Seminars in Cell & Developmental Biology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

snRNP proteins in health and disease

Michaela Krausová, David Staněk*

Department of RNA Biology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 27 July 2017
Received in revised form 9 October 2017
Accepted 12 October 2017
Available online xxx

Keywords:
Spliceosome
Mutations
Retinopathy
Congenital craniofacial disorders
Haematological malignancies

ABSTRACT

Split gene architecture of most human genes requires removal of intervening sequences by mRNA splicing that occurs on large multiprotein complexes called spliceosomes. Mutations compromising several spliceosomal components have been recorded in degenerative syndromes and haematological neoplasia, thereby highlighting the importance of accurate splicing execution in homeostasis of assorted adult tissues. Moreover, insufficient splicing underlies defective development of craniofacial skeleton and upper extremities. This review summarizes recent advances in the understanding of splicing factor function deduced from cryo-EM structures. We combine these data with the characterization of splicing factors implicated in hereditary or somatic disorders, with a focus on potential functional consequences the mutations may elicit in spliceosome assembly and/or performance. Given aberrant splicing or perturbations in splicing efficiency substantially underpin disease pathogenesis, profound understanding of the mis-splicing principles may open new therapeutic vistas. In three major sections dedicated to retinal dystrophies, hereditary acrofacial syndromes, and haematological malignancies, we delineate the noticeable variety of conditions associated with dysfunctional splicing and accentuate recurrent patterns in splicing defects.

Contents

* Corresponding author at: Vídeňská 1083, 142 20 Praha 4, Czech Republic.
E-mail address: david.stanek@img.cas.cz (D. Staněk).

https://doi.org/10.1016/j.semcdb.2017.10.011
1084-9521/

Please cite this article in press as: M. Krausová, D. Staněk, snRNP proteins in health and disease, Semin Cell Dev Biol (2017), https://doi.org/10.1016/j.semcdb.2017.10.011

1. Introduction

Removal of introns from pre-messenger RNA (pre-mRNA) is a central step in eukaryotic gene expression and a fundamental prerequisite for productive translation. Each splicing cycle is achieved in two stepwise trans-esterification reactions that are catalysed by the spliceosomal machinery. Most introns are processed by the major spliceosome consisting of essential RNA-protein complexes, namely the U1, U2, U4/U6, and U5 small nuclear ribonucleoprotein particles (snRNPs), whereas a small fraction of introns is recognized by the U12-dependent minor spliceosome comprised of U11, U12, U5, U4atac, and U6atac snRNPs. Intron removal and exon joining occurs in a series of orchestrated steps. Initially, U1 and U2 auxilliary factors interact with the pre-mRNA 5′-splice site (ss) and sequences at the 3′-ss, respectively: the polypyrimidine tract is recognized by the protein U2AF2, and the YAG/R 3′-ss consensus motif by U2AF1. U2AF2 forms a stable heterodimer with U2AF1 and interacts with SF1/mBBP which recognizes the pre-mRNA branch site (complex E). U2-associated protein complexes SF3a and SF3b subsequently navigate the U2 snRNP to the branch point sequence and displace SF1/mBBP to form the prespliceosomal complex A [1]. Binding of the U4/U6.U5 tri-snRNP to the A complex yields the pre-catalytic pre-B complex [2]. U1 is subsequently displaced by the activity of the RNA helicase yPrp28p (yPrp28p in the budding yeast *Saccharomyces cerevisiae*; DDX23 in humans) (B complex, Fig. 1) [3] and U4 snRNA and U4/U6 di-snRNP-specific proteins dissociate upon unwinding of the U4/U6 helix by yBrr2p helicase (SNRNP200 in humans) [4,5]. These structural rearrangements allow the binding of the NineTeen- and NineTeen-related complexes (NTC and NTR, respectively) to produce the activated B^{act} and catalytically active B^* complexes [6,7]. During these processes, U6 snRNA refolds to form an internal stem loop and to extensively base-pair with U2 snRNA [8]; both structures later contribute to the catalytic RNA core of the spliceosome [9,10]. The 5′-ss is shifted and tethered to the U6 snRNA ACAGAGA region, allowing for attack of the 2′-OH group of the branch point adenosine [11]. The first trans-esterification reaction produces a free 5′-exon 1 and intron lariat-3′-exon 2 intermediates [1]. The resultant C complex is further remodelled to C^*, where exons 1 and 2 are aligned by U5 snRNA for the second step of splicing [12,13]. Two DExD/H-box RNA helicases, namely yPrp2p (DHX16 in humans) [14] and yPrp16p (DHX38) [15], have been implicated in spliceosome rearrangements during catalytic steps, yielding B^* [16] and C^* [15], respectively. The ligated mRNA product is finally released by the yPrp22p (DHX8) helicase [17] and the residual intron-lariat spliceosome disassembled by yPrp43p (DHX15) [18], which allows recycling of snRNPs for repeated rounds of splicing.

In the recent years, the cryo-electron microscopy (cryo-EM) technique has allowed unprecedented depiction of the splicing machinery architecture. Initially, the structures of the yeast [19,20] and human [21] U4/U6.U5 tri-snRNP particle emerged. Subsequently, several research groups succeeded in resolving the yeast pre-catalytic complexes B [22] (Fig. 1) and B^{act} [23] structure, or captured the spliceosome assembly immediately after lariat formation (C complex) [24,25]. Also yeast and human spliceosomes stalled after yPrp16p/DHX38 remodelling (C^*) were recorded [26–29]. The major features of the available human structures (i.e. tri-snRNP and the C^* complex) are in general agreement with the yeast counterparts and reveal a rigid core of approximately 20 components [29]; we thus provide structural cues gleaned from the yeast models in cases the structure of the corresponding human complex has not been solved. In contrast, the human spliceosome contains several additional proteins along with the components of the Exon junction complex [29], and the position of SNRNP200/yBrr2p radically differs in the human and yeast tri-snRNP [21]. Together, the series of spliceosomal structures has provided remarkable insights into molecular features of splicing catalysis and supplied accurate structural hints on how pathological variants of splicing factors might compromise spliceosome assembly and catalysis or promote splicing errors.

2. Core spliceosome mutations in retinal dystrophies

Inherited retinal dystrophies comprise a complex set of hereditary conditions that feature degeneration of photoreceptor cells and dysfunction of retinal pigmented epithelium (RPE). To date, nearly 300 genes and/or loci have been found implicated in retinal disease (RetNet, https://sph.uth.edu/retnet/), dozens of which associate with non-syndromic retinitis pigmentosa (RP). RP is characterized by gradual decrease in both photoreceptor types (rods to a greater extent than cones) which triggers the migration of RPE cells to the inner retina [30]. Consistently, patients initially experience difficulties with dark adaptation and night blindness (rod-mediated achromatic vision in dim light), followed by the progressive loss of peripheral to central field vision (cone-related fine acuity vision in daylight and colour vision). With a worldwide prevalence of approximately 1:4000 individuals, RP accounts for the predominant form of familial blindness and follows several traits of inheritance [31]. RP manifests in diverse forms with varying genetic etiologies, but these can be roughly stratified according to the presumed gene function or the major biochemical pathway affected (e.g. components of the phototransduction cascade [32–34] or structural proteins [35]). In most cases, absent or defective gene products trigger photoreceptor cell death by formation of toxic aggregates, neglected cellular trafficking, misbalancing cell physiology [36] or disruption of paracrine interactions [37]. A set of RP patients harbours mutations in genes that encode ubiquitous RNA splicing factors (Fig. 2). These include three U5 snRNP proteins Prpf8 [38], SNRNP200 [39], and Prpf6 [40], and three U4/U6 di-snRNP constituents Prpf3 [41], Prpf4 [42], and Prpf31 [43]. RP, in addition, evolves due to defects in DEAH-box helicase DHX38 [44], and putatively also in the splicing factor PAP1 (encoded by the *RP9* gene [45]). Except for DHX38, pathogenic mutations in spliceosomal genes cause autosomal-dominant RP forms.

2.1. RP caused by impairment of U5 snRNP factors Prpf8, SNRNP200, and Prpf6

Prpf8 protein (encoded by the *PRPF8* gene) is the central scaffolding platform of both the U5 snRNP [46,47] and the whole spliceosome, where it orchestrates individual splicing steps [19,20]. yPrp8p constitutes of four major structural regions – the N-terminal NTD1 and NTD2 domains, the central core that includes reverse-transcriptase thumb/X (RT) and endonuclease (En) domains, the RNaseH-like domain (RH), and the Jab1/MPN domain. The N-terminal region anchors ySnu114p (EFTUD2 in humans), U5 snRNA, and the U5 Sm ring at the spliceosomal foot structural unit, whereas the C-terminal Jab1/MPN domain interacts with yBrr2p at the head corner of the spliceosome triangle [19,20] (Fig. 1). The architecture of the human Prpf8 in the tri-snRNP model is essentially similar except for the RH domain which is rotated by 180° in yeast compared to humans [21]. In the precatalytic state yPrp8p core region and RH domain stabilize interactions among U4/U6 di-snRNP members yPrp31p, yPrp3p, yPrp4p, ySnu13p, and yPrp6p (which is tri-snRNP specific in yeast [48], but tightly associated with the human U5 snRNP [49]), which altogether hold the U4/U6 snRNA duplex in an inactive conformation [20]. U4/U6 proteins are removed during spliceosome activation as the yBrr2p unwinds the U4/U6 duplex [50,51], and yBrr2p is again operational in the transition between the first and second catalytic steps [52]. SNRNP200/yBrr2p activity is tightly controlled by

Please cite this article in press as: M. Krausová, D. Staněk, snRNP proteins in health and disease, Semin Cell Dev Biol (2017), https://doi.org/10.1016/j.semcdb.2017.10.011