

Overcoming the chromatin barrier to end resection

Cell Research (2013) 23:317-319. doi:10.1038/cr.2012.148; published online 13 November 2012

Repair of double-strand breaks by homologous recombination requires 5'-3' resection of the DNA ends to create 3' single-stranded DNA tails. While much progress has been made in identifying the proteins that directly participate in end resection, how this process occurs in the context of chromatin is not well understood. Two papers in *Nature* report that Fun30, a poorly characterized member of the Swi2/Snf2 family of chromatin remodelers, plays a role in end processing by facilitating the Exo1 and Sgs1-Dna2 resection pathways.

DNA double-strand breaks (DSBs) are highly cytotoxic lesions that must be repaired appropriately to prevent the formation of deleterious chromosome rearrangements associated with tumorigenesis. Cells use two major pathways to repair DSBs: homologous recombination (HR) and non-homologous end joining (NHEJ). Repair by HR requires a homologous donor duplex and is considered a high-fidelity process, whereas the homology-independent end joining pathway involves re-ligation of the broken ends and is more error prone. A critical determinant of repair pathway choice that commits cells to HR instead of NHEJ is the initiation of 5'-3' resection of the DSB ends [1]. Genetic studies in *Saccharomyces cerevisiae* identified the Mre11-Rad50-Xrs2 (Xrs2 is known as NBS1 or NBN in human) complex and Sae2 as key factors in the initiation of resection by removing oligonucleotides from the 5' ends to form short 3' single-stranded DNA (ssDNA) tails, while the Exo1 exonuclease or the Sgs1 helicase functioning with the Dna2 endonuclease promote extensive

resection in a redundant manner [2-4]. Resection has been reconstituted *in vitro* with these proteins [5-7]; however, additional factors must be present *in vivo* to facilitate resection in the context of chromatin.

Previous studies have suggested that cells use both histone modifying and remodeling complexes to relax chromatin and hence facilitate DNA repair. After sensing of a DSB by the MRX complex, the Tel1 kinase (ATM in human) is activated and phosphorylates histone H2A over a large region from the break site, followed by histone acetylation that unwinds chromatin and facilitates the recruitment of remodeling complexes [8]. ATP-dependent remodelers are large multi-subunit complexes that couple ATP hydrolysis to movement of histones or nucleosomes, including exchange or incorporation of core histones or histone variants, eviction of histones or nucleosomes, and repositioning or sliding of nucleosomes, thereby modifying chromatin structure [9]. Several chromatin remodeling complexes, including INO80, SWR1, SWI/SNF and RSC in budding yeast, have been reported to participate in the DNA damage response. It has been proposed that INO80 facilitates the eviction or sliding of nucleosomes in the immediate vicinity of the break site to allow 5'-3' strand resection [10]. The SWR1 complex was suggested to exchange modified histones after repair, while SWI/SNF may facilitate clearing of nucleosomes surrounding the break site prior to Rad51-mediated strand invasion [8]. RSC is believed to affect resection initiation by facilitating Mre11 binding [9].

Two recent studies report that Fun30, a poorly characterized ATP-dependent chromatin remodeler, promotes DNA end resection in *Saccharomyces cerevisiae*. Both groups identified Fun30 by genome-wide screens for mutants with increased frequencies of recombination between a transformed linear DNA fragment and homologous chromosomal sequences. Chen *et al.* [11] found that deletion of *FUN30* caused increased gene targeting, while Costelloe *et al.* [12] found higher break-induced replication and gap repair efficiencies in the *fun30Δ* mutant, properties shared by the resection mutants *sgs1Δ* and *exo1Δ*. Using several different assays to monitor the formation of ssDNA at endonuclease-induced DSBs, both groups demonstrated that Fun30 promotes extensive resection by Exo1-dependent and Sgs1-Dna2-dependent pathways (Figure 1). Indeed, both the *fun30Δsgs1Δ* and *fun30Δexo1Δ* double mutants exhibited a more severe resection defect than any of the three single mutants [11, 12].

The effect of Fun30 on end resection could be direct or indirect. Evidence in support of a direct role was provided by both studies showing that Fun30 localized to DSBs and along the DNA from the break site with similar kinetics as Sgs1, Dna2 and Exo1 [11, 12]. Furthermore, Chen *et al.* [11] showed that Fun30 co-immunoprecipitates with RPA, Dna2 and Exo1, and enrichment of these resection factors at DSBs was reduced in the *fun30Δ* mutant. In addition, overexpression of Exo1 in the *fun30Δ* strain was able to rescue both resection and resistance to the topoisomerase I inhibitor camptothecin (CPT) [12].

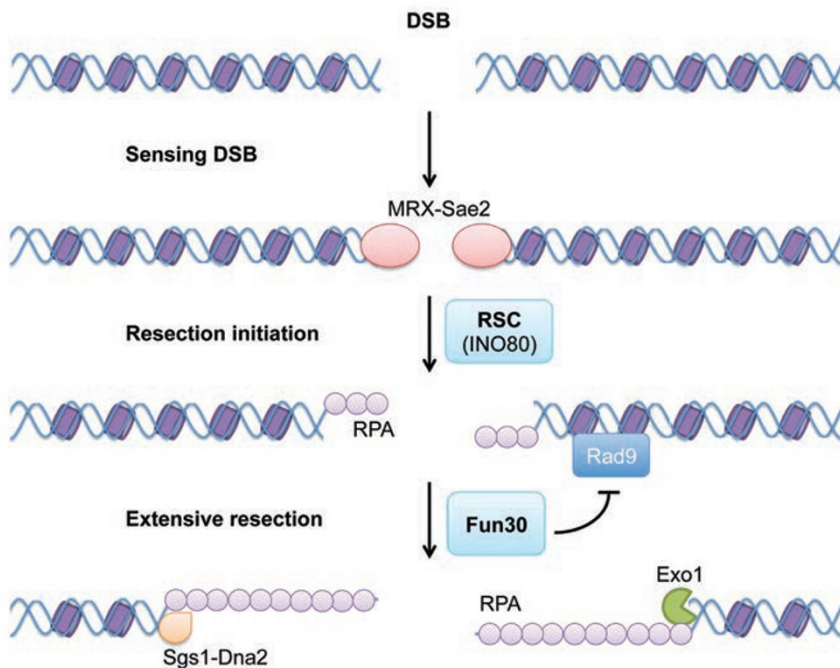


Figure 1 The involvement of chromatin remodelers in DSB end resection. Resection initiation is stimulated by RSC and to a lesser extent by INO80. Fun30 works with RPA, Dna2 and Exo1 to promote extensive resection, possibly through overcoming the resection barrier formed by Rad9-bound chromatin.

These data suggest a direct involvement of Fun30 in long-range end resection, possibly through its interaction with extensive resection factors. It remains to be determined whether Fun30 directly recruits the resection machinery, or Fun30-mediated chromatin remodeling facilitates access of resection proteins to ssDNA.

Importantly, the ATPase activity of Fun30, which is essential for its chromatin remodeling activity [13], was found to be required for efficient resection and resistance to CPT [11, 12], indicating a correlation between the two processes. Chen *et al.* [11] reported impaired recruitment of Fun30 to DSBs in the resection-defective *mre11Δ* and *sgs1Δexo1Δ* mutants, suggesting that Fun30-mediated chromatin remodeling is coupled with resection. Consistently, ChIP analysis of histone H3 and H2B occupancy around an endonuclease-induced DSB showed the same trend as resection in wild-type, *fun30Δ* and

sgs1Δexo1Δ cells [11, 12]. Further studies are needed to investigate which one is the causal process, histone eviction or resection. Although histone loss appeared to be slower in *fun30Δ* and *sgs1Δexo1Δ* than in wild-type cells, it could be due to impaired long-range resection. Thus Fun30 does not seem to function via evicting histones. It remains to be determined how Fun30 remodels chromatin structure to facilitate resection.

Costelloe *et al.* [12] extended their findings to human cells by showing that SMARCAD1, the potential human counterpart of Fun30, participates in end resection. SMARCAD1 co-localizes with γ H2AX to DSBs and the pattern of its accumulation at DSBs is similar to that of Exo1. Knockdown of SMARCAD1 caused a dramatic reduction in ionizing radiation-induced ssDNA formation and RPA loading, indicating impaired resection. Accordingly, cells depleted of SMARCAD1 displayed

hypersensitivity to genotoxic drugs and reduced HR.

Previous studies suggested that the ATP-dependent nucleosome remodeling complexes, INO80, RSC and SWR, affect resection. Chen *et al.* [11] sought to characterize the genetic interaction of these remodelers with Fun30 in promoting resection. Of all the single mutants, *fun30Δ* showed the strongest phenotype. Deleting components of the INO80 or RSC complexes together with Fun30 further delayed resection and elimination of all three remodeling factors resulted in a severe resection defect, indicating that Fun30 is the primary activity with RSC and INO80 playing redundant roles (Figure 1).

A further clue to the mechanism by which Fun30 promotes resection was revealed by its genetic interaction with Rad9, a histone-bound checkpoint mediator known to inhibit resection. Surprisingly, *rad9Δ* was able to suppress the resection defect of *fun30Δ* [11], suggesting that Fun30 is able to overcome the barrier to resection by Rad9-bound chromatin. Consistent with this hypothesis, elimination of Fun30 led to more Rad9 accumulation at DSBs. Understanding how Fun30 is recruited to DSBs and how it recruits other factors is likely to shed some light on its role in resection. γ H2A is required for the recruitment of INO80 and SWR [8], while recruitment of RSC absolutely requires Mre11 and partially depends on γ Ku70 [14]. It will be interesting to know whether Fun30 directly interacts with γ H2A and Rad9, since Rad9 is partly recruited by γ H2A. According to Chen *et al.*, recruitment of Fun30 and extensive resection factors to DSBs occurs in a mutually dependent manner. One possible explanation for this paradox is that some initial binding of extensive resection factors facilitates Fun30 localization, which in turn remodels the chromatin and makes it more accessible for more resection factors, forming a positive feedback loop.

As more ATP-dependent chromatin

remodelers with roles in DNA DSB repair are identified, more questions regarding their apparent functional redundancy are raised. Why do cells need so many complexes to remodel chromatin during DSB repair? How is their sequential recruitment to DSBs regulated and does the apparent redundancy reflect the ordered recruitment? Is recruitment of the early- and late-acting chromatin remodelers coordinated? Do the chromatin remodelers that facilitate end resection participate in later steps of repair, such as invasion of the donor locus by the Rad51-ssDNA complex and resolution of recombination intermediates?

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