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Cell Cycle News & Views

Cohesin complexes get more complex: The novel kleisin RAD21L

Comment on: Gutiérrez-Caballero C, et al. Cell Cycle 2011; 10:1477–87

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During meiosis, chromosomes acquire unique features: the two pairs of sister chromatids form axial elements (AEs) that synapse and form the synaptonemal complex (SC), telomeres attach to the nuclear membrane where they form a cluster that later disassembles, meiotic recombination between the two pairs of sister chromatids happens and chiasmata are formed as an obligatory structure that keeps the pairs connected in metaphase I. Mono-orientation of sister kinetochores and segregation of pairs of sister chromatids characterize the completion of meiosis I, while the reductional division of meiosis II is similar to a mitotic division.

Considering this astounding complexity, it is not surprising that meiocytes contain more than one type of cohesin complex (for cohesin reviews see refs. 1–3). The identification of REC8 as a meiosis-specific kleisin, i.e., a protein that closes the cohesin ring, followed by the description of the meiosis-specific SCC3-like SA3 (STAG3) protein and of a variant of the SMC1 protein called SMC1β, revealed a high variety among cohesin complexes. Most recently, three groups described yet another meiosis-specific cohesin subunit: RAD21L. Data mining by the groups of Yoshinori Watanabe,[4] Tatsuya Hirano[5] and, as reported in the May 1st issue of *Cell Cycle*, the group of Alberto Pendas led to the identification of this kleisin, which is expressed in spermatocytes and oocytes.

RAD21L localizes to emerging AEs at the entry into meiosis. Upon synapsis, RAD21L remains SC-associated until it gradually disappears towards the end of pachytene, at least on autosomes, even though the SC is still intact. This disappearance correlates with the dissolution of MSH4 foci and the appearance of MLH1 foci, indicators of sites of crossoves and chiasmata. Ishiguro et al. reported that in metaphase I spermatocytes, RAD21L is restricted to the centromere, while in metaphase I oocytes, RAD21L is not detectable. Staining beyond pachytene was not seen by Lee and Hirano and not reported by Guiterrez-Caballero et al. The staining pattern is roughly similar to that of REC8 but differs in important detail. REC8 may appear a bit later in leptotene, although several studies differ in this respect, and RAD21L may be involved in synapsis initiation. The two proteins localize in a mutually exclusive manner along pachytene chromosomes, as if RAD21L would support the chromosomal loop-axis structure at some locations and REC8 at others. RAD21 rarely colocalizes with RAD21L or REC8. Interestingly, the Hirano and Watanabe groups suggest loading of RAD21 complex(es) onto late pachytene chromosomes, which implies a replication-independent cohesin loading

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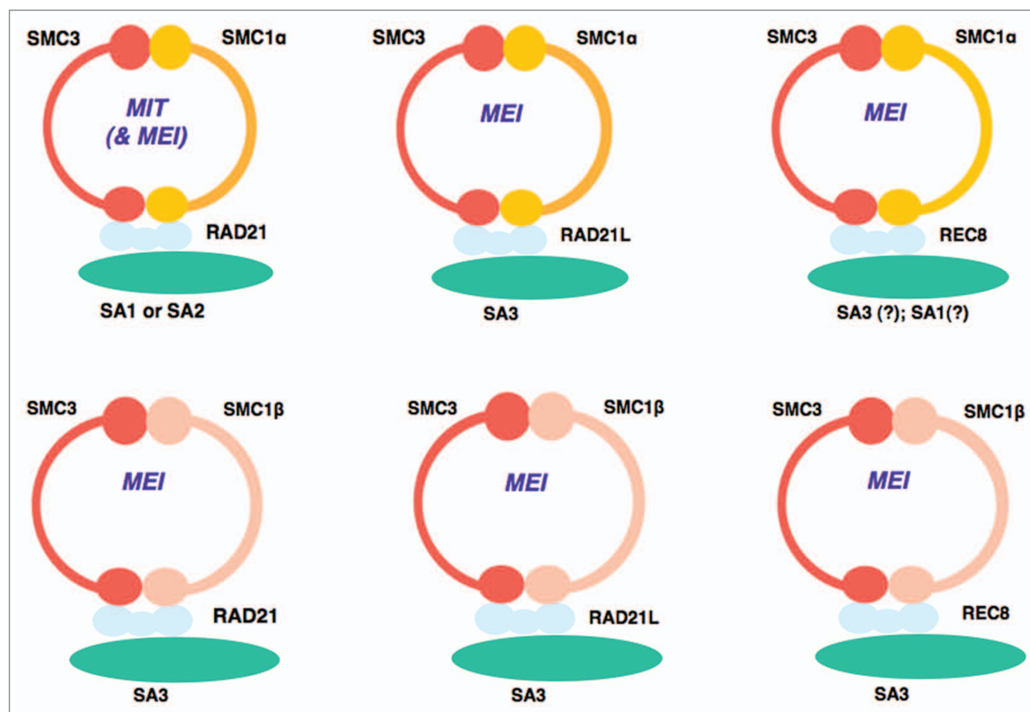

Figure 1. Cohesin complexes. Shown are cohesin variants for which evidence exists. The canonical cohesin complex is present in all somatic cells and likely in early meiocytes ("Mit & Mei"). All other complexes are meiosis-specific ("Mei"). There may be other cohesin variants as well.

mechanism. Since DNA double-strand breaks are largely repaired at that stage, breaks may not be required, which could be tested in a SPO11-deficient strain that lacks specific meiotic breaks.

How many cohesin complexes exist in meiocytes? Core to all cohesins is an SMC1/SMC3 heterodimer, and there are two types in vertebrates: SMC1α/SMC3 and SMC1β/SMC3. One of three different kleisins and of three different SCC3-type subunits may theoretically associate with either of them, yielding 18 different potential cohesin complexes. Even if only some of them are indeed formed in vivo, determining the composition and specific functions of all those complexes will keep researchers busy for years. Earlier data from a few labs suggested the existence of at least four types of cohesin complexes, and the Watanabe, Hirano and Pendas groups added some based on RAD21L (**Fig. 1**).

Precipitation experiments of RAD21L and other cohesin subunits either from primary testis cells or from cell lines that overexpress various cohesin proteins indicate that RAD21L associates with SMC1β/SMC3 or SMC1α/SMC3 and probably SA3. SA3 may be the only SCC3-type protein associating with meiosis-specific complexes. However, SA1 and SA2 can be observed in meiocytes, and whether they reflect a "leftover" mitotic cohesin complex only or are involved in genuine meiocyte complexes remains to be determined. It is obvious that none of these co-precipitation experiments proves the existence of these complexes in cells, since interactions can be indirect or could happen only in the lysate. Overexpression in cell lines may also force certain associations that may not occur under natural circumstances. Still, however, these data provide very valuable indications as to which type of complexes quite likely exist and act in meiocytes.

Much has been learned about the function of individual cohesin protein by generating mouse strains deficient in those proteins such as REC8[6,7] or SMC1β,[8] and more such mouse models will certainly be described in the near future. Only a combination of indirect protein interaction experiments, such as co-precipitations, direct interaction assays in vitro, cytogenetic localization studies throughout all relevant stages of gametogenesis, biochemical analysis and mouse genetics will, we hope, allow future research to appropriately approach the enormous complexity of cohesin complexes.

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Gene profiling for assessment of cell-based therapies

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Comment on: Soares MBP, et al. Cell Cycle 2011; 10:1448–55

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The paper by Soares et al.[1] in the May 1 *Cell Cycle* issue provides molecular evidence that adoptive transfer of bone marrow cells can modulate the expression of genes in an organ targeted by an infectious agent. Chagas disease is caused by infection with the protozoan parasite *Trypanosoma cruzi*, and approximately one-third of infected individuals develop a chronic cardiomyopathy. A mouse model of chronic Chagas disease was employed to test the hypothesis that transfer of bone marrow cells at the time of infection leads to changes in cardiac gene expression that coincide with reduction in disease pathology. In normal animals, the hearts of chronically infected mice display mononuclear cell infiltration, myocyte necrosis, fibrosis and edema, which is accompanied by upregulation in several thousand genes, including many of the immune response, as expected. Mice that were administered bone marrow cells showed a dampening of the expression of nearly all of these genes, with a increase in about a hundred others. What is unique about this study is the use of large-scale gene profiling to assess gene expression in the target organ. This unbiased approach may help to elucidate complex mechanisms of disease pathogenesis and suggest novel points of treatment, either via induction of protective genes or suppression of tissue-damaging inflammatory genes.

Although the unique feature of the paper is the use of gene profiling to monitor the effects of cell transfer, there are additional questions about cell-based therapy for Chagas disease raised by the study: does cell transfer reduce myocarditis if performed after initiation of disease? What types and numbers of cells within the bone marrow cell population are required for the cell-based therapy to be successful? What happens to gene expression in the heart and other organs of an uninfected animal upon cell transfer? Is parasitemia affected by cell transfer? These and many more questions will be answered in the coming years but monitoring bulk gene expression in this systematic way has many advantages over analysis of a small handful of potential mediators.

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Division of labor of the replication fork protection complex subunits in sister chromatid cohesion and Chk1 activation

Comment on: Smith-Roe SL, et al. Cell Cycle 2011; 10:1618–24

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Sister chromatid cohesion (SCC) is established during S phase near the replication fork. The cohesin complex, which has a major role in holding two sisters, consists of the Smc1, Smc3, Scc1/Mcd1 and Scc3 subunits (**Fig. 1**). It has been proposed that the cohesin complex forms a ring-like structure that is designed to entrap two sister chromatids. Since cohesin is loaded onto chromatin before DNA replication, the replication fork is thought to pass through cohesin rings as cells replicate chromosomes.[1-3] Considering that the replication fork contains the large replication machinery, cohesin rings may represent a significant obstacle for replication fork progression, leading to fork arrest at cohesin-bound chromosomal sites (**Fig. 1**). Accordingly, recent studies have focused on understanding how DNA replication is coordinated with the establishment of SCC.

It is widely understood that stalled forks activate the S-phase checkpoint. In response to stalled forks, the ATR kinase transduces a signal to phosphorylate the effector kinase Chk1 in a manner dependent on mediator proteins such as Claspin, Timeless and Tipin, resulting in cell cycle arrest to allow time for DNA repair (**Fig. 1**).[4] It has been proposed that S-phase checkpoint genes also safeguard sister chromatid cohesion in yeast.[5] However, how this checkpoint controls SCC is not known.

In a recent report, Smith-Roe et al.[6] carefully analyzed cohesion defects in the absence of Timeless and Tipin, which are known to form the replication fork protection complex (FPC).[7] Previous studies showed that the Timeless-Tipin FPC is involved in a variety of genome maintenance processes, including Chk1 activation, replication fork stabilization and SCC.[8] However, how the FPC coordinates such multiple mechanisms is enigmatic. Smith-Roe et al.[6] found that Timeless depletion causes a strong defect in SCC, whereas depletion of its partner Tipin has only minor effects on SCC. They also tested the involvement of other S-phase checkpoint factors, including ATR, Chk1 and Claspin. They found that Chk1 is not required for cohesion, while ATR and Claspin depletion only cause minor cohesion problems, similar to Tipin depletion. What are these results telling us? Why do these S-phase checkpoint factors have different influences on SCC?

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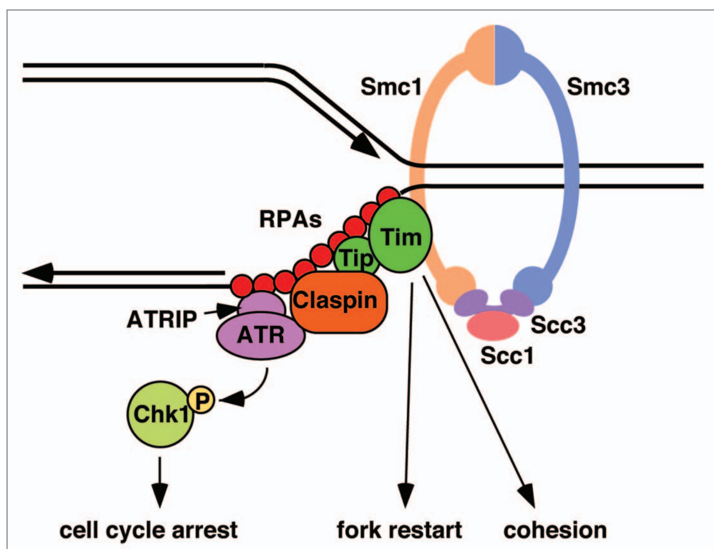

Figure 1. Separation of functions between Timeless (Tim) and Tipin (Tip). The replication fork may stall at cohesin-bound sites. ssDNA accumulated at stalled forks is coated by RPA bound by Tipin and ATRIP, which recruit Timeless and ATR, respectively. Tipin also recruits Claspin to mediate the ATR-Chk1-dependent checkpoint, resulting in cell cycle arrest. Timeless interacts with cohesins at the fork to help sister chromatid cohesion, while it facilitates the re-start of stalled replication forks.

It has been reported that Tipin binds replication protein A (RPA) and recruits Timeless to single-stranded DNA (ssDNA), an intermediate generated at stalled forks in response to replication stress.[9] Importantly, Tipin also recruits Claspin to ssDNA,[9] and Claspin is essential for the phosphorylation of Chk1 by ATR.[4] Since ATR is also recruited to ssDNA via interaction of its partner ATRIP and RPA,[4] it is suggested that Tipin-mediated Claspin recruitment to the fork promotes the phosphorylation of Chk1 by ATR, resulting in activation of Chk1 followed by cell cycle arrest (**Fig. 1**).[9] Then what is the role of Timeless? Timeless is also required for Chk1 activation.[8,9] However, its role in Chk1 activation may be stabilization of Tipin, as Timeless downregulation leads to the reduced level of Tipin.[10] These observations suggest that Timeless and Tipin have separate roles at the fork. Tipin seems to play an important role as a mediator of Chk1 activation by recruiting Claspin to ssDNA. In contrast, once recruited to the fork, Timeless appears to play a critical role in the establishment of SCC, which is independent of Tipin. Therefore, the work by Smith-Roe et al.[6] strongly suggests that there is a division of labor between Timeless and Tipin at the replication fork.

What would be the possible mechanisms for Timeless-dependent SCC? Interestingly, Leman et al. showed that Timeless interacts with cohesin subunits, whereas Tipin-cohesin interaction is weak. They also showed that cohesin subunits are dissociated from chromatin in Timeless-depleted cells.[7] It has been suggested that Ctf7/EcoI-dependent acetylation of cohesin loosens the ring to allow fast progression of the replication fork.[3] This situation could lead to temporal dissociation of cohesin from the chromatin, unless there is a mechanism to sustain cohesin subunits. Timeless seems to be in a perfect position to carry out this job, holding cohesin subunits

to prevent their dissociation from chromosomes when the fork passes through the ring. Since Timeless is also important for replication recovery after fork arrest,[7] it is also possible that Timeless regulates replisome assembly to allow for resumption of replication fork progression every time the fork stalls at cohesin sites. Further research would answer these questions and reveal the sophisticated mechanisms by which Timeless coordinates replication fork progression and SCC.

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Rae1: A new clue for nucleoporin leukemias

Comment on: Funasaka T, et al. Cell Cycle 2011; 10:1456–67

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Nuclear pore complexes (NPCs) are large proteinaceous assemblies that perforate the double membrane of the nuclear envelope and provide the only passageway into and out of the nucleus. NPCs are composed of ~30 distinct proteins, collectively termed nucleoporins or nups.[1] A striking correlation exists between mutations of nucleoporins that are involved in mRNA export and the development of various forms of leukemia. Oncogenic fusions of Nup98 share a common protein architecture, in which the N-terminal part of Nup98 is fused to a variety of partners, including various members of the homeodomain (HOX) transcription factor family.[2] The N-terminal part of Nup98 is composed of phenylalanine-glycine (FG) repeats and a 57-residue Gle2-binding sequence (GLEBS) and facilitates their binding to the transcriptional regulators histone deacetylase 1 (HDAC1) and CREB binding protein (CBP)/p300 as well as the mRNA export factor Ribonucleic acid export 1 (Rae1), respectively.[3-6] In addition to its role in mRNA export, Rae1 is involved in mitotic spindle pole assembly and chromosome segregation. Deregulated levels of Rae1 have previously been linked to aneuploidy.[7]

A prominent Nup98 fusion found in acute myelogenous leukemia (AML) is Nup98-HOXA9.[2] The oncogenic potential of this fusion was believed to result exclusively from transcriptional misregulation. This notion originated from the observation that the Nup98-HOXA9 fusion fails to localize to the nuclear envelope and interacts with the transcriptional modulators HDAC1 and CBP/p300, leading to the downregulation of HOX target genes.[6,8] Funasaka et al. have now added a twist to this model, and demonstrate that the overexpression of the Nup98-HOXA9 fusion also results in Rae1 mislocalization to the interior of the nucleus and, unexpectedly, to a reduced cellular concentration of Rae1.[9] Consistently, the authors demonstrate that the Nup98-HOXA9 fusion binds to the Rae1 promoter region and show that bone marrow samples derived from Nup98-HOXA9-positive leukemia patients display a substantial reduction of Rae1 mRNA levels. These results provide an exciting new link between Nup98 fusion-mediated leukemogenesis and the transforming properties of Rae1 in Nup98-HOXA9 patients. Future studies need to address whether such a direct link between mislocalization and downregulation of Rae1 and the leukemogenic potential of other oncogenic Nup98 fusions exists.

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A novel Rad18 ubiqitin ligase-mediated pathway for repair of camptothecin-induced DNA damage

Comment on: Palle K, et al. Cell Cycle 2011; 10:1625–38

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Translesion synthesis (TLS) is a mechanism that allows replication of DNA templates containing bulky lesions and is performed by specialized TLS DNA polymerases, such as DNA polymerase η (Polη). The Rad18 E3 ubiquitin ligase is a pivotal enzyme for regulation of Polη. Rad18 guides Polη to sites of replication stalling and monoubiquitinates PCNA at stalled replication forks, promoting a "polymerase switch" that replaces conventional replicative DNA polymerases with Polη or other TLS polymerases.[1,2]

Rad18-deficiency causes increased sensitivity to broad range of replication fork-stalling DNA lesions, including cyclobutane-pyrimidine dimers (CPD), benzo[*a*]pyrene and cisplatinum adducts, DNA inter-strand cross-links (ICLs) and DNA double-stranded breaks (DSB), arising from ionizing radiation or the cytotoxic quinoline alkaloid camptothecin (CPT).

Cellular responses to CPT and its analogs are of great interest therapeutically, because these compounds display anticancer activity and are used broadly in the clinic. The cellular target of CPT is the enzyme Topoisomerase I (TopI). CPT forms a ternary complex with TopI-DNA and prevents TopI-mediated DNA religation activity. The resulting ssDNA break is converted to DSB during DNA replication.

Defects in TLS polymerases do not confer DSB sensitivity. Therefore, the DSB-sensitivity of Rad18-deficient cells reflects Rad18 participation in non-TLS pathways. Indeed, TLS-independent roles for Rad18 in DNA repair have been described: Rad18 facilitates DSB tolerance during G_1 by ubiquitinating 53BP1 and promoting its retention at sites of DNA damage.[3] Additionally, Rad18 recruits Rad51 and its paralogs to DSB, thereby promoting Homologous Recombination (HR) in an E3 ubiquitin ligase activity-independent manner.[4] Thus, Rad18 repair activities are dissociable in terms of their dependence on E3 ubiquitin ligase activity. A new study by Palle and Vaziri identifies a role for Rad18 in another DNA repair process, namely the Fanconi Anemia (FA) pathway.

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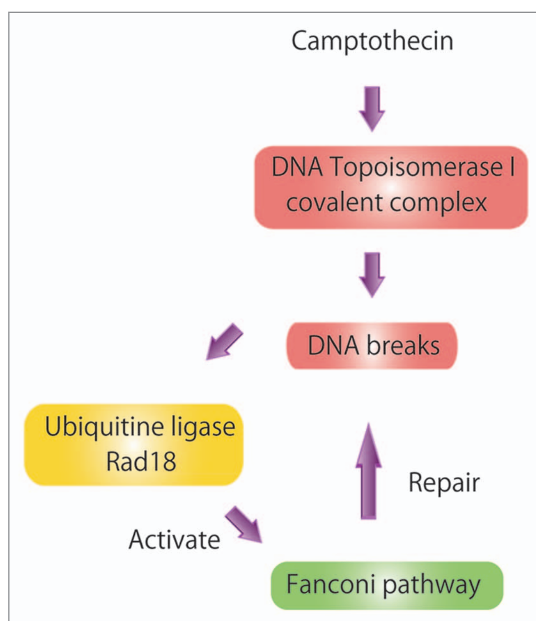

Figure 1. Camptothecin (CPT) forms a ternary complex with TopI and its DNA substrate. The CPT-bound ternary complex is stabilized and fails to religate TopI-induced single-stranded DNA nicks. Upon encountering DNA replication forks, the CPT-induced nicks are converted to DSB that activate Rad18. Rad18 E3 ligase activity toward unknown substrate(s) contributes to FA pathway activation and recruitment of Rad51 to replication-induced DSB, promoting HR and damage tolerance.

FA is a human genetic disorder characterized by congenital abnormalities and cancer predisposition. A hallmark of FA patient cells is hypersensitivity to agents that induce ICL (and moderate sensitivity to other genotoxins). Within the FA pathway, the multi-protein FA "core complex" acts as an E3 ubiquitin ligase that catalyzes monoubiquitylation of both FANCI and its binding partner FANCD2 (termed the "ID" complex). The ubiquitylated ID complex associates tightly with chromatin at sites of DNA damage and is a major effector of the FA pathway, apparently promoting repair of ICL and possibly other forms of DNA damage.[5]

Bulky DNA adducts and ICL induce PCNA monoubiquitylation and activate the TLS pathway via Rad18. Recent evidence indicates Rad18-mediated crosstalk between the FA and TLS pathways.[6-8] Thus, Rad18-mediated PCNA monoubiquitination is required for FA pathway activation via a mechanism involving recruitment of FANCL to chromatin and subsequent ID complex monoubiquitination.[6,7]

The report by Vaziri and Palle[9] indicates that a new PCNA ubiquitination-independent mechanism also links Rad18 to the FA pathway. Similar to previous studies with ICL and bulky DNA lesions, Palle and Vaziri show that CPT treatment induces FANCD2 monoubiquitination in a Rad18-dependent manner (**Fig. 1**). Rad18 and FANCD2 function in a common or partially overlapping pathway to confer CPT tolerance. Importantly, CPT does not induce detectable PCNA monoubiquitination. Thus, in response to CPT-induced DSB, Rad18 is unlikely to activate the FA pathway via a PCNA monoubiquitination-based mechanism. A PCNA-independent role of Rad18 in DSB repair has also previously been proposed by Chen and colleagues.[4] However, in those studies, Rad18 catalytic activity was dispensable for HR, whereas the new study shows that Rad18 E3 ubiquitin ligase activity is essential for FA pathway activation and damage tolerance after CPT treatment.

An important conclusion of the new study is that Rad18 substrate(s) other than PCNA are necessary for FA pathway activation in response to CPT.[9] Other known Rad18 substrates include RFC2 and 53BP1. Further work is necessary to determine whether ubiquitination of 53BP1, RFC2 or other putative Rad18 substrates contributes to FA pathway activation and CPT tolerance. The new work also invites questions regarding the mechanism(s) by which Rad18-FA pathway activation promotes repair or tolerance of CPT-induced DNA breaks. For example, it will be important to determine whether the choice of HR vs. lethal NHEJ-mediated mechanisms for DSB processing is influenced by Rad18. It will also be very interesting to determine whether RAD18 mutations (or other defects in Rad18 signaling) are associated with FA or FA-like disorders in human patients.

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