SNW1 enables sister chromatid cohesion by mediating the splicing of sororin and APC2 pre-mRNAs

Petra van der Lelij¹, Roman R Stocsits¹, Rene Ladurner¹, Georg Petzold¹, Emanuel Kreidl¹, Birgit Koch^{1,2}, Julia Schmitz^{1,†}, Beate Neumann², Jan Ellenberg² & Jan-Michael Peters^{1,*}

Abstract

Although splicing is essential for the expression of most eukaryotic genes, inactivation of splicing factors causes specific defects in mitosis. The molecular cause of this defect is unknown. Here, we show that the spliceosome subunits SNW1 and PRPF8 are essential for sister chromatid cohesion in human cells. A transcriptome-wide analysis revealed that SNW1 or PRPF8 depletion affects the splicing of specific introns in a subset of pre-mRNAs, including pre-mRNAs encoding the cohesion protein sororin and the APC/C subunit APC2. SNW1 depletion causes cohesion defects predominantly by reducing sororin levels, which causes destabilisation of cohesin on DNA. SNW1 depletion also reduces APC/C activity and contributes to cohesion defects indirectly by delaying mitosis and causing “cohesion fatigue”. Simultaneous expression of sororin and APC2 from intronless cDNAs restores cohesion in SNW1-depleted cells. These results indicate that the spliceosome is required for mitosis because it enables expression of genes essential for cohesion. Our transcriptome-wide identification of retained introns in SNW1- and PRPF8-depleted cells may help to understand the aetiology of diseases associated with splicing defects, such as retinosa pigmentosum and cancer.

Keywords cell cycle; mitosis; pre-mRNA splicing; sister chromatid cohesion

Subject Categories DNA Replication, Repair & Recombination; RNA Biology

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Introduction

Cell proliferation depends on the propagation of complete copies of the genome from one cell generation to the next. Eukaryotic cells

achieve this by first replicating all chromosomes, then biorienting them on the mitotic spindle and subsequently segregating chromosomes symmetrically into the forming daughter cells. The chromosome segregation process depends on physical connections between replicated DNA molecules because this sister chromatid cohesion resists the pulling forces of spindle microtubules and thereby enables the biorientation of chromosomes (Tanaka *et al*, 2000).

Cohesion is mediated by multi-subunit cohesin complexes which form large ring-like structures (reviewed in Remeseiro & Losada, 2013). These are thought to mediate cohesion by topologically embracing the DNA strands of sister chromatids (Haering *et al*, 2008). Cohesin is loaded onto DNA by the NIPBL-MAU2 loading complex (Ciosk *et al*, 2000; Murayama & Uhlmann, 2014) and establishes cohesion during DNA replication (Uhlmann & Nasmyth, 1998). Cohesion establishment depends on acetylation of SMC3 by the acetyltransferases ESCO1 and ESCO2 and subsequent recruitment of the protein sororin to cohesin (Lafont *et al*, 2010; Nishiyama *et al*, 2010). Sororin, encoded by a gene called *CDCA5* (Walker, 2001), is essential for cohesion (Rankin *et al*, 2005) because it antagonises Wapl, a protein that is able to release cohesin from DNA (Nishiyama *et al*, 2010), presumably by opening the cohesin ring (Chan *et al*, 2012; Buheitel & Stemmann, 2013; Eichinger *et al*, 2013). By inhibiting Wapl, sororin stabilises cohesin on DNA and thereby enables it to mediate cohesion from DNA replication until chromosomes are bioriented on the mitotic spindle (Schmitz *et al*, 2007).

The subsequent separation of sister chromatids in anaphase depends on removal of cohesin from chromosomes (reviewed in Peters *et al*, 2008). This process is initiated in prophase by mitotic kinases which phosphorylate both cohesin and sororin, thereby dissociating sororin from Wapl and enabling Wapl to release cohesin from chromosome arms (Hauf *et al*, 2005; Dreier *et al*, 2011; Zhang *et al*, 2011; Liu *et al*, 2013; Nishiyama *et al*, 2013). At centromeres, these phosphorylation reactions are antagonised by the protein phosphatase 2A (PP2A)-SGOL1 complex, which protects centromeric cohesin until all chromosomes have become bioriented. Once chromosome biorientation has been completed, that is in metaphase,

¹ IMP Research Institute of Molecular Pathology, Vienna, Austria

² EMBL Heidelberg, Heidelberg, Germany

*Corresponding author: Tel: +43 1797303002; E-mail: Jan-Michael.Peters@imp.ac.at

[†]Present address: Federal Joint Committee, Berlin, Germany

the ubiquitin ligase anaphase promoting complex/cyclosome (APC/C) becomes active and ubiquitylates B-type cyclins and the protein securin (reviewed in Peters, 2006). The subsequent degradation of these proteins by the 26S proteasome activates the protease separase. Separase then releases cohesin from centromeres by cleaving the SCC1 subunit and thereby initiates chromosome segregation.

Although it is well established that cohesin, the cohesin loading complex, the acetyltransferases ESCO1 and ESCO2, sororin and the PP2A-SGOL1 complex are essential for cohesion, it is not known if all proteins required for cohesion have been identified. We therefore searched for additional proteins that might be needed for cohesion. Because cohesion is required for chromosome segregation, we used for this purpose data from a genome-wide screen performed by the MitoCheck project which had identified genes required for mitosis in human cells (Neumann *et al*, 2010). By screening a subset of these genes, we identified SNW containing protein 1 (SNW1) as a protein essential for cohesion. SNW1 is known to be a component of the spliceosome which removes intronic sequences from pre-mRNAs before the mature mRNAs are exported from the nucleus. Splicing factors have previously been shown to be required for mitosis (Burns *et al*, 2002; Kittler *et al*, 2004; Carnahan *et al*, 2005; Pacheco *et al*, 2006; Xiao *et al*, 2007; Neumann *et al*, 2010; Song *et al*, 2010; Hofmann *et al*, 2013) (reviewed in Hofmann *et al*, 2010), and several reports have implicated a specific role for splicing factors in cell cycle progression (Huen *et al*, 2010; Ahn *et al*, 2011; Sharma *et al*, 2011). It has been suggested that some of the mitotic functions of splicing factors could be attributed to microtubule polymerisation and spindle assembly, but it is poorly understood how splicing factors contribute to spindle assembly and if these are the only roles of splicing factors in chromosome segregation (Biggins *et al*, 2001; Burns *et al*, 2002; Hofmann *et al*, 2013).

Here, we provide evidence that SNW1 is essential for sister chromatid cohesion because it is required for splicing of pre-mRNAs that encode sororin and the APC/C subunit APC2 (also called ANAPC2). Our data indicate that the main cause of cohesion defects in SNW1-depleted cells is a rapid reduction in sororin levels, which prevents the stabilisation of sufficient numbers of cohesin complexes on DNA during S and G2-phase. However, our results also reveal that SNW1 depletion leads to a reduction in APC/C activity. Unexpectedly, our data indicate that this reduction also contributes to cohesion defects, perhaps because reduced APC/C activity causes a mitotic delay which leads to the recently described phenomenon of “cohesion fatigue” (Daum *et al*, 2011; Stevens *et al*, 2011; Lara-Gonzalez & Taylor, 2012). Our study provides for the first time a molecular explanation for the essential role of splicing factors in mitosis.

Results

SNW1 is required for sister chromatid cohesion and mitotic progression

To search for proteins that are required for sister chromatid cohesion in human cells, we used data which had been obtained in an RNA interference (RNAi) screen performed by the MitoCheck consortium (Neumann *et al*, 2010; for a schematic outline of the screening procedures used in this study, see Supplementary Fig S1). In this screen, 22,612 human genes had been targeted by two to

three siRNAs each, and HeLa cells transfected with these siRNAs and expressing histone H2B tagged with green fluorescent protein (GFP) had been analysed by time-lapse microscopy. These experiments had identified 1,249 genes that might potentially be required for mitosis, of which 572 were validated by a number of criteria (Neumann *et al*, 2010). For the majority of these genes, the phenotypes caused by their inactivation had been annotated by a support vector machine (SVM) classifier (Walter *et al*, 2010), but initially, 207 of these genes were identified by manual annotation of a subset of the time-lapse microscopy data. Neither SVM classification nor manual annotation allowed the identification of cohesion defects due to the limited temporal and optical resolution of the primary data (recorded with a time lapse of 30 min using a Plan 10×, NA 0.4 objective). We therefore analysed selected candidate genes identified in the primary MitoCheck screen for their potential role in sister chromatid cohesion. For this purpose, we used the list of 207 manually annotated phenotypes because it was available before the list generated by the SVM classifier. The former list contained the known cohesion genes *SMC3*, *RAD21* (*SCC1*), *SGOL1* and *CDCA5*. The inactivation of these genes had caused chromosome alignment and segregation defects, mitotic delay and abnormal nuclear shapes (Neumann *et al*, 2010; www.mitocheck.org). We selected 18 other genes which, if inactivated by RNAi, were associated with similar phenotypes (Supplementary Table S1) and analysed if these are required for cohesion. Microscopic analyses of mitotic chromosome spreads revealed that inactivation of one of these genes, SNW1 (encoding SNW containing protein 1), by two distinct siRNAs resulted in complete loss of sister chromatid cohesion, that is the presence of single sister chromatids, in more than 50% of all mitotic cells, comparable to the phenotype obtained after inactivation of *SCC1* (Supplementary Table S1).

SNW1 was previously identified in yeast two-hybrid screens as a nuclear coactivator-62 kDa (NCoA62) protein interacting with vitamin D receptor (Baudino *et al*, 1998), as Ski interacting protein (Skip) interacting with v-Ski avian retroviral oncogene (Dahl *et al*, 1998), as a regulator of bone morphogenetic protein (BMP) during vertebrate embryogenesis (Wu *et al*, 2011) and as a protein required for proper telomere function (Lackner *et al*, 2011). It has been shown to interact with the U5 small nuclear ribonucleoprotein (snRNP) subcomplex of the activated spliceosome as part of the PRPF19/nineteen complex (NTC) (Neubauer *et al*, 1998; Makarov *et al*, 2004). SNW1 is also known as Prp45 and is orthologous to the splicing factor Bx42 in *Drosophila melanogaster* (Folk *et al*, 1996; Dahl *et al*, 1998). SNW1 inactivation has been reported to cause mitotic spindle and cytokinesis defects (Kittler *et al*, 2004), and the specificity of this phenotype has been confirmed by expression of RNAi resistant mouse *Snw1* (mSnw1) in human cells depleted of endogenous SNW1 (Kittler *et al*, 2005).

To test if also the cohesion defects observed in our experiments are caused by inactivation of SNW1, as opposed to “off-target” effects that the SNW1 siRNAs might have, we stably transfected mouse *Snw1* fused to a localisation and affinity purification (LAP) tag on a bacterial artificial chromosome (BAC) in HeLa cells. The expression of mSnw1-LAP prevented the cohesion defects otherwise observed after SNW1 RNAi, confirming that the cohesion defects were caused by inactivation of SNW1 (Fig 1A–C). To analyse how soon after SNW1 depletion defects in mitosis and cohesion occur, we transfected asynchronously growing HeLa cells with SNW1

siRNA and analysed at different time points their DNA content by fluorescence activated cell sorting (FACS; Fig 1D), SNW1 levels by immunoblotting (Fig 1E) and cohesion defects by chromosome spread staining (Fig 1F). We also determined the mitotic index (Fig 1G) by measuring the presence of condensed chromosomes stained with 4',6-diamidino-2-phenylindole (DAPI), the presence of histone H3 phosphorylated on serine 10 (H3S10P) and the accumulation of cyclin B1 in either the nucleus of prophase cells, or the cytoplasm of cells in prometaphase and metaphase (Fig 1H). Additionally, the steady-state levels of the latter two proteins were analysed by immunoblotting (Fig 1E). Chromosomes with parallel sister chromatids, indicative of defects in centromeric cohesion, were already observed 24 h after siRNA transfection when SNW1 had only been partially depleted and only a small increase in mitotic index could be observed. Complete loss of cohesion in the majority of cells was seen between 40 and 48 h when SNW1 levels became undetectable, and at these time points, between 20 and 30% of cells were in mitosis. These results indicate that SNW1 depletion rapidly leads to cohesion defects, possibly within one cell cycle, which takes about 24 h in HeLa cells under our culture conditions, and that SNW1 is required for progression through mitosis, at least in part because it is required for cohesion.

Depletion of SNW1 reduces the amount of stably chromatin-bound cohesin in G2 phase

To obtain insight into how SNW1 might contribute to cohesion, we analysed if SNW1 is required for the synthesis and assembly of cohesin complexes, for the loading of these complexes onto DNA, or for cohesin acetylation during S-phase and subsequent stabilisation of cohesin on DNA. Immunoblotting experiments did not reveal a detectable reduction in the levels of cohesin core subunits following SNW1 depletion (Supplementary Fig S2). Also, immunofluorescence microscopy analysis of cells from which soluble cohesin had been removed by pre-extraction showed that similar amounts of cohesin were bound to chromatin in the presence or absence of SNW1 (Fig 2A–C). The assay used in the latter experiment would have

Source data are available online for this figure.

been sensitive enough to detect a defect in cohesin loading onto DNA because we compared SNW1 proficient and SNW1-depleted cells directly side by side on the same cover slips, ruling out artefacts caused by inter-specimen variation [Fig 2B; for experimental details see Materials and Methods and (Kueng *et al*, 2006)], and because partial depletion of the SCC4 subunit of the cohesin loading complex did detectably reduce the levels of cohesin on chromatin in this assay (Fig 2B and C, *** indicates $P < 0.001$). Likewise, SNW1 depletion did not detectably reduce the levels of acetylated SMC3 as measured by immunoblotting (Fig 2D and E). Also in this case, our experiments could have revealed such a defect had it existed, as RNAi-mediated depletion of ESCO1 and ESCO2 greatly reduced the levels of acetylated SMC3 in our experiments (Fig 2D).

Cohesin acetylation during S-phase normally correlates with an increase in cohesin's chromatin residence time, which can be measured in inverse fluorescence recovery after photobleaching (iFRAP) experiments (Gerlich *et al*, 2006; Schmitz *et al*, 2007). To determine if SNW1 is required for the stabilisation of cohesin on chromatin during S-phase, we synchronised HeLa cells stably expressing a GFP-tagged version of Smc3 (mouse Smc3-LAP) by thymidine arrest release in S-phase, transfected these cells after a release from a first thymidine arrest with SNW1 or control siRNAs and analysed the cells 6–8 h after release from a second thymidine arrest by iFRAP (Fig 2F). DNA content analysis by FACS confirmed that most cells had been synchronised in G2-phase (Fig 2G), and immunoblot and chromosome spread analyses showed that SNW1 was partially depleted under these conditions (Fig 2H) to a degree that resulted in partial cohesion defects (Fig 2I).

The iFRAP kinetics could be fitted with a bi-exponential function (Fig 2J), confirming that in G2-phase two populations of cohesin exist on chromatin (Gerlich *et al*, 2006). Analysis of the iFRAP data indicated that in control cells, $64 \pm 2\%$ of cohesin complexes had a chromatin residence time of 16 min, while the remaining 36% bound chromatin much more stably with a residence of ~ 10 h (Fig 2K). SNW1-depleted cells also contained dynamically and stably bound cohesin complexes (Fig 2J), but the fraction of stably bound complexes was significantly reduced to 22% ($P < 0.01$; Fig 2K). A similar defect in cohesin stabilisation is known to be caused by sororin depletion (Schmitz *et al*, 2007), which under our

experimental conditions reduced the fraction of stably chromatin-bound cohesin to 16% (Fig 2K). We therefore analysed if SNW1 depletion affects the steady-state levels of sororin. Indeed, immunoblotting experiments revealed that sororin levels were reduced following transfection with SNW1 siRNAs (Supplementary Fig S2). Because sororin is required for cohesion in G2-phase (Schmitz *et al*, 2007), we analysed the proximity between sister chromatids by fluorescence *in situ* hybridisation (FISH) in cells synchronised in G2-phase. This revealed that in SNW1-depleted cells, chromosome arms were further separated than in control cells, although not as far as in sororin-depleted cells (Fig 2L and M). These results indicate that SNW1 is required for maintaining normal levels of sororin, proper stabilisation of cohesin on DNA and sister chromatid cohesion in post replicative cells.

Transcriptome-wide identification of introns whose splicing depends on SNW1

SNW1 could affect sororin levels by contributing to the splicing of the sororin pre-mRNA, or SNW1 could have a more direct role in cohesion. Consistent with the latter possibility, SNW1 has been reported to be located in the nucleus (Zhang *et al*, 2003) and to be recruited to specific sites on DNA (Chen *et al*, 2011) and has been detected together with the cohesin subunit SMC1 in the BdU1 spliceosome (Makarova *et al*, 2004). However, in mSnw1-LAP samples purified from HeLa cells, we could not detect cohesin by mass spectrometry, even though we had solubilised chromatin-bound proteins by nuclease digestion in this experiment and could detect numerous known spliceosomal interaction partners of SNW1 in the purified sample (Supplementary Table S2, www.mitocheck.org; Hutchins *et al*, 2010).

We therefore addressed if SNW1 contributes to sororin homeostasis by splicing the sororin pre-mRNA and analysed how many different pre-mRNAs are spliced in an SNW1-dependent manner. For this purpose, we transfected HeLa cells with SNW1 or control siRNAs, isolated polyA-containing RNAs 48 h later and analysed these by Solexa sequencing ("RNA-seq"). To exclude changes that could be caused by the siRNA transfection procedure *per se* or by "off-target" effects, we also analysed RNA from SNW1-depleted

Figure 2. Depletion of SNW1 reduces the amount of stably chromatin-bound cohesin in G2 phase.

- A The loading of cohesin was examined in a cell mixing experiment, in which HeLa cells with CenPA-GFP expression were transfected with control siRNA, while HeLa cells without CenPA-GFP were depleted for SCC4 or SNW1. Depletion after 48 h was analysed by Western blotting.
- B Twenty-four hours prior to harvesting, CenPA-GFP control-treated cells were mixed with SNW1- or SCC4-depleted HeLa cells in a 1:1 ratio to allow the quantification of cohesin levels by immunofluorescence on the same slide. Cells were classified using GFP intensity, that is control-treated (green encircled cells) versus SNW1-/SCC4-depleted cells (blue encircled cells + arrowhead), and cohesin (SCC1) fluorescence intensities were measured within both classes. Scale bar: 10 μ m.
- C Quantification of cohesin intensities of SNW1- or SCC4-depleted cells normalised to control-treated neighbouring GFP-positive cells ($n > 180$; error bars denote s.e.m., *** $P < 0.001$).
- D, E HeLa cells were transfected for 48 h with control, ESCO1 and ESCO2, or SNW1 siRNA, and chromatin fraction was analysed for the acetylation levels of SMC3 by Western blotting with an SMC3^{ac} specific antibody (D) and cell cycle profile was determined by FACS (E).
- F–I HeLa cells stably expressing mSmc3-LAP were synchronised by double thymidine arrest release and transfected with control, sororin or SNW1 siRNA at the first release. Synchronisation of cells was determined by FACS (G). Cells were collected 8 h after the second release for Western blot to assess knockdown efficiency (H) and for chromosome spreads ($n \geq 110$, error bars denote s.e.m.) (I).
- J, K Cells from the same experiment as (F–I) were photobleached 6 h after release, and fluorescence recovery was measured (J) to calculate the fraction of cohesin stably bound to chromatin ($n \geq 15$; error bars denote s.e.m.) (K).
- L, M Cells synchronised in G2 phase were transfected with control, sororin or SNW1 siRNA and analysed for inter-sister distances by FISH (L) using fluorescently labelled probes to loci on chromosomes 13 and 21. Scale bar: 5 μ m. Quantification of inter-sister FISH distances is shown in (M) ($n \geq 92$; error bars denote s.e.m., *** $P < 0.001$).

Source data are available online for this figure.

HeLa cells stably expressing the mSnw1-LAP construct that “rescues” the mitotic and cohesion phenotypes otherwise caused by SNW1 siRNAs (Fig 1A–C; Kittler *et al*, 2005). Manual inspection of the RNA-seq data aligned to the Integrated Genome Browser

(<http://bioviz.org/igb/>) revealed that SNW1-depleted cells more frequently contained intronic sequences than “rescued” cells expressing mSnw1-LAP or cells transfected with GL2 control siRNA. For example, SNW1 depletion led to an increase in intronic

Figure 2.

sequences derived from CDKN1A mRNA (Supplementary Fig S3A and B), which encodes the cyclin-dependent kinase inhibitor p21 and which has been reported to be spliced in an SNW1-dependent manner (Chen *et al.*, 2011). However, the splicing of other pre-mRNAs of cohesion genes, which are expressed in HeLa cells (ESCO1, ESCO2, PDS5A, PDS5B, RAD21, SGOL1, SGOL2, SMC1A, SMC3, STAG1, STAG2, WAPAL), was not detectably affected by SNW1 depletion (for examples, see Supplementary Fig S3C–F).

To identify SNW1 splice targets systematically, we developed an algorithm that allowed us to calculate how SNW1 depletion changes the abundance of intronic sequences in the human transcriptome (see Materials and Methods). For this, we compared the number of RNA-seq reads in SNW1-depleted cells with the corresponding number in SNW1-depleted cells that had been “rescued” by expression of mSnw1. This analysis revealed that out of 199,901 introns present in 20,250 genes, 24,583 introns (12.3%) were increased at least 2.83-fold after SNW1 depletion (Fig 3; $\log_2(\text{fc}) \geq 1.5$), indicating that their removal by splicing events normally depends on SNW1. These “upregulated” introns were present in 8,872 genes. These results imply that some introns are much more affected by our SNW1 depletion conditions than others, consistent with the previous report that SNW1 is required for splicing of CDKN1A pre-mRNA, but not for splicing of another pre-mRNA derived from the PUMA locus (Chen *et al.*, 2011). Similarly, we observed that among the upregulated introns, some were affected more strongly than others. For example, of the 24,583 introns that were increased at least 2.83-fold, 15,225 were upregulated fourfold or more (Fig 3A, $\log_2(\text{fc}) \geq 2$), and 3,618 were upregulated at least eightfold ($\log_2(\text{fc}) \geq 3$). A list of 1,200 upregulated introns, selected by stringent “filtering” criteria (see Material and Methods), is shown in Supplementary Table S3. We also identified intronic sequences which were reduced in abundance after SNW1 depletion (Fig 3A). Most of the latter sequences were present in transcripts which were expressed at low levels and whose abundance decreased further following SNW1 depletion. It is therefore possible that the apparent decrease in the abundance of these introns was caused by indirect effects which reduced the expression levels of the transcripts containing these introns.

The 1,200 SNW1-regulated introns on this “high confidence” list are present in 784 genes. To obtain insight into how SNW1 controls sister chromatid cohesion and mitotic progression, we compared this list of SNW1-regulated genes with the list of 572 validated “mitotic hits” from the genome-wide MitoCheck RNAi screen (Neumann *et al.*, 2010). Of the 572 mitotic genes, 36 contained at least one intron which was increased in abundance after SNW1 depletion (Supplementary Table S4), indicating that SNW1 depletion could affect mitosis by deregulating these genes. Among these, SNW1-regulated mitotic genes are nine with well-established mitotic functions (*ANAPC2*, *AURKB*, *BUB1B*, *CDC20*, *CDCA5*, *INCENP*, *KIF4A*, *PLK1*, *TACC3*) and two required for DNA replication (*CDT1*, *POLG*). Five of the proteins encoded by these genes (*AURKB*, *BUB1B*, *CDC20*, *INCENP* and *PLK1*) were not detectably reduced in their abundance 48 h after SNW1 depletion, as judged by immunoblotting (Supplementary Fig S2), whereas for three others (*KIF4A*, *POLG*, and *TACC3*), we were unable to obtain suitable antibodies. However, in the case of *CDT1*, *ANAPC2* and—as already described above—*CDCA5*, we observed that the proteins encoded by these genes (*CDT1*, *APC2* and sororin, respectively) were reduced after SNW1

depletion (Supplementary Fig S2). Because sororin and APC2 are known to have direct roles in chromosome cohesion and segregation, whereas *CDT1* depletion might affect these processes indirectly, we focused on sororin and APC2 in our subsequent experiments.

The depletion of these two proteins was specifically caused by SNW1 depletion, as opposed to “off-target” effects, because expression of mSnw1-LAP restored sororin and APC2 levels in cells depleted of endogenous SNW1 (Fig 3B). Analysis of the RNA-seq data revealed that SNW1 depletion increased predominantly the levels of the first intron in the sororin and APC2 mRNA (Fig 3C and D, sororin: $\log_2(\text{fc}) = 2.71$ and coverage = 0.641, APC2: $\log_2(\text{fc}) = 4.32$ and coverage = 0.128). This effect was reproducible and was specifically caused by SNW1 depletion as a control siRNA (GL2) or depletion of endogenous SNW1 in cells expressing the RNAi resistant mSnw1-LAP did not cause increased levels of these introns (RNA-seq data obtained in different experiments for the *CDCA5* locus are shown in Supplementary Fig S4) and could be confirmed by quantitative polymerase chain reactions (qPCRs; Fig 3E–G).

Because SNW1 depletion caused detectable splicing defects only in a subset of cellular transcripts, we tested if the stability of these transcripts inversely correlated with their sensitivity to SNW1 depletion. For this purpose, we treated HeLa cells with the transcription inhibitor actinomycin D and subsequently analysed the levels of selected mRNA at different time points by qPCR (Supplementary Fig S5). Over a time course of nine hours, we observed a decline in sororin mRNA levels with a half-life of approximately 2.5 h. In contrast, all other analysed transcripts, including the APC2 mRNA, were more stable. These results indicate that the sororin mRNA is short-lived and may therefore have to be re-synthesised at a high rate in every cell cycle. This could explain why retained introns can be detected in sororin transcripts rapidly after SNW1 depletion. However, our finding that the APC2 mRNA is not particularly short-lived implies that SNW1 depletion can also affect the splicing of more long-lived transcripts.

SNW1 is required for sister chromatid cohesion by splicing sororin and APC2 pre-mRNAs

To test if the cohesion defects in SNW1-depleted cells are caused by defective sororin splicing, we stably expressed a GFP-flag-tagged version of sororin from a cDNA, which is complementary to the mature sororin mRNA and therefore does not encode intronic sequences (Fig 4A). In the majority of HeLa cells expressing sororin from this construct, SNW1 depletion only caused cohesion defects at centromeres, resulting in “parallel” sister chromatids, but not a complete loss of cohesion (Fig 4B and C). Sororin can therefore partially restore the cohesion defects of SNW1-depleted cells. However, the ectopically expressed version of sororin was expressed at higher levels than endogenous sororin normally is (Fig 4A), raising the possibility that overexpressed sororin might have restored the cohesion defects in SNW1-depleted cells indirectly, for example by stabilising more cohesin complexes on chromatin than normally. To address this possibility, we depleted both SNW1 and Wapl from cells lacking the sororin cDNA. Sororin is an inhibitor of Wapl, and sororin is only essential for cohesion in the presence of Wapl (Nishiyama *et al.*, 2010). If SNW1 depletion caused cohesion defects by lowering sororin levels, Wapl depletion might therefore restore cohesion defects in SNW1 deficient cells. Figure 4 shows that this was indeed

Figure 3. SNW1 is involved in the splicing of a subset of introns, including those of sororin and APC2 mRNA.

A Transcriptome-wide sequencing was performed on mRNA isolated from HeLa cells with or without stable BAC expression of mouse Snw1-LAP after depletion of SNW1. An algorithm was developed to specifically identify and annotate introns and to analyse changes in intronic expression levels. Scatter plot showing changes in intronic aligned reads between SNW1 depleted and Snw1-LAP rescued cells plotted against coverage (reads/nucleotide). Significantly mis-spliced introns of sororin and APC2 are highlighted in blue.

B Sororin and APC2 expression were analysed by Western blotting of whole-cell extracts.

C, D Alignment of reads to the Integrated Genome Browser (IGB) confirmed the presence of intronic sequences after SNW1 depletion in the sororin (*CDCA5*) (**C**) and in APC2 (*ANAPC2*) mRNA transcripts (**D**) as indicated by the shaded box.

E Schematic representation of the primers used to analyse splicing by RT-qPCR.

F, G Quantification of the ratio of spliced versus unspliced sororin and APC2 transcripts, respectively, measured by RT-qPCR ($n \geq 2$, error bars denote s.e.m.).

Source data are available online for this figure.

partially the case, supporting the notion that a reduction in sororin levels contributes to the cohesion defect in SNW1-depleted cells.

These results indicate that sororin splicing defects are a major cause of cohesion defects in SNW1-depleted cells. However, neither sororin expression nor Wapl depletion could fully restore the

cohesion defects and increased mitotic index in these cell populations (see Fig 5 below). This was not caused by functional defects of GFP-flag-tagged sororin because this protein could largely restore cohesion defects in sororin-depleted cells (see Fig 5). We therefore analysed if the mis-splicing of other pre-mRNAs contributes to cohesion

Figure 4. Sororin cDNA expression or co-depletion of Wapl partially rescue SNW1 RNAi.

A Wapl was depleted, or GFP-flag-sororin was stably expressed in HeLa cells, both in combination with 48 h of SNW1 depletion and protein levels were analysed in a Western blot.

B Representative images of Giemsa-stained chromosome spreads, including an overcohesion phenotype. Scale bar: 10 μ m.

C Quantification of sister chromatid cohesion phenotypes after Wapl RNAi or sororin overexpression ($n \geq 150$; error bars denote s.e.m.).

Source data are available online for this figure.

defects in SNW1-depleted cells. As a candidate, we tested APC2 because prolonged inhibition of the APC/C, of which APC2 is an essential subunit, has been reported to cause gradual loss of cohesion (Daum *et al*, 2011; Stevens *et al*, 2011). This phenomenon has been called “cohesion fatigue”. First, we analysed if SNW1 depletion reduced APC2 levels to a degree that would detectably reduce APC/C activity. For this purpose, we immunoprecipitated APC/C from SNW1-depleted cells and analysed its ability to ubiquitylate Hsl1, a model substrate, *in vitro* (Fig 5A). Immunoblotting revealed that APC/C from SNW1-depleted cells contained reduced amounts of APC2 and its binding partner APC11 and that its ability to assemble ubiquitin chains on Hsl1 was indeed reduced compared to the activity of APC/C from SNW1 proficient cells.

Cohesion fatigue is known to be reduced by agents which interfere with microtubule dynamics, such as nocodazole or taxol, presumably because microtubule dynamics are needed to generate spindle pulling forces which cause a gradual loss of cohesion (Daum *et al*, 2011; Stevens *et al*, 2011). To test if cohesion fatigue contributes to cohesion defects in SNW1-depleted cells, we therefore analysed if nocodazole treatment reduces the residual cohesion defects that we had observed in SNW1-depleted cells expressing sororin from a cDNA. For this purpose, we removed mitotic cells by shake-off 36 h after transfection with SNW1 siRNAs and allowed the remaining interphase cells to enter mitosis over a period of 12 h in either the absence or presence of nocodazole and analysed sister

chromatid cohesion by chromosome spread analysis. Whereas SNW1-depleted cells expressing sororin from a cDNA that had entered mitosis in the absence of nocodazole showed mostly parallel sister chromatids, cells that were incubated in the presence of nocodazole showed normal sister chromatid cohesion (Fig 5B and C). This is consistent with the hypothesis that cohesion fatigue causes the residual cohesion defects observed in SNW1-depleted cells expressing sororin from a cDNA.

To test if this cohesion fatigue is caused by a reduction in APC2 levels, we next generated cell lines stably expressing either APC2-RFP from a cDNA alone, or together with the GFP-flag-sororin construct described above (Fig 5D). Expression of APC2-RFP alone restored cohesion defects in SNW1-depleted cells only to a small degree. In SNW1-depleted cells, only $6.0 \pm 1.6\%$ of mitotic cells retained partial cohesion (parallel sister chromatids), whereas in APC2-RFP expressing cells, this number was reproducibly increased to $26.6 \pm 3.4\%$ (Fig 5E). Despite being small, this effect was specific for SNW1-depleted cells, as APC2-RFP could not restore any cohesion in sororin-depleted cells. Interestingly, however, co-expression of APC2-RFP greatly increased the ability of GFP-flag-sororin to restore cohesion in SNW1-depleted cells. In parallel, the mitotic index was reduced to normal levels by the co-expression of GFP-flag-sororin and APC2-RFP (Fig 5F). The observation that residual cohesion defects in SNW1-depleted cells expressing sororin from a cDNA can be restored either by nocodazole treatment or by expression

Figure 5. The remaining partial cohesion defects in sororin-expressing cells depleted for SNW1 are rescued by inactivation of microtubules and APC2 cDNA expression.

A APC/C complexes were purified from HeLa cells after 48 h of control or SNW1 RNAi, and their ubiquitylation activity was analysed *in vitro* using recombinant Hsl1 as a substrate.

B, C HeLa cells and HeLa cells stably expressing GFP-flag-sororin were depleted for SNW1. After shaking off mitotic cells, the remaining interphase cells were grown for 12 h in the presence or absence of nocodazole, after which cells were analysed by chromosome spread (**B**; $n \geq 170$; error bars denote s.e.m.) and Western blot analysis (**C**).

D–F HeLa cells and HeLa cells stably expressing GFP-flag-sororin and/or APC2-RFP cDNA were transfected with control, SNW1 or sororin siRNA for 48 h, and expression levels were analysed by Western blotting (**D**). Chromosome spreads were obtained, and cohesion phenotypes were analysed (**E**) ($n \geq 170$; error bars denote s.e.m.). The mitotic index was obtained by counting the number of H3S10P-positive cells in IFM (**F**) ($n \geq 1,000$; error bars denote s.e.m.).

Source data are available online for this figure.

of APC2 from a cDNA is consistent with the hypothesis that a reduction in APC/C activity contributes to cohesion defects by causing a mitotic delay and cohesion fatigue. These results therefore indicate that SNW1 is required for sister chromatid cohesion by mediating the splicing of both sororin and APC2 pre-mRNAs.

PRPF8 is also required for splicing of sororin and APC2 pre-mRNAs and for sister chromatid cohesion

To test if other components of the spliceosome are also required for splicing of the sororin and APC2 pre-mRNAs, we analysed the consequences of depletion of PRPF8, a known subunit of the U5 subcomplex (reviewed in Will & Luhrmann, 2011), which was also found to be required for mitosis (Neumann *et al*, 2010). Depletion of PRPF8 also caused cohesion defects, a reduction in sororin and APC2 levels, and an increase in sororin and APC2 introns as detected by RNA-seq and qPCR, although to a lesser degree than observed after depletion of SNW1 (Fig 6). These results support the notion that SNW1 is required for cohesion and mitotic progression because it is required, as part of the spliceosome, for splicing of sororin and APC2 pre-mRNAs. As in SNW1-depleted cells, we found in the RNA-seq experiments that only a subset of introns was retained in PRPF8-depleted cells (Supplementary Table S5). Interestingly, of the introns which we found to be upregulated by stringent filtering criteria in PRPF8-depleted cells, 51% were identical with introns that were increased in abundance in SNW1-depleted cells (Fig 6). These results imply that the removal of these introns is particularly dependent on normal levels of the NTC and U5-subcomplexes of the spliceosome, of which SNW1 and PRPF8 are subunits, respectively.

Discussion

In eukaryotes, most primary transcripts from protein-coding genes have to be spliced to generate mRNAs which serve as functional templates for protein synthesis (reviewed in Will & Luhrmann, 2011). RNA and protein components of the spliceosome are therefore essential for virtually any cellular process, as are transcription and translation. Surprisingly, however, several genome-wide RNAi screens in *Drosophila* and human cells as well as other studies have revealed that numerous splicing factors are essential for chromosome biorientation and segregation (Kittler *et al*, 2004; Somma *et al*, 2008; Hofmann *et al*, 2010; Neumann *et al*, 2010). For example, 27 components of the human spliceosome were identified in the MitoCheck screen (Hofmann *et al*, 2010; Neumann *et al*, 2010). These defects have been proposed to be caused by defective splicing of pre-mRNAs required for mitosis (Biggins *et al*, 2001; Burns *et al*, 2002; Pacheco *et al*, 2006; Huen *et al*, 2010; Song *et al*, 2010; Ahn *et al*, 2011; Sharma *et al*, 2011). However, the spliceosomal complex Prp19 has also been found to be required for proper spindle assembly in *Xenopus* egg extracts, in which mitotic processes occur independently of transcription and splicing (Hofmann *et al*, 2013). For this reason, and because depletion of spliceosomal proteins leads to mitotic defects very rapidly, it has also been proposed that splicing factors have more direct roles in mitosis (Kittler *et al*, 2004; Hofmann *et al*, 2013). It therefore remained unknown if splicing factors have direct or indirect roles in mitosis, and in the latter case, which splicing events are essential for this function.

Our results reveal that even a partial reduction in the levels of the splicing factor SNW1 causes rapid loss of sister chromatid cohesion and that a reduction in the protein levels of sororin is largely responsible for this defect. It is well established that sororin is essential for cohesion (Rankin *et al*, 2005), because it antagonises the cohesin release factor Wapl (Nishiyama *et al*, 2010) and thus stabilises cohesin on DNA (Schmitz *et al*, 2007). Furthermore, sororin mRNA levels are fluctuating during the cell cycle (Walker, 2001), the sororin mRNA is short-lived (this study) and the protein is degraded during mitotic exit and G1-phase because it is a substrate of APC/C-CDH1, a form of the APC/C which is only active during these phases of the cell cycle (Rankin *et al*, 2005; Nishiyama *et al*, 2010). The short-lived nature of sororin mRNA and protein could explain why even partial defects in splicing of the sororin pre-mRNA could rapidly lead to cohesion defects.

However, expression of sororin from an intronless cDNA did not completely restore cohesion defects in SNW1-depleted cells, even though sororin expression from the same cDNA could largely restore cohesion defects caused by depletion of endogenous sororin. These results imply that the SNW1-dependent expression of other proteins is also required for proper sister chromatid cohesion. Our data indicate that APC2 is such a protein because SNW1 depletion reduced the levels of APC2, decreased the ubiquitylation activity of APC/C and because expression of both sororin and APC2 from cDNAs was sufficient to restore cohesion in SNW1-depleted cells. Two previous studies also reported a role of splicing factors in maintaining proper levels of APC/C by showing that the fission yeast protein Dim1p is required for splicing of the pre-mRNA encoding the APC/C subunit Lid1p (an ortholog of APC4 in human cells; Carnahan *et al*, 2005) and that depletion of the human U2AF35 protein causes defects in the splicing of the pre-mRNA encoding the APC/C subunit APC3/CDC27 (Pacheco *et al*, 2006). However, our experiments did not reveal defects in APC3 or APC4 splicing in SNW1-depleted cells.

How a reduction in APC2 levels could contribute to a cohesion defect is not immediately obvious given that APC/C activity is required to initiate sister chromatid separation, that is to abrogate cohesion, and not to maintain it. However, recent studies have shown that a prolonged mitotic arrest caused by inhibition of the APC/C can also cause gradual loss of cohesion (Daum *et al*, 2011; Stevens *et al*, 2011; Lara-Gonzalez & Taylor, 2012). This phenomenon, called “cohesion fatigue”, is thought to be caused by the disruption of cohesin complexes which are under physical tension by pulling forces generated by the mitotic spindle. It is therefore conceivable that SNW1 depletion causes a major cohesion defect by limiting the number of cohesin complexes that are able to mediate cohesion and that this residual population of “cohesive” cohesin complexes is further decreased once chromosomes become bioriented and are exposed to mitotic spindle pulling forces. In wild-type cells, chromosome biorientation would lead to silencing of the spindle assembly checkpoint, activation of the APC/C, degradation of securin and B-type cyclins and subsequent activation of separase (Peters, 2006). However, in SNW1-depleted cells, partial cohesion defects could delay proper biorientation of all chromosomes and could thus prevent APC/C activation with normal kinetics. At the same time, reduced APC2 levels could further contribute to a defect in APC/C activation, leading to a mitotic delay. In those

Figure 6. Similar to SNW1 RNAi, depletion of the splicing factor PRPF8 results in cohesion defects and mis-splicing of sororin and APC2 mRNA.

A, B To assess specificity of PRPF8 RNAi, mouse Prpf8-LAP was expressed from a BAC in PRPF8-depleted HeLa cells (A), and sister chromatid cohesion was analysed in chromosome spreads (B; $n \geq 150$; error bars denote s.e.m.).

C To compare which introns are retained following depletion of either PRPF8 or SNW1, HeLa cells were transfected with siRNAs targeting either PRPF8, or as a control GL2 and RNA were analysed 48 h later by RNA-seq. Results from two independent experiments were pooled and analysed for the presence of retained introns, as described in Materials and Methods. Under these conditions, using stringent filtering criteria, 2,189 and 3,884 introns were found to be upregulated in PRPF8- and SNW1-depleted cells, respectively. 1,113 of these were identical. Note that for SNW1-depleted cells, this number is higher (3,884) than the number (1,200) obtained in the experiment shown in Fig 3. This difference could be due to differences in experimental design or SNW1 depletion efficiency.

D–G Mis-splicing at the *CDCA5* (sororin) and *ANAPC2* (APC2) loci was visualised on the IGB (D, F) and quantified by RT-qPCR (E, G; $n \geq 2$, error bars denote s.d.). Note that retention of intron 1 in the *ANAPC2* transcript following PRPF8 depletion could be detected by RT-qPCR (G) but not by RNA-seq (F). However, the RNA-seq data show clear retention of another intron (number 9, marked by an arrow in F) in both SNW1- and PRPF8-depleted cells. This short 67-bp-long intron is not listed in Supplementary Tables S3 and S4 because the data for these tables were generated by 100-bp paired-end sequencing runs which were too long to detect such a short intron.

Source data are available online for this figure.

chromosomes which come at least transiently under tension, this delay could further decrease cohesion, especially at the microtubule attachment sites, via the reported “cohesion fatigue” phenomenon. Although speculative, this hypothesis can explain why either

depolymerisation of microtubules by nocodazole or expression of APC2 from a cDNA can largely restore the residual cohesion defects that are otherwise seen in SNW1-depleted cells that are expressing sororin from a cDNA.

Because SNW1 is a subunit of the spliceosome, it is conceivable that other splicing factors are also required for mitotic progression because they are needed for the splicing of pre-mRNAs that encode cohesion proteins such as sororin. Our observation that depletion of PRPF8, another component of the spliceosome, also leads to a reduction in sororin levels and cohesion defects is consistent with this possibility. Furthermore, in the accompanying manuscript, Sundaramoorthy *et al* (2014) have identified 26 different pre-mRNA splicing factors that are required for sister chromatid cohesion and have shown for four of these factors that the cohesion defects caused by their depletion can be restored by sororin expression. These results indicate that a key function of the general splicing machinery in enabling progression through mitosis is to mediate cohesion, presumably predominantly by catalysing splicing of the sororin pre-mRNA.

Unexpectedly, our RNA-seq analysis revealed that depletion of SNW1 and PRPF8 affected overlapping but relatively small subsets of splicing reactions in the transcriptome. Similar observations have been made in yeast, where inactivation of individual splicing factors reduced the splicing of different transcripts to various degrees (Pleiss *et al*, 2007). This could imply that SNW1 and PRPF8 are not essential for the splicing of all cellular transcripts, even though both proteins are thought to be constitutive components of the general splicing machinery and PRPF8 is known to be required for spliceosome activation (Maeder *et al*, 2009; Mozaffari-Jovin *et al*, 2013). Alternatively, it is possible that these proteins were simply not depleted to sufficiently low levels in our RNAi experiments to affect all splicing reactions. It is also possible that pre-mRNAs whose splicing was not detectably affected are more long-lived than those in which we could detect intronic sequences. Our observation that the sororin mRNA is particularly short-lived and therefore presumably has to be re-synthesised in every cell cycle is consistent with this possibility. However, our mRNA stability measurements indicate that the APC2 mRNA is as stable as mRNAs which are not detectably affected by SNW1 or PRPF8 depletion, suggesting that differences in mRNA longevity alone cannot explain why the splicing of some pre-mRNAs is particularly sensitive to reductions in SNW1 or PRPF8 levels. Finally, it is possible that many transcripts retaining intronic sequences are difficult to detect by RNA-sequencing because they are rapidly degraded by nonsense mediated decay (NMD; Baker & Parker, 2004; Jaillon *et al*, 2008).

Even more surprisingly, we also noticed that in those pre-mRNAs whose splicing was affected by SNW1 or PRPF8 depletion, only some intronic sequences were detected, whereas others were not. For example, in the sororin pre-mRNA, intron 1 was readily and reproducibly observed in RNA-seq and qPCR experiments after SNW1 depletion, whereas only small or no detectable change in the levels of other sororin introns or transcript levels could be seen. The retained intronic sequences were, depending on the affected pre-mRNA, found at different positions within the primary transcript and had a highly variable length distribution (see Supplementary Tables S3, S4 and S5). These results raise the possibility that these spliceosome subunits are more rate limiting for some splice reactions than for others within one and the same pre-mRNA species. Alternatively, it is also in this case conceivable that only subsets of introns were detected by our analyses because transcripts containing other introns may have been rapidly degraded by the NMD pathway.

Even though it is presently unclear why the splicing of some introns is particularly sensitive to a reduction in SNW1 and PRPF8 levels, we expect that the transcriptome-wide identification of these introns reported here will be useful for the interpretation of cellular and organismal phenotypes that have been observed after spliceosome inactivation. For example, we found that SNW1 depletion leads to the retention of intronic sequences in the mRNAs encoding the bone morphogenetic protein 2 (BMP2; Supplementary Table S3, Supplementary Fig S6). This observation could help to explain why SNW1 is required for BMP signalling during early vertebrate development (Wu *et al*, 2011). Interestingly, mutation of PRPF8 and other splicing factors have also been linked to human diseases such as pancreatic cancer (Furukawa *et al*, 2011), myeloid neoplasms (Haerlach *et al*, 2014; Kurtovic-Kozaric *et al*, 2014) and retinosa pigmentosa, a hereditary syndrome which leads to progressive degeneration of the retina and blindness (reviewed in Liu & Zack, 2013). Our identification of splicing reactions which are particularly sensitive to PRPF8 inactivation might in the future be useful for understanding the aetiology of these diseases at the molecular level.

Materials and Methods

Plasmids and antibodies

The following antibodies were used: SMC3 (hSMC3 727 Peters laboratory ID 845), mouse anti-cyclin B1 (Santa Cruz sc-245), rabbit anti-H3S10P (Millipore 06570), goat anti-H3 (Santa Cruz sc-8654), rabbit anti-SCC4 (SCC4 294 Peters laboratory ID 974), chicken anti-GFP [used in IF (Abcam ab13970)], mouse anti-SCC1 (Rad21 Millipore 05-908), rabbit anti-ESCO1 (Peters laboratory ID 782M), mouse anti-myc (myc (9E10), Peters laboratory ID A668), rabbit anti-APC2 (Cell Signalling 12301), rabbit anti-sororin (sororin 1809M Peters laboratory ID 953), rabbit anti-SMC1 (Bethyl A300-055A), rabbit anti-INCENP (Peters laboratory ID 733M), rabbit anti-SA1 (Peters laboratory ID 1047G), goat anti-SA2 (Bethyl, A300-158A), rabbit anti-BubR1 (Peters laboratory ID 920), mouse anti-PLK1 (Zymed, 33-1700), rabbit anti-CDT1 (Bethyl, A300-786A), rabbit anti-APC5 (Peters laboratory ID 802G), mouse anti-Aurora B (BD, 611083), mouse anti-CDC20 (Santa Cruz E-7), rabbit anti-PDS5A (Bethyl A300-089A), rabbit anti-PDS5B (Peters laboratory ID 770G), rabbit anti-securin (Peters laboratory ID 892) and rabbit anti-PRP8 (Fisher Scientific PA5-28721). Rabbit anti-SNW1 was a gift from R. Lührmann, goat anti-GFP (used in Western blot (Fig 2A)) was a gift from T. Hyman, mouse anti-acetyl SMC3 was a gift from K. Shirahige, guinea pig anti-ESCO2 was a gift from J. de Winter and rabbit anti-shugoshin was a gift from Y. Watanabe. GFP-flag-sororin cDNA was transcribed from a pIRESpuro vector which was kindly provided by M. Petronczki. hAPC2 cDNA was PCR amplified and cloned via Gateway cloning (Invitrogen) into a pIRESneo3 vector with *AflIII* and *NotI*.

Cell culture, RNAi and cDNA transfection and chromosome spreads

HeLa, HeLa mSnw1-LAP, HeLa CENP-A-EGFP, HeLa mSmc3-LAP, HeLa GFP-flag-sororin, HeLa APC2-RFP and HeLa mPrpf8-LAP cells were cultured in DMEM supplemented with 10% FCS, 0.2 mM L-glutamine and antibiotics (all Invitrogen). Cells were transfected

for 48 h with 20 nM siRNA purchased from Ambion (Firefly luciferase control siRNA (GL2): 5′ – CGUACGCGGAUACUUCGAtt – 3′, SNW1: ID# 108321 and s22716, ESCO1: 5′ – GAGAAUAAUUUCCAGGUtt – 3′, ESCO2: 5′ – GAAAGAACGUGUAGUAGCAtt – 3′, Wapl: 5′ – GGCTTC CGAGGAGAATATGtt – 3′) and Thermo Scientific (sororin: D-015256-06) by RNAiMAX according to manufacturer's instructions (Invitrogen). For synchronisation, cells were treated by a double thymidine block (2 mM) for 16 h with siRNA transfection at the first release of 8 h. To inactivate microtubule pulling forces, mitotic cells were removed by shake-off 36 h after depletion, and remaining cells were incubated in the presence or absence of 100 ng/ml nocodazole for the last 12 h of depletion. For chromosome spreading and Giemsa staining, nocodazole was added to the medium for 45 min at 100 ng/ml. Cells were harvested and hypotonically swollen in 40% medium/60% Vienna tap water for 5 min at room temperature. Cells were fixed with freshly made Carnoy's solution (75% methanol, 25% acetic acid), and the fixative was changed several times. For spreading, cells in Carnoy's solution were dropped onto glass slides and dried. Slides were stained with 5% Giemsa (Merck) for 4 min, washed briefly in tap water and air-dried. In every experiment, two slides were blindly counted per condition as technical replicate. In addition, every experiment was biologically replicated at least once, resulting in a total of minimally four counted slides per condition.

Cell extracts for immunoblotting

Cell pellets were resuspended in extraction buffer [25 mM Tris–HCl pH 7.5, 100 mM NaCl, 5 mM MgCl₂, 0.2% NP-40, 10% glycerol, 1 mM NaF, 10 mM sodium butyrate, Complete protease inhibitor mix (Roche), benzonase (VWR)] and lysed on ice (whole-cell extract; WCE). To obtain chromatin-bound proteins for analysis of acetylated SMC3 (Fig 2D), the homogenate was spun at 10,000 g and washed three times with extraction buffer and resuspended in extraction buffer. WCE and chromatin fraction were resuspended in SDS sample buffer, sonicated in a waterbath and heated to 95°C.

Mass spectrometry

Cell pellets were resuspended in extraction buffer (25 mM Tris pH 7.5, 100 mM NaCl, 5 mM MgCl₂, 0.2% NP-40, 10% glycerol, 10 mM sodium butyrate, Complete protease inhibitor mix and benzonase (250 U/ml Novagen)) and lysed on ice by passing through a hypodermic needle. Insoluble material was removed by centrifugation. Supernatant extract was added to crosslinked anti-GFP antibody beads (Chromotek), incubated, washed and eluted with 0.1 M glycine pH 2.0. Eluates were processed for in-solution digest and mass spectrometry as described (Gegan *et al*, 2007). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (<http://proteomecentral.proteomexchange.org>) via the PRIDE partner repository (Vizcaino *et al*, 2014) with the data set identifier PXD001249 and DOI: 10.6019/PXD001249.

Immunofluorescence, FISH and FACS

Cells grown on coverslips were fixed with 4% paraformaldehyde in PBS for 20 min, with 2 min pre-extraction with 0.1% Triton X-100 in PBS prior to fixation in case of cohesin SCC1 staining. Cells were permeabilised with 0.1% Triton X-100 in PBS for 5 min and blocked

with BSA in PBS containing 0.01% Triton X-100. Cells were incubated with primary and secondary antibodies (Alexa 488, Alexa 568 and Alexa 647; Molecular Probes), and DNA was counterstained with DAPI. Coverslips were mounted onto slides with ProLong Gold (Molecular Probes). Images were taken with an Axioplan 2 microscope (Zeiss) using a CoolSnap HQ camera (Photometrics) and processed with MetaMorph (Universal Imaging). For FISH, cells were spun onto glass slides, pre-extracted and fixed, and fluorescence *in situ* hybridisation with DNA probe kit XA 13/18/21 (Metasystems) was performed as described (Schmitz *et al*, 2007). Quantifications were processed with Microsoft Excel 2007 and GraphPad Prism 5. Significance levels were quantified using unpaired *t*-test. Cells for FACS were fixed in PBS with 75% methanol for at least 2 h at –20°C and washed with PBS, DNA was stained with PI buffer (50 µg/ml propidium iodide, 10 mM Tris pH 7.5, 5 mM MgCl₂, 200 µg/ml RNase A) for 1 h at 37°C, and cell cycle distribution was obtained with FACSCanto (BD) and analysed using FlowJo (Tree Star).

Inverse fluorescence recovery after photobleaching (iFRAP)

Cells for FRAP experiments were grown in 8-well Labtek II chambered coverglass (Nunc). Fifteen minutes prior to imaging, Hoechst was added at a final concentration of 0.5 µg/ml to visualise DNA and cycloheximide was used at 1 µg/ml to inhibit protein synthesis. Live-cell images were acquired on an LSM5 Duo (Zeiss) confocal microscope using a 40× EC Plan-Neofluar oil objective and open pinhole. Twenty-four cells were imaged simultaneously using the Autofocus Screen macro developed by Zeiss and the Jan Ellenberg group at EMBL Heidelberg (D). After acquiring 4 pre-bleach images, the field of view except for a nuclear region was photo-bleached three times with 100% laser intensity (100 mW diode 488). Images were acquired every 2–3 min. The data were processed using ImageJ, and the turboreg plugin (<http://bigwww.epfl.ch/thevenaz/turboreg/>) was used to correct for stage or cell movements based on Hoechst staining. Data normalisation was performed in Microsoft Excel 2007, and curves were fit to a bi-exponential function using Berkeley Madonna software. The calculated values for residence times and stable fraction size as well as resampled intensity difference curves were plotted and compared using GraphPad Prism. For live-cell imaging, signal intensities were quantified using ImageJ (<http://rsb.info.nih.gov/ij/>). Quantifications were processed with Microsoft Excel 2007 and GraphPad Prism 5. Significance levels were quantified using unpaired *t*-test.

mRNA-seq, qPCR and mRNA stability assays

For mRNA sequencing experiments, total RNA was obtained by TRIzol, and mRNA was isolated using Dynabeads (both Invitrogen) following manufacturer's instructions. mRNA was fragmented in fragmentation buffer (40 mM TrisOAc pH 8.2 mM, 100 mM KOAc 30 mM MgOAc) for 3 min at 94°C, and RNA quality was analysed using an RNA 6000 Pico kit (Agilent Technologies). The fragmented mRNA was reversely transcribed with Superscript III kit (Invitrogen), followed by second-strand synthesis (5× Second-strand buffer, dUTP, random hexamers, DNA Pol I, DNA Ligase and RNase H, all Invitrogen). Double-stranded DNA concentrations were measured by Picogreen (Invitrogen), and samples were

submitted to Solexa sequencing at CSF facility, NGS Unit (<http://csf.ac.at/>), Vienna, Austria. For the comparison of introns retained in SNW1-depleted cells which did or did not express mSnw1-LAP from a BAC, 100-bp paired-end sequencing was used. For the comparison of introns retained in cells transfected with PRPF8, SNW1 or, as a control, GL2 siRNAs, 50-bp single-end sequencing was used. qPCR was performed with primers designed as depicted in Fig 3E using Platinum Sybr green (Invitrogen). The RNA-seq data from this publication have been submitted to the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>) and assigned the identifier GSE61071. For mRNA stability assays, 5 µg/ml actinomycin D was added to the medium for different time periods and total RNA was isolated using TRIzol reagent (both Invitrogen). cDNA was synthesised by Superscript II (Invitrogen) and analysed for transcript abundance by qPCR.

Transcriptome-wide identification of retained introns

Mapping of Illumina HiSeq2000 derived and quality-controlled paired-end reads (length 100 bases) was performed using tophat version 1.3.1 with the standard Illumina library type against the Homo sapiens hg19 genome. The insert size distribution was estimated from aligned paired-end reads, and the expected mean inner distance between mate pairs was set to values at about 200; standard deviation for the distribution on inner distances was 128. Minimum length of cut segments was 18, and maximum allowance of mismatches was the default of 2. For annotation of intronic regions, a snapshot of the refGene table from UCSC was taken (RefSeq genes track within the group of Genes and Gene Prediction Tracks; assembly GRCh37/hg19), after which available (annotated) transcript variants were merged into gene locations by fusion of all regions which are marked as coding (exons). The rationale behind this procedure is to identify and annotate those intronic segments that are not covered by any transcript variant. As a unique identifier for introns within the hereby generated list of 119,901 introns, a name string was built for each intron consisting of the alternate gene name resp. transcript identification strings combined with an incremental count that is sorted regardless of the transcript's strandedness. S. Anders' htseq-count algorithm was applied for read counting in which the intersection-nonempty or union modes would give no different results due to the nature of our intron list. For a stringent degree of reliance, (A) the fold change of reads per intron between SNW1 depleted and rescued cells was calculated, as well as (B) each intron's overall coverage in both conditions as the count of reads per nucleotide: (A) fold change = # reads depleted + 1/# reads rescued + 1, B): coverage = Σ reads (depleted + rescued)/intron length, and a minimum of sum of reads of 20 was taken. To avoid graphical artifacts, a minimum of the sum of reads of 30 was selected to display introns in Fig 3A. Cut-off values of a log₂ (fc) of ≥ 1.5 and coverage of ≥ 0.05 were used for Supplementary Tables S3, S4 and S5.

APC/C activity assay

APC/C activity assay was described in Buschhorn *et al* (2011).

Supplementary information for this article is available online: <http://emboj.embopress.org>

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Author contributions

Experiments were designed and data interpreted by PvdL, RS, RL, GP, EK, BK, JS and J-MP. BN and JE manually selected 207 mitotic hits. BK and JS performed the secondary screen and LAP-rescue. PvdL analysed the mitotic phenotype, cohesin loading and acetylation upon SNW1 RNAi. JS and PvdL did FISH experiments. RL performed and analysed iFRAP. RS developed software to analyse mRNA-seq data for splicing defects, PvdL and RS interpreted mRNA-seq data. PvdL performed qPCR experiments, generated HeLa cells expressing GFP-flag-sororin and APC2-RFP and carried out chromosome spreading experiments. GP performed Hsl1-ubiquitilation assay. PvdL, RL and J-MP wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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