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THE EXPANDING UNIVERSE OF COHESIN FUNCTIONS: A NEW GENOME STABILITY CARETAKER INVOLVED IN HUMAN DISEASE AND CANCER

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ABSTRACT

Cohesin is responsible for sister chromatid cohesion, ensuring the correct chromosome segregation. Beyond this role, cohesin and regulatory cohesin genes seem to play a role in preserving genome stability and gene transcription regulation. DNA damage is thought to be a major culprit for many human diseases, including cancer. Our present knowledge of the molecular basis underlying genome instability is extremely limited. Mutations in cohesin genes cause human diseases such as the Cornelia de Lange syndrome and the Roberts syndrome/SC phocomelia, and all the cell lines derived from affected patients show genome instability. Cohesin mutations have also been identified in colorectal cancer. Here, we will discuss the human disorders caused by the alterations of cohesin function, with emphasis on the emerging role of cohesin as a genome stability caretaker.

Key words: Cohesin, genome instability, cancer, Cornelia de Lange syndrome, Roberts syndrome

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OVERVIEW OF COHESIN FUNCTIONS

Canonical role of cohesin

The discovery of the cohesin complex has led to a breakthrough in clarifying the "cohesive force" in play during mitosis in holding the sister chromatids together and ensuring correct chromosome segregation. Cohesin is composed of four subunits, a pair of SMC proteins, SMC1A (MIM# 300040) and SMC3 (MIM# 606062), which are members of the Structural Maintenance of Chromosome family, and two non-SMC proteins, RAD21/Scc1 (MIM# 606462) and STAG/Scc3/Sa (Table 1) [Michaelis et al., 1997]. In vertebrates, there are two closely related Scc3 homologs, called STAG1 (MIM# 604358) and STAG2 (MIM# 604359) [Losada et al., 2000; Sumara et al., 2000]. In addition, a meiotic isoform is also present, named STAG3 (MIM# 608489) [Prieto et al., 2001]. SMC proteins are characterized by a globular hinge domain flanked by two α-helical domains (Figure 1A) which fold back on themselves at the hinge, forming a long antiparallel α-helical coiled coil arm that brings the N and C termini together. The N-terminal contains the Walker A box (or P-loop) which binds ATP. The C-terminal holds the Walker B, binding to DNA. SMC1A and SMC3 dimerize at the hinge domains, forming a V-shaped structure through hydrophobic interactions. Basically, the cohesin complex acts as a tripartite ring in which SMC1A and SMC3 are connected by their hinge domains on one side, and RAD21 closes the ring by connecting the SMC1A and SMC3 head domains on the other side [Anderson et al., 2002; Haering et al., 2002] (Figure 2). The cohesin ring shape supports the model where interaction between cohesin and DNA is topological, the so-called "ring" or "embrace" model. According to this model, cohesin topologically encircles sister chromatids. The cohesin opens at the head domain followed by the sister chromatid entering the ring and

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becoming topologically entrapped when RAD21 locks the ring [Gruber et al., 2006; Gruber et al., 2003; Haering, et al., 2002; Haering et al., 2004; Ivanov and Nasmyth, 2007]. Transient dissociation of SMC hinge domains also seems to contribute towards the entry of DNA into the cohesin ring [Gruber et al., 2006]. Several alternative models, as well as the bracelet, oligomerization and snap models have been proposed as the molecular basis of sister chromatid cohesion [Diaz-Martinez et al., 2008; Guacci 2007; Huang et al., 2005; Milutinovich and Koshland, 2003]. These models have stimulated the research in this field and the true mechanism may be a combination of those proposed. The cohesin binding to chromatin is dynamic and occurs during the G1/S phase in the budding yeast or in the telophase of the previous cell cycle in vertebrates [Haering et al., 2004]. In yeast, cohesin binds along chromosome arms every 10-20 kb, while its density is higher at centromeric regions [Glynn et al., 2004; Kogut et al., 2009; Lengronne et al., 2004]. By contrast, in *Drosophila* the cohesin binding regions cover from a few kilobases up to 100 kb in length [Misulovin et al., 2008]. Though the chromosome arm dissociation process has yet to be fully understood, removal of cohesin begins in the prophase and is completed by the anaphase. In the first step, RAD21 and STAG subunits are phosphorylated by Polo-like kinase 1, causing the dissociation of the cohesin only along the chromosome arms [Hauf et al., 2005], while the bulk of centromeric cohesin remains unchanged [Waizenegger et al., 2000]. Once chromosomes are bi-oriented on the mitotic spindle at anaphase, cohesin is completely removed from chromosomes by the separase protein (MIM# 604143) that cleaves RAD21, triggering sister chromatid disjunction. Initially, separase is inactivated through an association with securin (MIM# 604147), which is released after degradation through the ubiquitin dependent proteolysis pathway [Hauf et al., 2001; Uhlmann et

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al., 2000]. This model suggests that cohesin, preserved from degradation by separase during the prophase, can be loaded onto chromatin just after the restoration of the nuclear membrane.

Cohesin regulatory factors

Cohesin interacts with several other proteins contributing towards its function (Table 1).

The loading of cohesin onto chromatin requires NIPBL activity (MIM# 608667, homolog of fungal Scc2 and *Drosophila* Nipped-B). It has been speculated that NIPBL may be involved in the activation of SMC ATPase and chromatin remodeling [Arumugam et al., 2003; Jahnke et al., 2008]. PDS5A (MIM# 613200) and PDS5B (MIM# 605333) proteins are essential for the association of cohesin with chromatin and sister chromatid cohesion. Their amino acid sequences are conserved during evolution and are characterized by HEAT repeats which promote protein interaction [Panizza et al., 2000]. The presence of two PDS5 and STAG proteins renders possible the existence of four different types of cohesin containing either STAG1 or STAG2 and either PDS5A or PDS5B [Losada et al., 2005; Sumara et al., 2000]. Cohesin is also associated with WAPL; and like the PDS5 proteins, WAPL (MIM# 610754) also contains motifs which promote protein-protein interaction. In fact, it has been shown that the FGF motifs of the WAPL protein allow the physical interaction with PDS5 and cohesin subunits [Shintomi and Hirano, 2009]. It is thought that WAPL is involved in the process of removing cohesin from chromatin [Gandhi et al., 2006; Kueng et al., 2006]. ESCO2 (MIM# 609353) is involved in the establishment of bridges between chromatid sisters during the cell cycle [Ivanov et al., 2002; Skibbens et al., 1999; Toth et al., 1999]. It is also involved in the SMC3 acetylation [Heidinger-Pauli et al., 2009; Zhang et al., 2008a], necessary for replication fork progression [Terret et al., 2009]. Sororin (MIM# 609374) is the last identified protein known to be involved in cohesin activity.

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Data regarding this protein are limited; however, it has been hypothesized that it is involved in sister chromatid cohesion [Rankin et al., 2005; Schmitz et al., 2007]. It is clear that cohesin activity is tightly regulated and it is possible that new regulatory proteins will be identified in the near future.

NON CANONICAL ROLE OF COHESIN: GENE EXPRESSION REGULATION

There is increasing experimental evidence suggesting that cohesin plays a part in the regulation of gene expression. The first proof that cohesin factors regulate gene expression comes from studies on the *Drosophila Ultrabithorax* and *Cut* genes which are involved in many developmental processes [Rollins et al., 1999]. In fact, the expression depends on Nipped-B which might facilitate the interaction between promoter and remote enhancers possibly by regulating chromosome architecture. Since Nipped-B acts as a cohesin regulatory protein, it was hypothesised that cohesin would have the same effect as Nipped-B on the gene expression regulation. Surprisingly, the inhibition of *Smc1a*, *Rad21* and *Sa* led to an increase of *Cut* expression [Dorsett et al., 2005; Rollins et al., 2004]. These findings highlighted a possible role of cohesin as an insulator, inhibiting enhancer-promoter communication in *Cut,* and suggested that *Nipped-B* promotes enhancer-promoter interaction by altering cohesion binding to chromatin [Rollins et al., 2004] . Notably, cohesin contributes to the insulator activity of the CCCTC binding factor (CTCF), a zinc finger DNA-binding protein that is evolutionary conserved [Parelho et al., 2008; Rubio et al., 2008; Stedman et al., 2008; Wendt et al., 2008]. However, recent data suggest that the manner in which cohesin regulates gene expression is more complex. The fact that the depletion of either *Rad21* or *Smc3* in Zebrafish results in the reduced expression of *Runx* genes, involved in the activation of hematopoietic and neurogenic genes has been

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demonstrated [Horsfield et al., 2007]. Studies in *Drosophila* show that cohesin can exert opposing effects, both positive and negative, on gene expression [Schaaf et al., 2009]. These results are consistent with gene expression data obtained in Cornelia de Lange syndrome (CdLS; MIM#s 122470, 300590, 610759) cell lines and in *Nipbl*$^{+/-}$ mutant mice where many genes are down- and up-regulated [Kawauchi et al., 2009; Liu et al., 2009b]. Beyond its role in sister chromatid cohesion, emerging data show that the activity of cohesin and cohesin regulatory factors is critical for the proper development of organisms by regulating gene expression in multiple ways.

Mutations in *NIPBL*, *SMC1A* and *SMC3* cause CdLS while mutations in *ESCO2* are associated with Roberts syndrome (RBS; MIM# 268300) /SC phocomelia (MIM# 269000) [Deardorff et al., 2007; Krantz et al., 2004; Musio et al., 2006; Schule et al., 2005; Tonkin et al., 2004; Vega et al., 2005]. CdLS and RBS are collectively termed "cohesinopathies" [reviewed in Liu and Krantz, 2008]. CdLS is the most frequent disease among those due to cohesion pathway defects. Though the molecular pathobiology of CdLS is largely unknown, it is noteworthy that the dysregulation of gene expression likely represents the underlying pathogenesis of CdLS. It has recently been discovered, by using a genome-wide approach to study gene expression, that alteration of a restricted number of genes which can be considered as CdLS biomarkers are capable of differentiating CdLS from non-CdLS samples. Their expression levels also correlate with the phenotypic severity of the disorder [Liu et al., 2009b]. In addition, small changes in Nipbl activity affect appropriate development in the *Nipbl*$^{+/-}$ mouse model. In particular, gene expression profiling demonstrates that *Nipbl* deficiency leads to modest but significant transcriptional dysregulation of many genes [Kawauchi et al., 2009].

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Cohesinopathy cell lines show both spontaneous and induced genome instability. In addition, mutations or dysregulation of cohesin and regulatory cohesin genes are associated with human cancer (see below). These observations suggest that the cohesin pathway may be involved in genome stability maintenance and tumorigenesis. This review focuses specifically on the emerging role of cohesin as a new genome stability caretaker involved in human disease and cancer.

COHESIN AND GENOME INSTABILITY

Genome instability has been discovered as one of the major factors responsible for human diseases, including cancer. It manifests as point mutations, gene amplifications, aneuploidies (i.e. the gain or loss of entire chromosomes), deletions and translocations. The genomic stability depends on an intricate machinery of DNA repair mechanisms, correct chromosome segregation pathways and the activation of cell cycle checkpoints in order to arrest cell cycle progression. The initial confirmation that cohesin is involved in genome stability came from studies in *S. pombe*. *Scc1* mutants are hypersensitive to DNA damage [Birkenbihl and Subramani, 1992], suggesting that cohesin plays an important function in the DNA damage response. In recent years, ample experimental evidence supports the notion that cohesin is also involved in genome stability maintenance in human cells. SMC1A was shown to be the target of the ATM kinase with phosphorylation of SMC1A-Ser957 and SMC1A-Ser966, occurring in an ATM dependent manner after ionizing irradiation [Kim et al., 2002; Yazdi et al., 2002]. Subsequently, it has been shown that NBS1 and BRCA1 are required for the recruitment of activated ATM at the sites of DNA double strand breaks, and that this process is necessary for the phosphorylation of SMC1A by ATM following irradiation [Kitagawa et al., 2004]. We showed that SMC1A phosphorylation

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can be ATM-independent, depending on the type of DNA damage. Moreover, we found that SMC1A is phosphorylated on Ser966 by ATR after replication stress induced by aphidicolin, while no phosphorylation occurred on Ser957 [Musio et al., 2005]. The finding that ATM is not involved in the SMC1A phosphorylation following replication stalling is not surprising because it seems to be specifically involved in response to irradiation [Kim et al., 2002; Yazdi et al., 2002]. These observations suggest that cohesin participates in two different pathways, triggering a specific response to different DNA damage types that challenge genome stability.

The heterodimer SMC1A-SMC3 is also a member of a mammalian protein complex, called recombination complex RC-1, which promotes DNA recombination [Jessberger et al., 1996; Stursberg et al., 1999]. There are three additional members belonging to the RC-1 complex: DNA ligase III, DNA polymerase ε, and unidentified structure-specific DNA endonuclease. It is thought that the RC-1 complex catalyses several recombination-related reactions such as the homology-dependent transfer of DNA between two dsDNA molecules, DNA single-strand re-annealing, and the repair of gaps or deletions in dsDNA substrates [reviewed in Jessberger, 2002].

Following DNA damage, cohesin accumulates in a large 50 kb domain that surrounds the damage site [Strom et al., 2004; Unal et al., 2004] and SMC1A-SMC3 are phosphorylated [Kim, et al., 2002; Luo et al., 2008; Yazdi et al., 2002]. Cohesin's ability to establish cohesion *de novo* is essential for DNA damage repair [Strom, et al., 2004; Unal et al., 2004] and we found that fork stalling induced an increase in SMC1A synthesis levels [Musio et al., 2005]. These findings suggest that the cohesin increase and its accumulation can stabilize the broken sites, acting as scaffolding to improve the recruitment of DNA repair machinery. Furthermore, the

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phosphorylation of cohesin proteins may be required for activation of cell cycle checkpoints, allowing cell cycle arrest and DNA repair.

COHESIN AND CANCER

Genome instability is thought to play a pivotal role in the tumorigenic process and it is considered a hallmark of cancer cells. So far, only a limited number of genes thought to be involved in the maintenance of genome instability have been identified as having somatic mutations in human cancer. The recent finding that human colorectal cancers cells carry mutations in cohesin genes (Figure 3) further support the notion that cohesin is involved in genome instability and cancer. Eleven somatic mutations have been identified in more than 130 colorectal cancer cases. Six of them map to three core cohesin genes (*SMC1A*, *SMC3* and *STAG3*), four to a regulatory cohesin gene (*NIPBL*) and one to *RNF20*, a non related cohesin gene [Barber et al., 2008]. *SMC1A* and *NIPBL* account for 8 out of 10 mutations (Figures 3A, 3B). All *SMC1A* mutations are missense (Supp. Table S1): c.1186T>C, c.1300C>T, c.1680C>G and c.3556G>A leading to Phe396Leu, Arg434Trp, Ile560Met and Val1186Ile changes, respectively. The first two mutations map to the coiled-coil domain, the third to the hinge domain and the last to carboxy terminal P-loop NTPase domain (Figure 3A). It is interesting to note that *SMC1A* mutations are only missense in both *SMC1A*-mutated CdLS patients (see below) and in cancer cells, suggesting that frameshift and nonsense mutations are lethal. The four mutations in *NIPBL* are: c.1435C>T, c.2967_2968 insT, c.1660C>T, c.5378T>A leading to Arg479X, Val992fsX, Gln664X and Met1793Lys changes (Figure 3B, Supp. Table S1). Finally, *SMC3* and *STAG3* show the mutations c.2635C>T and c.24117C>T (Supp. Table S1), which cause the Arg879X (Figure 3C) and Ile795Thr (Figure 3D) amino acid changes, respectively.

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The majority of these mutations are heterozygous nonsense mutations which disrupt protein function through the creation of stop codons. This finding raises the possibility that nonsense mutations might be more predisposed to cancer than missense mutations**.** These mutations could dysregulate cohesin activity by preventing correct protein association, by altering the SMC ATPase activity or affecting the dynamic displacement of cohesin from chromatin. Colorectal cancer cells are characterized by chromosomal instability, resulting in chromosome gain or loss. It is possible to argue that abnormal cohesion pathway activity leads to chromosome missegregation and chromosome instability. This hypothesis is supported by the observation that colorectal cancer cells exhibit up to 100-fold higher rates of missegregation than normal cells [Lengauer et al., 1997]. Therefore chromosomal instability in colorectal cancer cells could be a direct consequence of cohesin mutations.

In addition to core cohesin genes, there is increasing evidence also linking cohesin regulatory genes to tumorigenesis. The overexpression of *WAPL* correlates with the progression of cervical cancer malignancy (Table 1), and cells overexpressing *WAPL* develop tumors after injection into nude mice [Oikawa et al., 2004]. Separase is found to be significantly overexpressed in human breast cancer [Zhang et al., 2008b], osteosarcoma and prostate cancer (Table 1) [Meyer et al., 2009]. Its induction leads to premature separation of chromatids, lagging chromosomes with specific chromosome aneuploidies [Zhang, et al., 2008b]. Finally, *ESCO2* is up-regulated in more aggressive melanomas [Ryu et al., 2007], Securin (also known as proto-oncogene pituitary tumor transforming gene, *PTTG*) is overexpressed in pituitary tumors [Zou et al., 1999] and *RAD21* is overexpressed in breast [Atienza et al., 2005; Oishi et al., 2007] and prostate cancer (Table 1) [Porkka et al., 2004]. *ESCO2* and Securin promote cohesin function while *WAPL* and Separase antagonize this function. Therefore the fact that those genes are overexpressed in

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cancer suggests that cohesin and positive and negative regulatory proteins have a complex relationship with tumorigenesis. Indeed, in some cases these genes might act as oncogenes and in others as tumor suppressor genes. At present, the genetic factors which determine these opposite effects are not known.

Cancer is thought to be a multistep process in which abnormalities in proto-oncogenes and tumor suppressors predispose cells to the acquisition of many other genetic changes which are responsible for the acquisition of the full malignant phenotype. Cohesin could play an important role in genome instability and tumorigenesis, and chromosome aneuploidy due to defects in correct chromosome segregation could cause the loss of heterozygosity of tumor suppressor genes or the improper activation of proto-oncogenes. SMC1A associates with mitotic microtubules at the spindle pole, and SMC1A imbalance leads to multipolar spindles [Wong and Blobel, 2008]. Furthermore, the inhibition of cohesin genes by RNA interference or antisense oligonucleotide causes aneuploidy and micronuclei formation in dividing cells [Barber, et al., 2008; Musio et al., 2003]. These observations suggest that the association of SMC1A with microtubules is required for proper segregation, and its dysfunction leads to aneuploidy. In addition, cohesin mutations could dysregulate the expression of proto-oncogenes or tumor suppressor genes, triggering uncontrolled cell proliferation and the development of malignant cells. In human cells, cohesin depletion leads to changes in transcription at the imprinted *H19/IGF2* locus [Nativio et al., 2009; Wendt et al., 2008]. Several lines of evidence support the hypothesis that the altered imprinting process at *H19/IGF2* locus is important in tumorigenesis [Feinberg, 1999; Pollak 2008]. It is therefore possible that defects in cohesin affect this imprinted domain and the genes within it.

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COHESINOPATHIES AND GENOME INSTABILITY

CdLS consists of characteristic facial features, hirsutism, gastroesophageal reflux, upper extremity malformations ranging from small hands to severe forms of oligodactyly and truncation of the forearms and mental retardation. Approximately 60% of CdLS probands carry a *NIPBL* mutation, most of which are point mutations or small insertions and deletions in coding regions or splice junctions. It is thought that these mutations produce either abnormal full length or truncated NIPBL proteins [Gillis et al., 2004]. A subset of CdLS probands carry mutations in the *SMC1A* and *SMC3* genes [Deardorff et al., 2007; Musio et al., 2006]. *SMC1A* is responsible for 5% of CdLS cases whereas up until now only a single proband has been found to have a mutation in *SMC3*. The *PDS5B* gene could be an additional CdLS gene. Moreover, the *Pds5B* knockout mice show several features reminiscent of the CdLS phenotype, and the genetic screening of a large cohort of CdLS patients allowed the identification of a patient carrying a mutation in the *PDS5B* gene. However, the same mutation has been found in unaffected relatives making the role of this mutation in CdLS less clear [Zhang et al., 2009].

Collectively, a total of 23 mutations in the *SMC1A* and *SMC3* core cohesion genes have been identified in CdLS (Supp. Table S1). All mutations are missense or small in-frame deletions (3 or 15 nucleotides deletion) [Deardorff et al., 2007; Liu et al., 2009a; Mannini et al., 2010]. The SMC3 amino acid change maps to the coiled coil domain (Figure 1B) whereas the SMC1A amino acid changes encompass all gene domains (Figure 1C) and do not affect the synthesis of the SMC1A protein [Liu et al., 2009a; Musio et al., 2006; Revenkova et al., 2009]. *SMC1A* maps to Xp11.2 which escapes X chromosome inactivation [Brown et al., 1995; Tsuchiya and Willard, 2000]. Both hemizygous male and heterozygous female CdLS patients have been identified [Deardorff et al., 2007; Liu et al., 2009a; Musio et al., 2006]; it is likely that *SMC1A* mutations

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act as a dominant negative in females [Liu et al., 2009a; Mannini et al., 2010; Revenkova et al., 2009].

RBS is an autosomal recessive developmental disorder characterized by pre-and post-natal growth retardation, tetraphocomelia, and craniofacial abnormalities. The causative gene of RBS is *ESCO2* which encodes a protein belonging to the highly conserved *ECO1*/*CTF7* family of acetyltransferase which is involved in regulating sister chromatid cohesion [Vega et al., 2005]. Most *ESCO2* mutations are expected to cause stop codons that result in truncated protein or mRNA instability due to nonsense-mediated mRNA decay [Gordillo et al., 2008].

All cohesinopathy cell lines are characterized by genome instability. A number of chromosomal rearrangements (balanced de novo translocations, duplications, deletions) have been reported in CdLS probands. The molecular characterization of the chromosomal rearrangement t(5;13)(13.1;q12.1) allowed the identification of the *NIPBL* gene [Krantz et al., 2004]. CdLS cell lines show a statistically significant increase of both spontaneous and induced chromosome aberrations through genotoxic treatments such as irradiation and Mitomycin C [Revenkova et al., 2009; Vrouwe et al., 2007]. It is possible that dysregulation of cohesion activity triggers spontaneous genome instability and confers sensitivity to genotoxic treatments. Increased DNA damage seems to be a common feature of CdLS, suggesting that genome instability analysis and genotoxic agent treatments can be a useful diagnostic assay and can serve as a classification tool. We found that spontaneous genome instability analysis is able to discriminate among the different types of *SMC1A* mutations. In fact, CdLS patients carrying mutations in the head domain show higher frequency of chromosome aberrations than patients with mutations in the other domains [Revenkova et al., 2009].

RBS cell lines also display genome instability occurring as chromosome aneuploidies

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andmicronuclei formation, in addition to poor growth, reduced plating efficiency and lower density for confluent cultures [Jabs et al., 1991; Musio et al., 2004]. Cells from RBS patients exhibit a characteristic abnormality of their constitutive heterochromatin, described as precocious sister chromatid separation and puffing of the peri- and para-centromeric regions of chromosomes 1, 9 and 16. Less clear is the situation in CdLS; a study reported precocious sister chromatid separation [Kaur et al., 2005; Musio, et al., 2004], however more recent studies showed no cohesion defects in CdLS cell lines [Castronovo et al., 2009; Liu et al., 2009b; Revenkova et al., 2009; Tonkin et al., 2004; Vrouwe et al., 2007]. Similar observations have also been reported in *Nipbl*$^{+/-}$ mice [Kawauchi et al., 2009] and *Nipped-B* mutant fly [Rollins et al., 2004].

To date, no mutations have been identified in other cohesin pathway genes, their effect on cohesion can be inferred only by *in vitro* treatments. The depletion of *WAPL* prevents the resolution of sister chromatids, while its overexpression induces premature sister chromatid separation [Gandhi et al., 2006; Kueng et al., 2006]. The inactivation of Sororin leads to precocious sister chromatid separation [Schmitz et al., 2007] and the inhibition of Separase prevents sister chromatid separation leading to diplo- or quadruplochromosomes, indicating that sister chromatids were not disjoined in the previous cell cycle [Wirth et al., 2006].

Cohesinopathy probands have a low rate of cancer; up till now only a few cases of cancer have been described. In fact, four RBS patients developed oculomotor nerve cavernous angiomas, malignant melanoma and rhabdomyosarcoma [Feingold 1992; Ogilvy et al., 1993; Parry et al., 1986; Wenger et al., 1988] while some CdLS patients developed esophageal adenocarcinoma [reviewed in Kline et al., 2007]. It is possible to speculate that genomic instability in CdLS leads to the formation of certain tumors. Consistent with this notion is the observation that trisomy 21

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promotes the development of leukemia while the frequency of solid cancers is low in Down syndrome patients [Satgé et al., 1998]. Further epidemiologic studies will address the argument as to whether cohesinopathies lead to cancer development.

CONCLUSION

It is becoming clearer that the role of cohesin is not limited to holding sister chromatids together and ensuring correct chromosome segregation. More recent works have revealed that the universe of cohesin functions is rapidly expanding. In fact, cohesin is essential for gene expression regulation, cell cycle control, DNA damage response and genome stability maintenance. Mutations or dysregulation of cohesin and regulatory cohesin genes are associated to cohesinopathies and cancer. In addition, cohesinopathy cell lines show both spontaneous and induced genome instability, and in some cases it is responsible for the CdLS etiopathology. However, whether genome instability is a cause or a consequence of cohesinopathies is still open to debate. The dysfunction of cohesin pathways can cause missegregation, leading to chromosome aneuploidy, a hallmark of cancer cells, and improper activation or inhibition of proto-oncogene or tumor suppressor genes, respectively. However, it has recently been shown that aneuploidy represents a barrier towards cancer development [Williams et al., 2008]. It is possible that within a population of genomic-deregulated cells those with tolerated aneuploidy and high proliferative capacity are selected, leading to cancer development [Williams et al., 2008]. Altogether this evidence supports the intriguing notion that cohesin may represent a new genome stability caretaker in mammalian cells involved in human disease and cancer. It is becoming increasingly clear that cell lines derived from cohesinopathy probands suffer from

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defects in surveillance mechanisms, leading to genome instability. Cohesin defects and/or genome instability, in addition to dysregulation of gene expression resulting from mutations in cohesin genes could account for the growth retardation as well as developmental defects by affecting the number of cells in developing tissue, leading to specific cohesinopathy phenotypes.

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FIGURE LEGENDS

Figure 1. SMC core cohesin protein organization and CdLS. (A) Schematic organization of SMC proteins. They contain five distinct domains: N-terminal (or P-loop NTPase), a hinge motif flanked by two coiled coil regions and a C-terminal (or P-loop NTPase); (B) Mapping of the amino acid change identified in a CdLS patient in the SMC3 protein; (C) Localization onto the SMC1A protein of the twenty two amino acid mutations identified in CdLS.

Figure 2. Structure of cohesin complex. The somatic vertebrate core cohesin complex consists of two SMC subunits, namely SMC1A and SMC3, and two non SMC subunits, RAD21 and STAG. Each SMC subunit forms a rod shaped protein with a globular hinge domain at one end and an ATP nucleotide binding domain at the other. SMC1A and SMC3 are connected by their hinge domains on one side and the RAD21 closes the ring by connecting SMC1A and SMC3 head domains on the other side, creating a huge tripartite ring.

Figure 3. Cohesin and regulatory cohesin proteins and colorectal cancer. Localization of the four amino acid changes identified in both SMC1A (A) and NIPBL (B), and the single change

detected in SMC3 (C) and STAG3 (D). The protein length is not in scale.

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Table 1. Components of cohesin pathway and their involvement in human diseases

Gene	OMIM	Role	Disease
SMC1A	300040	Core cohesin member	CdLS Colorectal cancer
SMC3	606062	Core cohesin member	CdLS Colorectal cancer
RAD21	606462	Core cohesin member	Breast cancer Prostate cancer
STAG1	604358	Core cohesin member	
STAG2	604359	Core cohesin member	
STAG3	608489	Core cohesin member	Colorectal cancer
ESCO2	609353	Cohesin regulatory, sister chromatid cohesion	RBS Melanoma cancer
NIPBL	608667	Cohesin regulatory, cohesin loading	CdLS Colorectal cancer
PDS5A	613200	Cohesin regulatory, sister chromatid cohesion	
PDS5B	605333	Cohesin regulatory, sister chromatid cohesion	CdLS

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PTTG1 (Securin)	604147	Cohesin regulatory, sister chromatid dissociation	Pituitary cancer
ESPL1 (Separase)	604143	Cohesin regulatory, sister chromatid dissociation	Breast cancer Osteosarcoma Prostate cancer
CDCA5 (Soronin)	609374	Cohesin regulatory, sister chromatid cohesion	
WAPL	610754	Cohesin regulatory, cohesin dissociation	Cervical Cancer

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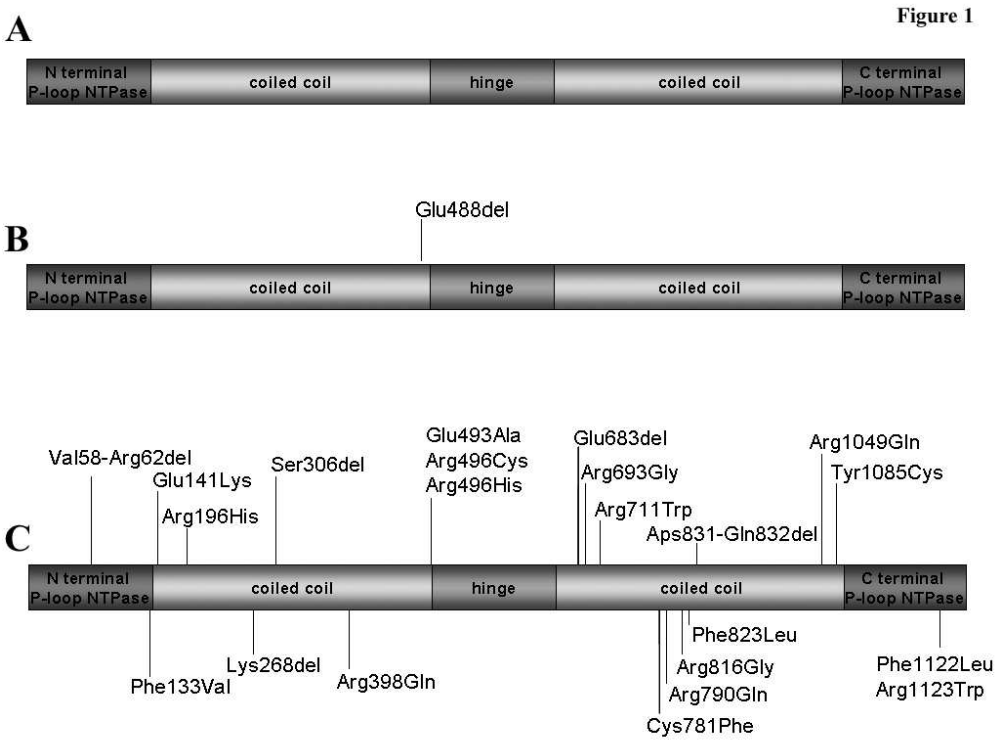

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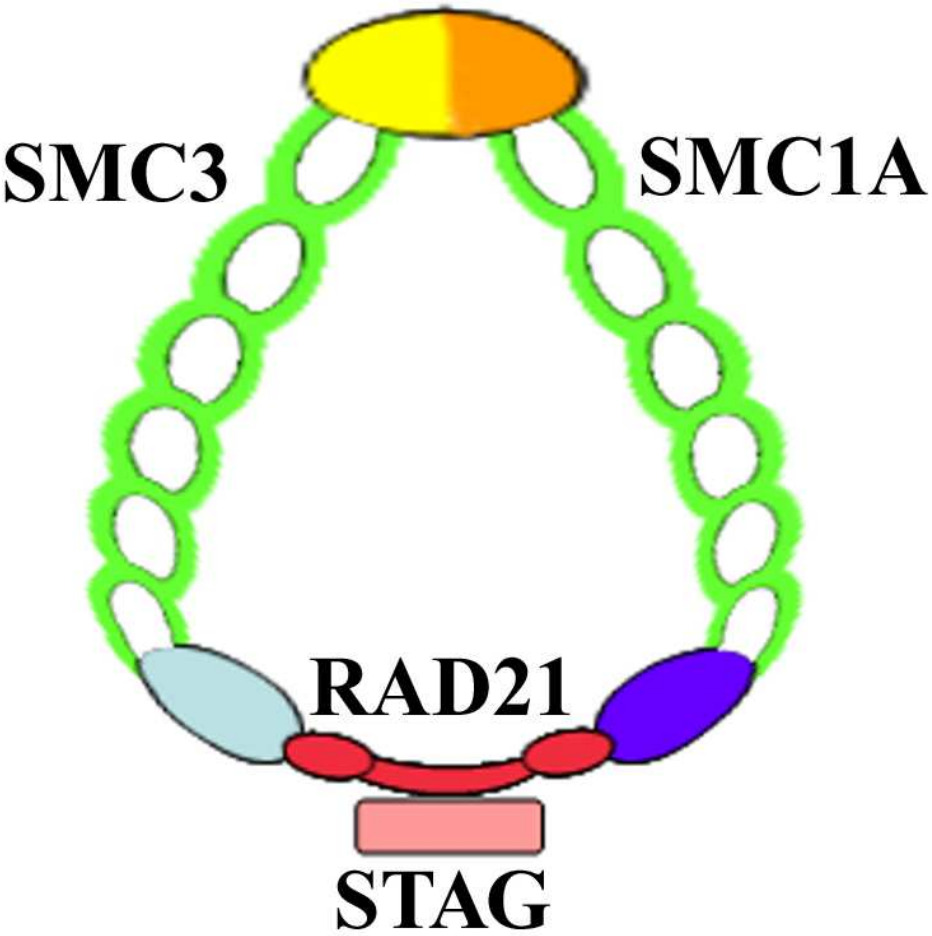
Figure 2



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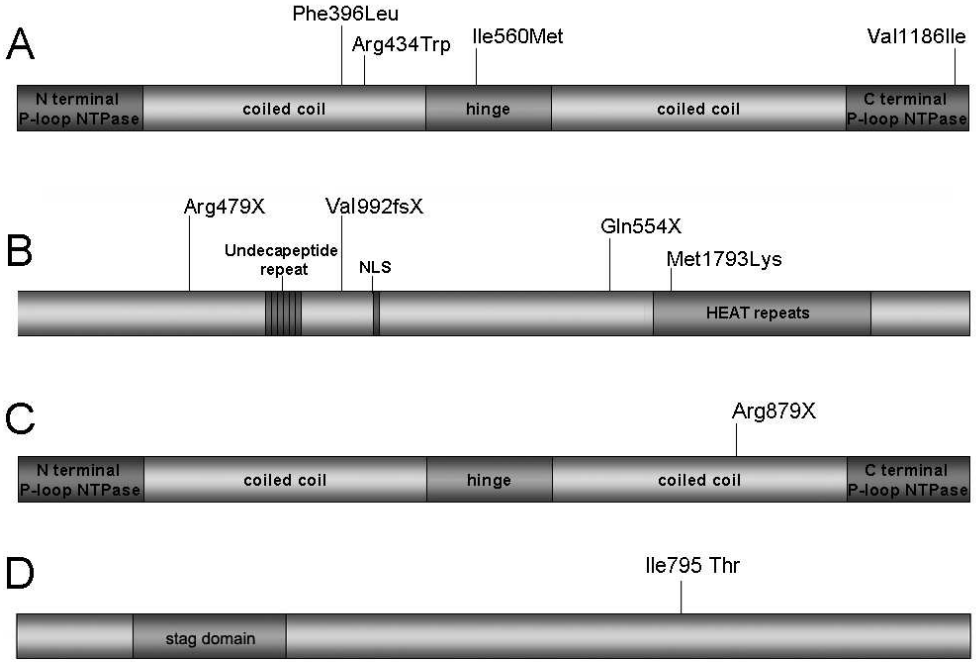

Figure 3

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Supp. Table S1. Mutational spectrum of the *SMC1A*, *SMC3*, *STAG3* and *NIPBL* in human disease

Gene	Nucleotide changes*	Effect on amino acid	Disease
SMC1A	c.173_187del15	p.Val58-Arg62del	CdLS
	c.397T>G	p.Phe133Val	CdLS
	c.421G>A	p.Glu141Lys	CdLS
	c.587G>A	p.Arg196His	CdLS
	c.802_804del3	p.Lys268del	CdLS
	c.916_918del3	p.Ser306del	CdLS
	c.1193G>A	p.Arg398Gln	CdLS
	c.1478A>C	p.Glu493Ala	CdLS
	c.1486C>T	p.Arg496Cys	CdLS
	c.1487G>A	p.Arg496His	CdLS
	c.2046_2048del3	p.Glu683del	CdLS
	c.2077C>G	p.Arg693Gly	CdLS
	c.2131C>T	p.Arg711Trp	CdLS
	c.2342G>T	p.Cys781Phe	CdLS
	c.2369G>A	p.Arg790Gln	CdLS
	c.2446C>G	p.Arg816Gly	CdLS
	c.2467T>C	p.Phe823Leu	CdLS
	c.2493_2495del3	p.Asp831-Gln832del	CdLS
	c.3146G>A	p.Arg1049Gln	CdLS
	c.3254A>G	p.Tyr1085Cys	CdLS
	c.3364T>C	p.Phe1122Leu	CdLS
	c.3367C>T	p.Arg1123Trp	CdLS
	c.1186T>C	p.Phe396Leu	Colorectal cancer
	c.1300C>T	p.Arg434Trp	Colorectal cancer
	c.1680C>G	p.Ile560Met	Colorectal cancer
	c.3556G>A	p.Val1186Ile	Colorectal cancer
SMC3	c.1464_1466del3	p.Phe488del	CdLS
	c.2635C>T	p.Arg879X	Colorectal cancer
STAG3	c.2384T>C	p.Ile795Thr	Colorectal cancer
NIPBL	c.1435C>T	p.Arg479X	Colorectal cancer
	c.2967_2968insT	p.Val992fsX	Colorectal cancer
	c.1660C>T	p.Gln554X	Colorectal cancer
	c.5378T>A	p.Met1793Lys	Colorectal cancer

* Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequences NM_006306.2 (for *SMC1A*), NM_005445.3 (for *SMC3*), NM_012447.2 (for *STAG3*), NM_015384.3 (for *NIPBL*) according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

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