Splicing together sister chromatids

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Splicing of pre-mRNAs is a necessary step for expression of the majority of genes in higher eukaryotes, and its regulation through alternative splice site selection shapes their proteomes. Defects in multiple splicing factors result in aberrant mitotic progression, although the molecular basis for this observation has remained elusive. Recent papers in *The EMBO Journal* and *EMBO Reports* reveal that expression of sororin, a critical regulator that stabilizes cohesin rings in sister chromatids, is exquisitely sensitive to defects in the splicing machinery, thus explaining the striking link between spliceosome function and chromosome segregation.

See also: **S Sundaramoorthy *et al*** (November 2014), **P van der Lelij *et al*** (November 2014), **Y Oka *et al*** (September 2014) and **E Watrin *et al*** (September 2014)

Removal of introns from mRNA precursors in the cell nucleus (pre-mRNA splicing) is an essential process for the generation of translatable mRNAs. The splicing machinery (also known as the spliceosome) is composed of small nuclear RNAs (snRNAs), and about 150 proteins involved in the recognition and removal of introns (Wahl *et al*, 2009). The spliceosome is believed to assemble on pre-mRNAs in a stepwise manner, with early events involving the recognition of the 5' and 3' ends of the intron by U1 and U2 small snRNA-protein (snRNPs) complexes, respectively. Subsequent assembly of the U4/5/6 tri-snRNP brings together the splice sites, and further dynamic rearrangements, including those induced by the PRP19/CDC5 (NTC) complex, help to catalyze the two phosphotransfer reactions that lead to intron removal and splicing together of the flanking exons (Wahl *et al*, 2009). As expected from the

general requirement of splicing for proper gene expression, defective function of spliceosome molecules has been linked to multiple cellular abnormalities and human pathologies (Cooper *et al*, 2009).

Several observations in the last years have linked defective splicing with aberrant chromosome segregation during mitosis. Depletion of multiple spliceosome components results in prometaphase delay and chromosome misalignment, suggesting either defective splicing of critical mitotic components or additional direct functions of these spliceosome factors in chromosome segregation (Hofmann *et al*, 2010). Four new articles in *The EMBO Journal* and *EMBO Reports* now propose that sister chromatid cohesion (SCC), a process required for the proper alignment of chromosomes and their ordered segregation, is especially sensitive to alterations in the splicing machinery (van der Lelij *et al*, 2014; Oka *et al*, 2014; Sundaramoorthy *et al*, 2014; Watrin *et al*, 2014). SCC is mediated by the cohesin ring (Losada, 2014), a four-subunit complex that entraps DNA fibers, topologically preventing the separation of sister chromatids after DNA replication (Fig 1). The establishment of cohesion is mediated by acetylation of core cohesin proteins and binding of sororin, a protein that displaces the unloading factor WAPL, thus stabilizing the cohesin rings in chromatin (Losada, 2014). During prophase, cohesin is first released from chromosome arms, generating the typical 'X' shape of chromosomes. The cohesin rings that remain at the centromeric regions are later proteolytically cleaved leading to the ordered segregation of sister chromatids during the metaphase-to-anaphase transition (Fig 1).

Knockdown of several components of the spliceosome results in premature loss of SCC, thus preventing the stable attachment

of microtubules and the bi-orientation of chromatids (van der Lelij *et al*, 2014; Sundaramoorthy *et al*, 2014; Watrin *et al*, 2014). Some of these factors, such as SNW1 (a component of a NTC-related subcomplex), PRPF8 (a key coordinator of splicing catalysis associated with U5 snRNP) or MAFK1 (which associates transiently in the context of spliceosomal rearrangements previous to catalysis), are believed to have core functions in the splicing process. Their impact on cell division, however, raised the possibility that they display specific functions in mitotic progression different from splicing (Hofmann *et al*, 2010). To address this issue, Petronczki and colleagues analyzed in detail a set of 33 spliceosome components previously reported to alter mitosis when downregulated (Neumann *et al*, 2010). In this new study, knockdown of 26 out of these 33 splicing factors tested result in defective SCC (Sundaramoorthy *et al*, 2014). Downregulation of UBL5, an ubiquitin-like modifier involved in pre-mRNA splicing, also results in similar defects in SCC in a separate study (Oka *et al*, 2014). These factors belong to different subcomplexes and are involved in a variety of steps, from early spliceosome assembly (e.g. U2AF2, SF3B1) to catalysis (e.g. PRPF8), suggesting that a general role of pre-mRNA splicing in SCC is more likely than splicing-independent function of all these factors and subcomplexes.

Why is SCC specifically sensitive to defective splicing? All these four studies provide a similar answer. Whereas alteration of the splicing machinery does not alter protein levels or acetylation of core components of the cohesin ring, it invariably results in decreased levels of sororin and defective stable association of cohesin with chromatin (van der Lelij *et al*, 2014; Oka *et al*, 2014; Sundaramoorthy *et al*, 2014;

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Figure 1. The control of soririn levels by the spliceosome determines proper sister chromatid cohesion during mitosis.

The protein levels of soririn, encoded by the *CDCA5* gene in humans, depend on the proper splicing of *CDCA5* pre-mRNAs in interphase. After replication (S-phase), the cohesin ring maintains sister chromatids together. The stabilization of these cohesin complexes depends on soririn (arrows), which prevents premature WAPL-dependent removal of cohesion from chromatin. These soririn-containing cohesin rings maintain sister chromatids together during mitosis (M) until cohesin is removed from chromosome arms during prophase and from the centromeric region during the metaphase-to-anaphase transition. Under conditions of limiting spliceosome function, increased intron retention (IR) in *CDCA5* transcripts is observed, resulting in decreased soririn protein levels. This results in a WAPL-dependent release of cohesin rings from chromatin, impaired bi-polar alignment of chromosomes and mitotic arrest. The defects in spliceosome function may affect additional targets not shown here. MT, microtubules.

Watrin *et al*, 2014). The decreased levels in soririn proteins correlate with increased intron retention in transcripts of the *CDCA5* gene encoding soririn (Fig 1), but not in other cohesion-related transcripts, likely resulting in inefficient translation, translational frameshifts leading to truncated proteins and/or RNA degradation via the nonsense-mediated decay pathway. Consistent with this concept, premature loss of cohesion is significantly rescued by expression of intron-less and therefore splicing-insensitive soririn cDNAs. Importantly, co-depletion of WAPL significantly reverts the phenotype induced by downregulation of spliceosome components (van der Lelij *et al*,

2014; Oka *et al*, 2014; Sundaramoorthy *et al*, 2014; Watrin *et al*, 2014). This is in line with the major contribution of soririn to cohesion, that is, the displacement of the cohesin-releasing factor WAPL, strongly arguing that precocious SCC induced by defective pre-mRNA splicing is mostly the consequence of soririn downregulation (Fig 1).

The fact that soririn is short-lived and requires synthesis at a high level in every cell cycle may at least partially explain why its pre-mRNA is particularly sensitive to depletion of spliceosome components (van der Lelij *et al*, 2014; Sundaramoorthy *et al*, 2014). Alternatively, certain soririn introns may have evolved a particular sensitivity

to reduced spliceosome activity although testing this possibility would require further research. Yet, additional mitotic factors are likely influenced by splicing deregulation. The results by Peters and colleagues suggest that APC2 (also known as ANAPC2), a core component of the anaphase-promoting complex (APC/C) involved in the removal of cohesin at the metaphase-to-anaphase transition, is also affected by splicing defects. Depletion of SNW1 or PRPF8 results in reduced APC2 levels and impaired APC/C activity (van der Lelij *et al*, 2014) resulting in cohesion fatigue, a process in which sister chromatids separate in the presence of pulling forces. In fact, co-expression of

intron-less forms of sororin and APC2 cooperates in rescuing SCC defects, and expression of sororin alone is more efficient in preventing loss of SCC in conditions in which microtubules are destabilized, thus preventing cohesion fatigue (van der Lelij *et al*, 2014).

Restoration of sororin levels efficiently rescues defective SCC but not other mitotic defects observed in the presence of impaired spliceosome function, suggesting additional targets required for proper chromosome segregation (Sundaramoorthy *et al*, 2014; Watrin *et al*, 2014). The mitotic phosphatase CDC25 has been previously reported as a target of spliceosome defects in *Drosophila* and human cells (Hofmann *et al*, 2010), and a detailed analysis of intron-retaining transcripts or alternative splice selection in cells with impaired spliceosome function may eventually provide additional targets further linking splicing with the control of the cell division cycle.

Cohesin plays additional roles in the DNA damage response, DNA replication and repair and gene regulation (Losada, 2014), suggesting that the intriguing link between splicing function and SCC may have relevant implications beyond mitosis. Collectively, these new findings open the possibility that physiological regulation of spliceosome function (e.g., through cell cycle-dependent changes in the expression or in the activity of splicing factors) can act as checkpoints to

influence cell division or genome stability. The identification of anti-tumor drugs targeting components of the spliceosome and the frequent mutation of the same factors in a variety of cancers may be relevant in this context (Bonnal *et al*, 2012). Given the importance of splicing and cohesin in cancer and other human diseases (Cooper *et al*, 2009; Losada, 2014), the connection between these two processes deserves further exploration in the upcoming years.

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