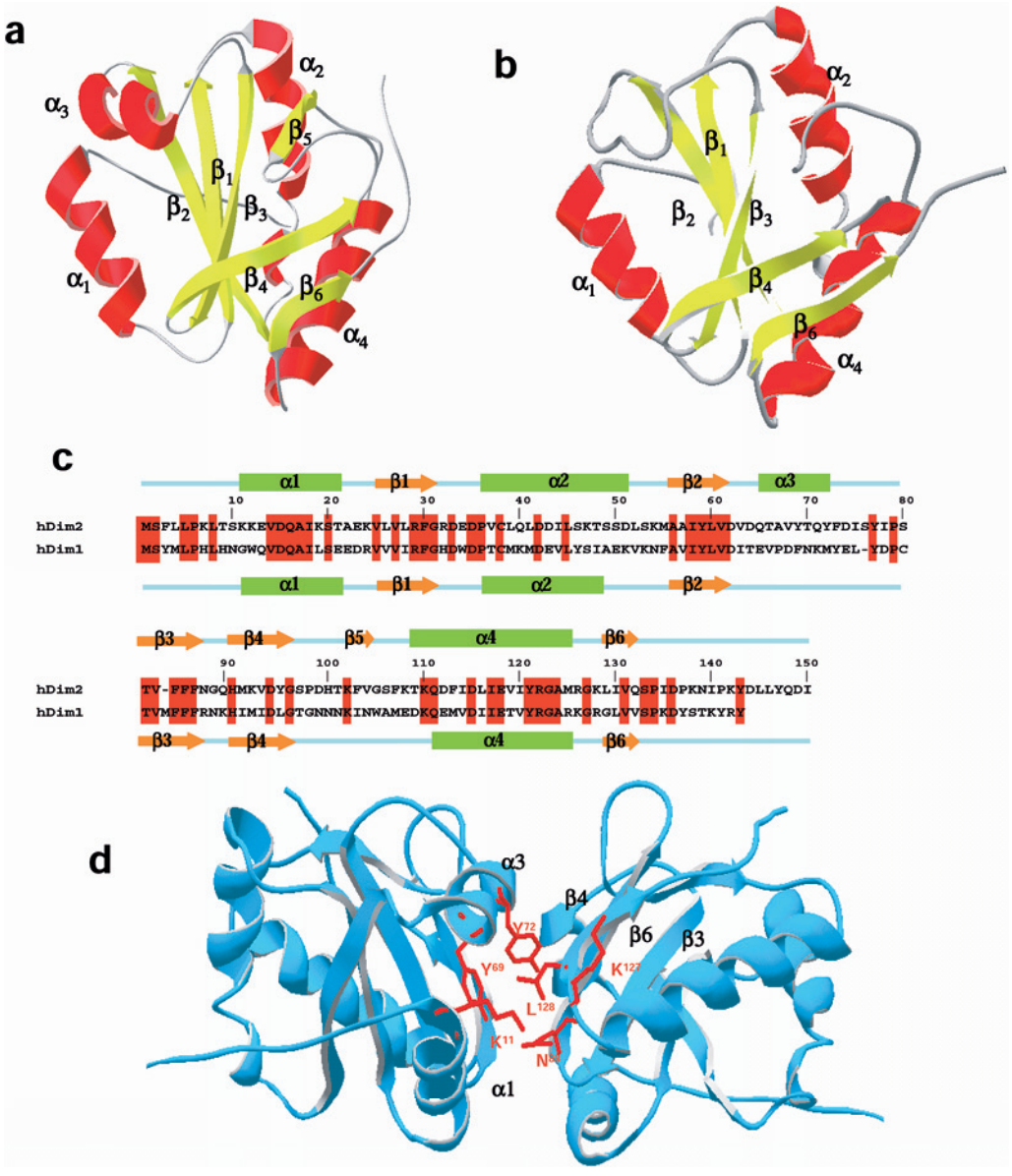

Figure 3. Structural comparison of the three-dimensional structures of hDim2 and hDim1. Ribbon representation of hDim1 (*a*) and hDim2 (*b*) structures. Secondary structure elements are shown (alpha helices, red; beta sheets, yellow; turns, grey). (*c*) Amino acid sequences and secondary structure alignments of hDim2 (PDB:1XBS) and hDim1 (PDB:1QGV). Identical residues are highlighted in red. (*d*) Ribbon representation of the crystallographic dimer of hDim2. The residues involved in the dimer interface are reported in red (Tyr^{72}, Tyr^{69} and Lys^{11} with Asn^{88}, Leu^{128} and Lys^{127}).

central core of six β-strands (β1–β6), surrounded by four α-helices (α1–α4) (Fig. 3b). As in the Dim1 structure, the β6 strand is involved in an additional interaction with the β4 strand, causing a displacement of the latter in comparison with the structure of thioredoxin. Superimposition of the structures of hDim1 and hDim2 highlights two major differences: hDim2 harbors an additional α-helix (α3) that connects strands β2 and β3, similar to what is observed in the structure of thioredoxin [78]; moreover, hDim2 contains an additional β strand (β5) connecting α4 and β4, which plays an essential role in the stabilization of the C-terminal region of the protein. Stabilization of the structure of the C-terminal region of Dim2 involves a cluster of aromatic residues, including Phe^{85}, Phe^{103}, Phe^{107}, and Phe^{112}, together with Pro^{99}, residues that are highly conserved and specific to the Dim2 family. The contacts between β4 and β5 and the loop connecting these two strands form a hydrophobic cleft that stabilizes the structure of Dim2 mainly through stacking of the Phe residues. Interestingly, potential phosphorylation sites (Ser^{97}, Ser^{80}, Thr^{81}, Ser^{105}) that are specific to the Dim2 family and that

may be involved in the regulation of Dim2 function or Dim2/partner interactions are rendered accessible by this major conformational change [75].

Structural comparison between Dim1 and Dim2

Common structural organization of Dim1 and Dim2. In higher eukaryotes, the Dim1 and Dim2 protein families exhibit about 37% sequence identity with 65% homology. Determination of the structures of Dim1 and Dim2 revealed several highly conserved key residues and domains that may be relevant at the functional level and associated to certain extent with the redundant function of these proteins in the spliceosome. The structures of both hDim1 and hDim2 are characterized by a thioredoxin-like fold and present a dipole organization. In both proteins, one side is essentially negatively charged, and the major negatively charged residues include Glu^{34}, Asp^{42}, Asp^{33}, Asp^{62}, Asp^{64}, Asp^{35}, Asp^{43}, Asp^{74}, and Asp^{93}, which are evolutionarily conserved in the Dim1 and Dim2 families. There are two other important common motifs in Dim1 and Dim2: one is located in the β1 strand and in the β1–β2 loop (residues 30–40 in hDim2), which contain the highly conserved "RFG" sequence and stabilize both structures through hydrophobic interactions (Fig. 3c); the second motif corresponds to the hydrophobic domain forming the β3 and β4 strands (residues 90–101), which are highly conserved in Dim2 and have been postulated to constitute an interface for partner binding.

Dim1-specific structural features. Structural analyses of Dim1 and Dim2 have also pointed out major differences that can explain the different behaviors of these two proteins at the functional level within the cell. Based on surface analysis of the hDim1 crystal structure [50], putative protein/protein and protein/RNA interaction domains have been proposed, and several areas that are not conserved in the Dim2 family have been mapped in both yeast and human orthologs [49, 61, 66, 77]. The basic patch of Dim1 involving Arg^{86}, Lys^{88}, Arg^{124}, Lys^{125}, and Arg^{127} surrounding Glu^{126} has been proposed to be involved in interactions with both protein partners and RNA; in hDim2, the second part of the basic motif, including Arg^{86} and Lys^{88} in the β3–β4 loop, is replaced with the polar residues Asn^{86} and Gln^{88}. Both Arg^{86} and Lys^{88} have been shown to be essential for the biological function of hDim1, as their mutation yields variants that fail to rescue *dim1–35* [77]. The function of hDim1 associated with this domain as a potential RNA-binding site is not maintained in hDim2, which probably does not interact with RNA.

A second critical domain in hDim1 is a motif in the N-terminal domain that interacts with different spliceosome-related proteins (hnRNP-F and Npw38/pQBP-1) involved in pre-mRNA splicing [75, 77]. The interfaces between Dim1 and Np/PQ or hnRNP-F involve residues 21–24 and 40–43, which are not conserved or only partially homologous, respectively, in Dim2. Moreover, the two negatively charged residues (Glu^{65} and Asp^{68}) shown to be essential for binding of both Np/PQ and hnRNP-F partners are also not conserved in Dim2. The lack of interaction of Dim2 (Dim2/DLP) with NP/pQ and hnRNP-F was confirmed by biochemical analysis [75].

Dim2 is dimeric in its native state. In contrast to its homolog Dim1, Dim2 is able to form stable homodimers in solution. The dimeric nature of Dim2 in its native state was determined in vitro by size exclusion chromatography and cross-linking experiments. Thermodynamic studies demonstrated that the native structure of Dim2 is strongly stabilized by its dimeric organization, which does not occur through inter-chain disulfide bond formation, and that the monomer/dimer transition is associated with significant unfolding of the monomeric structure of Dim2. In contrast, although Dim1 exhibits structure and folding similar to Dim2, it is a stable monomer in its native state. Structural analysis has highlighted potential motifs (accessible for interactions with partners) on the surface of hDim2 that are not conserved in hDim1. These motifs may be involved in either the dimerization interface or in an interaction with other protein partners. A protein-binding site of hDim2 is located on the α3 helix and corresponds to a patch of exposed polar residues (Gln^{65}, Thr^{66}, Thr^{70}, Tyr^{69}, Gln^{71}, and Tyr^{72}). Analysis of the crystallographic 2-fold dimer of Dim2 revealed that the dimer interface in the crystal lattice is formed mainly by contacts between the α1 and α3 helices and the β3–β4 and α4–β6 loops (Fig. 3d). The major interactions involve polar and charged residues, which are highly conserved in Dim2 (Lys^{11}, Tyr^{69}, Tyr^{72}, Leu^{128}, Lys^{127}, and Ile^{129}). Mutagenesis of residues Tyr^{69} and Tyr^{72} is sufficient to abolish dimer formation [75]. Finally, identification of the dimeric form of Dim2 in cellulo by mass spectroscopy analysis further supports its biological relevance and points to a potential role in the regulation of Dim2 (Simeoni and Divita, unpublished results).

Conclusions

The spliceosome machinery is a dynamic macromolecular organization that includes several proteins

able to shuttle from a macrostructure to another and several accessory proteins that control its mechanism and stability. Dim proteins, which have been classified into Dim1 and Dim2 families, lie at the interface between the cell cycle and the splicing machinery. The Dim protein families are important actors in the structural and the functional dynamics of the spliceosome. In higher eukaryotes, Dim proteins play an essential role in splicing and cell progression. Although the Dim proteins exhibit a thioredoxin-like fold, they lack the disulfide bond required for the thioredoxin redox activity. Thioredoxin-like motifs can have a large diversity of functions, including chaperone-like protein folding assistance and protein complex stabilization.

Dim1 and Dim2 proteins exhibit major differences in their biological and structural properties. Dim1 is evolutionarily conserved among different species and constitutes an essential protein that is indispensable for both cell viability and splicing in *S. pombe*, *S. cerevisiae,* and *C. elegans*. In human, Dim1 has been isolated as a structural component of the U5 snRNP complex of the spliceosome. In contrast, the function of Dim2 remains poorly defined. It is specific to higher eukaryotes and is probably associated with several cellular pathways. Although Dim2 has not been isolated as a spliceosome component, it has been shown to interact with Prp6 protein, the most important bridge between the U5 and U4/U6 snRNPs that form the tri-snRNP. Dim2 is not a component of the core splicing protein; however, given the dynamic nature of the splicing process, Dim2 can be envisioned to associate transiently with the spliceosome.

Preliminary data on the subcellular localization of hDim2 in the cytoplasm support an involvement of this protein in other cellular pathways. The structure of hDim2, in comparison to its Dim1 homolog, contains an extra α-helix (α3) and a β-strand (β5) that stabilize the protein as well as Dim2-specific domains potentially involved in interactions with partners. Dim2 forms stable homodimers in solution. The role of multimerization *in cellulo* and the function of the thioredoxin fold in the Dim protein family are not yet known. Future studies will be required to elucidate the biological roles of these intriguing proteins and to highlight novel cellular pathways.

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