SCIENTIFIC REPORT

Sororin pre-mRNA splicing is required for proper sister chromatid cohesion in human cells

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Abstract

Sister chromatid cohesion, which depends on cohesin, is essential for the faithful segregation of replicated chromosomes. Here, we report that splicing complex Prp19 is essential for cohesion in both G2 and mitosis, and consequently for the proper progression of the cell through mitosis. Inactivation of splicing factors SF3a120 and U2AF65 induces similar cohesion defects to Prp19 complex inactivation. Our data indicate that these splicing factors are all required for the accumulation of cohesion factor Sororin, by facilitating the proper splicing of its pre-mRNA. Finally, we show that ectopic expression of Sororin corrects defective cohesion caused by Prp19 complex inactivation. We propose that the Prp19 complex and the splicing machinery contribute to the establishment of cohesion by promoting Sororin accumulation during S phase, and are, therefore, essential to the maintenance of genome stability.

Keywords: Prp19 complex; sister chromatid cohesion; Sororin; pre-mRNA maturation; genome stability

Subject Categories: Cell Cycle; RNA Biology

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Results and Discussion

Prp19 complex is required for sister chromatid cohesion in mitosis

To address whether Prp19 complex functions in SCC, we investigated consequences of depleting Prp19 complex core components on mitotic SCC and on progression through mitosis in HeLa cells. To assess phenotype specificity, we used a HeLa cell line that constitutively expresses murine Cdc5L fused to a localisation-and-affinity-purification (LAP) tag containing green fluorescent protein (GFP) (Supplementary Fig S1A, B and D) and resistant to hCdc5L siRNAs (Fig 1D and Supplementary Fig S1C). Cells were transfected with control, Scc1, Cdc5L or Prp19 small interfering RNAs (siRNAs), and we analysed SCC status in mitoses 24, 48 and 72 h later, by observation of spread chromosomes (Fig 1A). In both cell lines, most of control cells displayed normal cohesion when a separation of sister chromatids 72 h after transfection was observed in up to 90% of cells depleted of Cdc5L or Prp19, similar to depletion of cohesin itself (Fig 1B and C). Consistently, most of mCdc5L-LAP cells depleted of Scc1 and Prp19 displayed cohesion defects (Fig 1C). By contrast, Cdc5L depletion had almost no effect on SCC in these cells, demonstrating that reduced level of endogenous Cdc5L protein caused SCC defects. Western blot analyses revealed that total amounts of Prp19 and Cdc5L were reduced, respectively, in Cdc5L- and in Prp19-depleted cells (Fig 1D), possibly because one becomes unstable in the absence of the other. Consistent with the cohesion defects in mitosis, inactivation of Prp19 complex resulted in an arrest in prometaphase, with the chromosomes failing to align on the metaphase plate (Supplementary Fig S1E–G). Importantly, in
these cells enrichment of the centromeric region in the chromosome passenger protein Aurora B was maintained (Fig 1E), demonstrating that cohesion loss was not due to an untimely cell entry into anaphase. Thus, the depletion of either Cdc5L or Prp19 leads to the premature separation of sister chromatids implying that Prp19 complex is required for normal SCC in mitosis. We also noticed that Cdc5L- and Prp19-depleted cells displayed aberrant, collapsed mitotic spindles (Supplementary Fig S1E and F) that were more pronounced in Cdc5L- than in Prp19-depleted cells, presumably because of differences in depletion efficiency. The appearance of these abnormal spindles is unlikely to be an indirect consequence of defective SCC, as this was not observed in Scc1-depleted cells. Rather, it could indicate a distinct and specific role of Prp19 complex in mitotic spindle assembly, as reported recently [24].

Prp19 complex is required for accumulation of Sororin protein through splicing of Sororin pre-mRNA and for interphase cohesion

As Prp19 complex functions in pre-mRNA splicing, its inactivation may affect splicing of pre-mRNA(s) encoding protein(s) essential to SCC, and thus induce cohesion defects in an indirect manner. Consistent with this possibility, we observed that depletion of two splicing factors distinct from Prp19 complex, SF3A120 and U2AF65 [25,26], as well as chemical inhibition of splicing using spliceostatin A (SSA, [27]) also triggered defective mitotic cohesion (Fig 2A and B). These treatments also increased mitotic indices, except for SF3A120 depletion where only few cells were in mitosis possibly because of an additional role of SF3A120 (Fig 2C). This
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Figure 2. Depletion of the splicing factors SF3a120 and U2AF65 or chemical inhibition of the splicing machinery cause premature separation of sister chromatids in mitosis.

A B HeLa cells were transfected with control and SF3a120 (A) or U2AF65 (B) siRNAs, and chromosome spreads were prepared 24 and 48 h after transfection. Percentages of prometaphase cells displaying defective cohesion were determined (middle panels, n > 100 per condition). Depletion efficiency was analysed by immunoblotting with the indicated antibodies (left panels). Percentages of prometaphase cells in each condition are shown in the right panels.

C HeLa cells were treated with 5 nM of SSA or with methanol for 24 h. RT-PCR reactions were performed to detect intron retention in p80-coilin and β-actin pre-mRNAs and analysed on agarose gel (left panels). In parallel, cells were left untreated or treated for 24 h with methanol, 0.5 or 2 nM of SSA and processed as in (A). Percentages of prometaphase cells displaying defective cohesion are shown (middle panel, n > 100 per condition), and percentages of prometaphase cells are shown in the right panel.

indicates that SCC defects are common early consequence of splicing deficiency and suggests that Prp19 complex involvement in SCC is an indirect consequence of its function in splicing. If true, Prp19 complex inactivation should then affect the production of protein(s) required for normal cohesion. To test this possibility, we analysed cellular levels of known proteins involved in cohesion after Prp19 complex inactivation. HeLa cells treated with control, Cdc5L or Prp19 siRNAs were synchronised at the G1/S transition by double thymidine arrest. Before the second release and 4 h later, total protein extracts were prepared and analysed by Western blotting experiments (Fig 3A). In control cells, level of Sororin, which is essential to SCC [9,10], increased during the course of the experiment, consistent with its accumulation during S phase [11], and similar to the accumulation of its mRNA during this period (Supplementary Fig S2C). By contrast, Sororin accumulation was greatly reduced in cells where Cdc5L and Prp19 were depleted (Fig 3A and Supplementary Fig S2A), when level of other known interphase cohesion factors was unaffected (Supplementary Fig S2B). Similarly, reduced accumulation of Sororin was also observed upon SF3a120 or U2AF65 depletion and SSA treatment (Fig 3B). These results indicate that splicing inactivation leads to reduced accumulation of Sororin, possibly by perturbing the splicing of its pre-mRNA. Consistent with this possibility, increased retention of Sororin RNA introns 1 and 2, as well as to a lesser extent that of intron 5 of GUSB RNA used as a control, could be observed in Prp19- and Cdc5L-depleted cells when compared to control cells (Fig 3C). By contrast, no particular increased intron retention in Smc1, Smc3, Scc1, SA1, SA2, Esco1 and Esco2 could be observed (Supplementary Fig S3D), indicating that Sororin splicing was selectively affected by Prp19 complex inactivation. Similar observations were also made upon SF3a120 or U2AF65 depletion and SSA treatment (Fig 3D and Supplementary Fig S2F), although SSA had a stronger overall impact on RNA splicing. This indicates that Prp19 complex and spliceosome inactivation leads to accumulation of unspliced Sororin pre-mRNA and demonstrates that Prp19 complex is essential for the accumulation of Sororin protein as a consequence of its function in splicing. This also suggest that the described interaction between Prp19 complex and cohesin [23],

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known to act in gene expression, could reflect a common function of these complexes in transcription regulation, RNA maturation, or in the coupling between these two processes.

Sororin is a protein conserved from fly to human, that is degraded as cells exit from mitosis [10] and accumulates during S phase [11]. If Sororin reduction accounts for cohesion defects...
observed upon Prp19 complex inactivation, then this inactivation should produce phenotypes similar to those of Sororin depletion. Consistent with this prediction, we observed that cohesion complexes were still associated with chromatin upon Cdc5L depletion (Supplementary Fig S3E) indicating that Prp19 complex, similar to Sororin [9], is dispensable for the association of cohesion with chromatin. Next, we assessed interphase SCC by DNA fluorescence in situ hybridisation (FISH) using a probe recognising a region that is trisomic in HeLa cells (Fig 3E)[9,28]. We addressed cohesion status in cells depleted of Cdc5L, Prp19 or Sccl by measuring distances between paired dots (Fig 3F). In control cells, the mean distance between paired FISH signals was 0.4 µm and increased to 0.67 µm in Sccl-depleted cells, and to 0.64 µm and 0.65 µm in Cdc5L- and Prp19-depleted cells, respectively. Thus, similar to Sororin, Prp19 complex is dispensable for cohesion loading onto chromatin and is required for interphase cohesion.

Correction of endogenous Sororin protein level counteracts SCC deficiency caused by Prp19 complex inactivation

These observations altogether strongly suggest that reduction in Sororin protein accounts for defective cohesion caused by Prp19 inactivation. This implies that correcting Sororin protein level should rescue the cohesion defects caused by Prp19 complex inactivation. To test this prediction directly, we generated an intron-free Sororin coding sequence, expression of which in cells does not depend on splicing. We used this sequence to establish a cell line expressing Sororin in fusion with GFP (Sororin-GFP), which is expressed at a level close to that of endogenous Sororin, and is resistant to siRNAs (Fig 4A). Both cell lines were treated with control, Sororin, Sccl, Cdc5L and Prp19 siRNAs, and mitotic chromosome spreads were prepared and analysed at different times after transfection. As shown in Fig 4B, the proportion of wild-type cells exhibiting defective cohesion upon Sororin depletion reached 84% 48 h after siRNA transfection. In Sororin-GFP cells, this number was reduced to 44%, indicating that Sororin-GFP compensates partially for endogenous Sororin depletion. This incomplete rescue might be due to uneven expression levels between cells or to Sororin not being fully functional when tagged with GFP. By contrast, the expression of Sororin-GFP had no effect on defective cohesion observed after Sccl depletion (Fig 4B, upper panel) indicating that Sororin-GFP only rescues cohesion defects caused by reduced Sororin level. Finally, proportions of cells exhibiting defective cohesion upon Cdc5L and Prp19 depletion reached 84% and 68%, respectively, in wild-type cells, and decreased to 51% and 46% in Sororin-GFP cells (Fig 4B, lower panel). These results indicate that expression of Sororin-GFP is sufficient to rescue Cdc5L and Prp19 depletions, at least partly. This strongly supports the possibility that defective cohesion caused by inactivation of Prp19 complex directly originated from reduced level of Sororin protein, although we cannot rule out that reduced level of another, as yet unidentified, cohesion factor could also participate in this phenotype. We also observed that, even though ectopic expression of Sororin was able to correct defective SCC caused by Prp19 complex inactivation, most mitotic cells in which cohesion had been restored still displayed abnormal spindles (unpublished observation, EW). Consequently, number of mitotic cells was similarly high in both cell lines upon Prp19 complex inactivation (Fig 4C). This indicates that Sororin reduction, and therefore defective cohesion, is not the sole consequence of Prp19 complex inactivation and suggests that Prp19 complex, or spliceosome activity, also impacts on proper assembly of mitotic spindle, in agreement with a previous report [24]. This observation also illustrates pleotropic involvements of spliceosome in cell progression though mitosis.

Finally, we took advantage of the anti-cohesive property of the protein Wapl. RNAi-mediated depletion of Wapl is able to rescue defective cohesion caused by Sororin depletion [11]. Thus, if defective cohesion observed upon inactivation of Prp19 complex was caused by a reduction in Sororin level, depleting Wapl at the same time should then restore normal SCC. To test this prediction, cells treated with control, Sororin, Cdc5L and Prp19 siRNAs were eventually depleted of Wapl by RNAi (Fig 4D), and SCC was assessed by chromosome spreading. As shown before [7], Wapl depletion prevented opening of chromosome arms in more than 80% of prometaphase cells. Furthermore, the proportion of prometaphase cells exhibiting uncohered sister chromatid upon Sororin depletion was reduced from 90% to 12% by the co-depletion of Wapl, as previously reported [11]. Finally, Wapl depletion was also able to rescue cohesion defects caused by Cdc5L and Prp19 depletions, further supporting the notion that cohesion defects originated from reduced level of Sororin. Altogether these results indicate that reduced expression of Sororin consequent to defective splicing of its pre-mRNA accounts for defective cohesion upon Prp19 complex inactivation. Why would Sororin pre-mRNA be so sensitive to splicing inactivation? In contrast to other known interphase cohesion factors, present at similar cellular level throughout the cell cycle, Sororin is degraded at every cell exit from mitosis. Hence, robust SCC relies on...
de novo production of Sororin proteins during S phase and would thus be particularly sensitive to defective processing of Sororin pre-mRNA, as compared to that of pre-mRNAs encoding other cohesion factors. In addition, we showed that splicing of Sororin was more sensitive to spliceosome inactivation than any other well established interphase cohesion factors, although the molecular basis for this difference remains to be determined.

Interestingly, genome-wide RNAi studies have shown that depletion of 30 distinct splicing factors, including factors studied here, resulted in perturbed mitosis [22,29], consistent with defective SCC. Mark Petronczki and his collaborators made the striking discovery that depletion of any of these 30 splicing factors resulted in defective cohesion in mitosis (personal communication), identical to results we reported here, indicating that inactivation of various splicing
factors causes remarkably similar SCC defects and strongly suggesting that defective cohesion, possibly due to reduced level of Sororin, is a common feature of altered splicing machinery. Mutations in SCC apparatus genes are associated with cancers, including myeloid neoplasms [30], bladder cancer [31], glioblastoma, melanoma and Ewing’s sarcoma [32] and trigger aneuploidy and chromosome instability in glioblastoma cells [32]. Recent work has suggested that acquisition of mutations in SCC components is an important step in oncogenic process [33]. Similarly, mutations in genes encoding splicing factors are also found in cancers such as myeloid neoplastic disorders [34] and chronic lymphocytic leukaemia [35]. Based on our findings that perturbation of the splicing machinery alters cohesion and SCC regulation, it is possible that deleterious mutations in splicing genes participate in oncogenesis by promoting genome instability though defective cohesion functions, at least in some cancer types. Further molecular investigations in different cancers will be essential to determine whether and how splicing machinery deficiencies participate in tumourisation through defective cohesion.

Materials and Methods

RNA extraction, reverse transcription, PCR and quantitative PCR analyses

Total RNAs were extracted from HeLa cells using the RNeasy kit (Qiagen). 1 μg of total RNAs served as matrix in reverse transcription reaction using Superscript II reverse transcriptase (Life Technologies). PCR reactions were performed using primer pairs that specifically span from exon 2 to exon 3 of p80-coilin pre-mRNA or amplify the junction between exon 3 (e3) and intron 3 (i3) of β-actin pre-mRNA (sequences were described in [26,27], respectively). PCR products were analysed by electrophoresis on 1% agarose gel. Quantitative real-time PCR analyses were performed using PowerSybr mix on a 7700 QPCR ABI following the manufacturer’s instructions. For intron retention, the relative abundance of pre-mRNA over total RNA (pre-mRNA/mRNA) was calculated as follows: DCT = (Cpre-mRNA – CTotal RNA). The relative abundance of total RNAs was calculated as: DCT = (Cexon – CtotalGUS). All measures were done in triplicates. Intron-spanning primer pairs were used for mature mRNAs, primers amplifying exon–intron or intron–exon junctions were used for pre-mRNAs, and pairs within one exon were used for total RNAs (Supplementary Table S1).

Cell culture, synchronisation and RNAi

HeLa cells were grown in DMEM (Invitrogen) supplemented with 10% foetal bovine serum (PAA), 0.2 mM L-glutamine and antibiotics (Invitrogen). Single mCdc5L-LAP cell clones were isolated from mCdc5L-LAP cell pool, selected in medium containing 800 μg/ml genetin (PAA) and maintained in medium supplemented with 400 μg/ml genetin. Sororin-GFP cell lines were obtained by transfection (Transfection). The sequences of the control, Scc1, Wapl and Sororin siRNAs used here have been described elsewhere [7,9,28]. The sequences of Cdc5L, Prp19 and SF3a120 have also been described in previous studies [20,26]. Sequence of U2AF65 siRNA used in this study was 5’-AAGGCGCGAAGAUU-ACUUGGAACdTdT-3’. Cells were used at the indicated times after transfection. The small inhibitor spliceostatin A was kindly provided by Minoru Yoshida [27].

Immunofluorescence and chromosome spreads

Immunofluorescence staining was performed as previously described [36]. Immunofluorescence analyses were performed using a fluorescent microscope (DMRXA2 Leica) with a 40× oil immersion objective lens, and images were processed using MetaMorph software (Molecular Devices). For chromosome spread, cells were treated for 30 min with 100 ng/ml of nocodazole. Cells were collected and resuspended in 1 ml of medium, 1.5 ml of tap water was added. Six minutes later, 7 ml of Carnoy fixative (3:1, methanol: glacial acetic acid). Cells were then spread on glass slide, dried, stained with Giemsa stain and mounted in Entellan. Mitotic chromosome spreads observations were made using a light microscope (DM2000 Leica) with a 40× dry objective.

Supplementary information for this article is available online: http://embor.embopress.org

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Author contributions

MD, TW and ZH performed experiments and discussed results, CP discussed results, EW conceived the project, designed and performed the experiments, analysed the data and wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References


