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Sir-Ku-itous Routes to Make Ends Meet

Minireview

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Barbara McClintock first noticed that the normal ends of chromosomes were different from ends created by chromosome breakage. We now understand that normal ends are capped by proteins that bind to the repeating DNA sequences at telomeres; this apparently protects them from engaging in end-to-end fusions and other recombination events that frequently occur at the ends of a double-strand break made elsewhere in a chromosome. How then are we to explain that many of the proteins that seem to be essential for normal telomere structure and function are also implicated in the end fusion process? Two papers studying the response to DNA damage in yeast (Martin et al., 1999; Mills et al., 1999) have focused attention on this fascinating question and revealed even more layers of complexity: the Ku and Sir proteins residing at telomeres relocalize in response to DNA damage, and this process is under the control of the cell's DNA damage checkpoint genes.

Ku and Sir Proteins at Telomeres

The telomeres of *Saccharomyces* have an irregular repeating sequence of $(TG)_{1-6}TG_{2-3}$, to which are bound a number of proteins critical for their replication and maintenance. Also found are the silent information regulator proteins Sir2, Sir3, and Sir4 that establish heterochromatic chromatin structures not only near telomeres but at the silent mating-type loci *HML* and *HMR* (Grunstein, 1998). Deletion of one of the *SIR* genes causes the derepression of epigenetically silenced genes adjacent to telomeres.

Also in residence at telomeres are the yeast homologs of the DNA end–binding Ku proteins, yKu70 and yKu80. There is some thought that Ku proteins help protect telomere ends. For example, when another telomere-binding protein, Cdc13, is defective, the absence of either yKu70 or yKu80 is lethal (Nugent et al., 1998).

Chromatin immunoprecipitation of DNA sequences associated with yKu80p confirmed that the Ku proteins are associated with telomeres (Gravel et al., 1998). Immunofluorescent marking of yKu80p confirms that it colocalizes with Sir proteins and Rap1p at the several clusters of telomeres at the periphery of the yeast nucleus (Martin et al., 1999). Moreover, yKu70p was shown to bind to Sir4p by a two-hybrid assay (Tsukamoto et al., 1997). Similar to *sir* mutants, deletion of *YKU70* or *YKU80* causes the loss of the silencing of telomere-adjacent genes (Boulton and Jackson, 1998; Laroche et al., 1998; Nugent et al., 1998), but unlike the *sir* mutants, the absence of Ku proteins causes the delocalization of telomeres from the nuclear periphery (Laroche et al., 1998).

Now, Martin et al. (1999) have gone further and made a surprising discovery. By quantitative chromatin precipitation analysis, they show that the Ku proteins are not simply found at the chromosome end, but along the silenced adjacent region, as are the Sir proteins (Figure 1). But while the absence of Sir proteins does not cause the loss of Ku proteins from telomeres (though it does reduce Ku association with telomere-adjacent DNA sequences), the absence of Ku proteins results in the loss of telomere-associated Sir proteins. This gives Ku proteins a more prominent role in heterochromatin assembly at telomeres than previous models would have predicted.

Before turning to the roles of Sir and Ku proteins in DNA damage repair, we should also acknowledge the participation of another family of proteins that play roles at both telomeres and during DNA repair. Among their many functions (reviewed by Haber, 1998), the Mre11-Rad50-Xrs2 family of proteins affect telomere maintenance; their absence also causes telomere shortening, but no loss of telomere silencing.

Therefore, a completely normal telomere depends on yKu70, yKu80, Mre11, Rad50, Xrs2, Sir2, Sir3, and Sir4. Surprisingly, all of these proteins have now been implicated in the repair of broken DNA ends.

Ku and Sir Proteins during NHEJ

A chromosome suffering a double-strand break (DSB) can be repaired either through homologous recombination or by nonhomologous end joining (NHEJ) (reviewed by Jeggo, 1998). NHEJ is quite evident in mouse cells lacking telomerase, where telomeres shorten and frequently engage in end-to-end fusions (Lee et al., 1998a). In mammals, end joining is quite efficient, and even blunt-ended DNA molecules, transfected into cells, are joined together. An analysis of the junctions shows that most ends have been joined at sites where one or a few base pairs could be made, often resulting in various deletions or sometimes in small insertions. In both yeast and mammals, NHEJ requires DNA ligase IV and its associated XRCC4 protein.

In yeast, and probably in mammals, there are actually several related, but distinct, end joining processes. Rejoining of 4 bp complementary ends, created in vitro or in vivo by endonucleases, is surprisingly efficient, with 30%–50% of the molecules being rejoined, and almost all of them precisely religated. But when DNA ends are not complementary, or when the HO endonuclease is continually expressed in vivo (so that simply religating the ends will lead to their recutting), repair is inefficient. There are two alternative outcomes: small insertions, resulting from misalignment and filling-in of the ends, or deletions that remove a few or a few thousand base pairs (Moore and Haber, 1996). Interestingly, the deletion pathway in yeast occurs at all stages of the cell cycle, whereas the filling-in process is restricted to the S and/or G2 stages (Moore and Haber, 1996). Moreover, deletion events are largely independent of Mre11, Rad50, and Xrs2, but both filling-in and the efficient religation mechanisms depend on these proteins.

Ku proteins bind to DNA ends and facilitate end joining in vitro. Yeast Ku proteins are required for end joining by any of the three pathways. Mammalian Ku proteins are similarly essential in V(D)J rearrangements of the

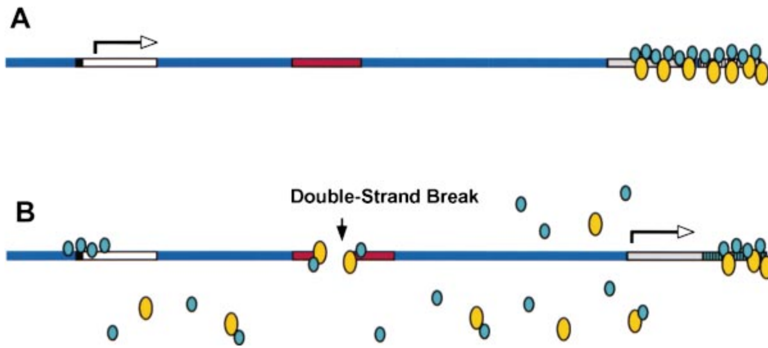

Figure 1. Relocalization of Ku and Sir Proteins after DNA Damage

(A) In the absence of chromosomal DSBs, Ku proteins (yellow) and Sir proteins (aqua) are localized at telomeric and subtelomeric regions, where genes are epigenetically repressed. This can be demonstrated by chromatin immunoprecipitation, in which DNA sequences cross-linked to proteins are immunoprecipiated with antibodies against a protein, purified, and identified by PCR.

(B) When a DSB is introduced within the chromosome, Ku and Sir proteins delocalize from the telomeres and are found throughout the nucleus, but they also are localized to sites of DSBs. Genes near telomeres that were epigenetically silenced by Sir and Ku proteins are now more strongly transcribed, but genes with adjacent silencer sequences, located more internally on the chromosome, become more repressed.

immune system or for the joining of linear, transfected DNA. In mice and humans, efficient end joining also requires the Ku-associated protein kinase DNA-PK (reviewed by Jeggo, 1998). Although yeast have several PI-kinase-related proteins, none seems to fulfill the role of DNA-PK; rather, Mec1 and Tel1 are more related to the mammalian ATM and ATR proteins involved in DNA damage checkpoints. In mammals, damaged DNA ends are also bound by poly(ADP-ribose) polymerase, whose action may stabilize ends or open up chromatin for repair; but again this activity has not been found in yeast (reviewed by Lindahl et al., 1995).

As surprising as the fact that Ku proteins play a decisive role at telomeres was the report by Tsukamoto et al. (1997) that *SIR* proteins were as important as Ku or Mre11 for efficient plasmid religation. This led to the speculation that a key step in NHEJ might be to prevent extensive resection of the ends by nucleases, by making a heterochromatic structure involving the Sir proteins and thus facilitate end joining mediated by Ku and other proteins. Alternatively, Sir proteins might act indirectly, for example by regulating the expression of other genes that play a role in DNA repair. One way to address this would be to see whether Sir proteins actually associate with DSBs. By chromatin immunoprecipitation, both Mills et al. (1999) and Martin et al. (1999) have shown that Sir3p does indeed show up at broken chromosome ends.

Ku and Sir Respond to DNA Damage

Mills et al. (1999) and Martin et al. (1999) provide fascinating cytological and molecular evidence that both Ku and Sir proteins respond to the presence of even a single DSB, by delocalizing from telomeres and binding to the ends of DSBs. Both labs examined the effect of growing yeast in the presence of the DNA-damaging agents MMS and bleomycin. Both labs also take advantage of a galactose-induced HO endonuclease gene in a strain where there are no homologous sequences for homologous recombination and hence the DSB can only be repaired by NHEJ (Moore and Haber, 1996).

In addition, Mills et al. (1999) examine cells where the restriction enzyme EcoRI is expressed and chromosomes are cleaved at many sites. Previously Lewis et al. (1999) showed that nearly 100% of cells could survive such damage, despite the fact that an analysis of purified DNA showed that every chromosome had been cut several times. EcoRI creates a strange kind of DSB damage, in which the ends must never fly apart, else lethal deletions between adjacent EcoRI sites would have been expected. Repair is Ku dependent and largely independent of the homologous recombination pathway.

In the presence of any of these types of damage, four important changes are noted. First, both Ku and Sir proteins partially dissociate from telomeres, and especially from subtelomeric regions, and can be detected diffusely all over the nucleus (Figure 1B). This is even the case when a single, unrepaired DSB is induced by HO endonuclease cleavage of a unique locus. Thus, the proteins are not simply being titrated to sites of DNA damage. Delocalization does not occur after UV damage, which does not generate DSBs.

One striking result reported by Mills et al. (1999) is that Sir3p delocalization is cell cycle dependent. Thus, both with MMS treatment or after EcoRI cleavage, cells have a punctate (telomere-localized) pattern for Sir3p in G1 cells, but the protein delocalizes as cells progress into S phase. The punctate staining reappears as cells enter G2. In a logarithmically growing population, only 20% of cells show delocalization. The restoration of punctate localization is less evident after some types of DNA damage. Why might the damage response be S phase dependent? Mills et al. suggest that both MMS and EcoRI damage might only be "revealed" when a replication fork passes the damaged or cleaved site.

A second major finding is that DNA damage causes a moderate derepression of telomere-silenced markers, again suggesting the loss of Sir proteins from telomeres, though not nearly as profoundly as when yKu80 is deleted.

Third, by chromatin immunoprecipitation analysis, both groups confirm that there is a loss of a substantial portion of both Ku and Sir3p from telomeres. Loss of Sir3p and Ku is more complete at telomere-adjacent sites than at the telomere itself.

Fourth, after DNA damage by EcoRI or HO endonuclease, the Ku and Sir proteins can be found at or near

the new DSB ends (Figure 1B). Martin et al. report that yKu80p appears at sites of DNA damage 2 hr earlier than Sir3p. This might mean that Sir proteins are simply coming along for the ride because of the association of Sir4p with yKu70p. It should be possible to carry out a kinetic analysis of NHEJ to see whether it is delayed until the time Sir proteins arrive on the scene.

Relocalization of Ku and Sir Is under the Control of DNA Damage Checkpoint Genes

The other key finding in these two papers is that the loss of Ku and Sir3p from telomeres, especially subtelomeric regions, after DNA damage is under the control of the checkpoint genes *RAD9* and *MEC1*. Normally after a cell suffers an unrepaired DSB, it pauses for many hours at the G2/M checkpoint, but there is no arrest in *rad9* or *mec1* cells (Lee et al., 1998b). Now, in addition to controlling the onset of mitosis, checkpoint genes are implicated in communicating with proteins sequestered at telomeres. There is not much delocalization of either yKu80 or Sir3p after DNA damage in checkpoint-defective cells. This important observation raises many questions about how the checkpoint apparatus is linked to the machinery that causes relocalization of telomere-associated proteins.

Yet, the importance of this checkpoint-dependent regulation in terms of DNA repair is not yet established. When chromosomes are assaulted by EcoRI, their repair is strongly *RAD9* dependent as well as Ku dependent (Lewis et al., 1998). But this does not demonstrate that the death of *rad9* cells is attributable to the failure to delocalize most of the Ku or Sir proteins from telomeres. Death could result from a failure to arrest in G2/M, so that cells do not have sufficient time to repair DNA before damaged or broken chromosomes are segregated during mitosis. With less extensive damage, such as repair of a single chromosomal DSB or the ligation of transformed linear DNA, a *rad9* mutation reduces repair by no more than a factor of two (Lee et al., 1999; Mills et al., 1999).

Because Sir and Ku protein delocalization can be triggered by a single DSB, we can rule out the idea that they are all bound at sites of DNA repair. Normally cells do not experience massive numbers of DSBs, so the teleology of telomere reorganization is hard to imagine on that score. In fact, yeast are quite sensitive to the amount of DNA damage; they will arrest at G2/M but then adapt and grow when the cell has suffered a single unrepaired DSB, but they become permanently arrested with as few as two such breaks (Lee et al., 1998b).

Is the Effect of sir *Mutations on NHEJ Indirect?*

Recent results have raised the possibility that the effect of Sir proteins on NHEJ is largely a reflection of their role in regulating mating-type gene expression (Åström et al., 1999; Lee et al., 1999). When *SIR* genes are deleted, haploid yeast cells express the normally silent mating-type cassettes, *HML*α and *HMR***a**, causing the haploid cell to have the nonmating phenotype of a *MAT***a**/*MAT*α diploid. Cells expressing both *MAT***a** and *MAT*α genes turn off a set of haploid-specific genes and turn on other diploid-specific genes. Thus, *MAT***a**/*MAT*α diploids are more X-ray resistant than diploids expressing a single mating type and have higher levels of both spontaneous and DSB-induced recombination (Heude and Fabre, 1993; Lee et al., 1999). Yeast cells apparently "know" they are diploid or haploid by their mating type: in nature, haploids mate and diploids don't.

It makes sense that haploid cells would wish to upregulate NHEJ, since in the G1 stage of the cell cycle they must rely on NHEJ to repair breaks, whereas diploid G1 cells always have a homologous chromosome present to act as a template for homologous recombination. Two observations support the idea that *sir* mutants predominantly affect NHEJ by affecting mating type (Åström et al., 1999; Lee et al., 1999). First, haploid *sir2* or *sir3* or *sir4 MAT***a** cells that lack *HML*α, and thus remain a mating, are as efficient in NHEJ as Sir$^+$ strains. Similarly, a *MAT***a**/*MAT*α diploid exhibits reduced end joining, compared to a diploid expressing only *MAT*α.

However, other data argue that Sir proteins actually do play at least a small role in DNA repair, even in *MAT***a**/*MAT*α cells. Sir$^-$ *MAT***a**/*MAT*α cells are more defective in DNA end joining and are more sensitive to MMS than Sir$^+$ strains (Lee et al., 1999; Martin et al., 1999; Mills et al., 1999). The importance of Sir proteins is still not fully resolved, as there are significant differences among the results from several labs, some of which could reflect strain differences or the nature of the DNA breaks. Thus, NHEJ of a single HO-induced DSB on a chromosome is reduced no more than 2-fold in a *sir* mutant, whereas it is reduced 200-fold in a *yku70* mutant (Lee et al., 1999). In contrast, Mills et al. (1999) show that a *sir2* strain—independent of the cell's mating type—is as sensitive to EcoRI cleavage as one deleted for *YKU70*.

In general the evidence does not support the idea that Sir proteins could make regions near the DSB heterochromatic, possibly inhibiting 5′ to 3′ degradation of DNA by exonucleases and facilitating end joining (Jackson, 1997; Tsukamoto et al., 1997). The absence of yKu70 does cause a 2-fold increase in exonuclease resection of DSB ends (Lee et al., 1998); *sir* mutants haven't been tested. But there is no evidence that more extensive resection poses a problem for yeast to complete NHEJ, since many repair events are deletions that remove thousands of base pairs.

Martin et al. (1999) offer an alternative explanation for the movement of Sir proteins: the Sir proteins may be dispatched to sites of newly assembled chromatin, just as the chromatin assembly factor CAF1 is recruited to sites of UV damage in mammalian cells. Sir proteins would then be involved in reestablishing chromatin structure at sites of DNA damage. UV damage doesn't cause a change in Sir protein localization (Mills et al., 1999), but the idea merits further study.

Finally, there is the possiblity that there may be a few other genes besides *HML* and *HMR* and those near telomeres that could be subject to Sir repression. Martin et al. (1999) report that an internally located *ADE2* gene with an adjacent silencer becomes *more* repressed after DNA damage. This raises the possibility that there are normal genes that could be repressed by Sir proteins in response to DNA damage—a kind of DNA damage stress response. Microarray technology is now available to ask whether there are other *SIR*-regulated genes. We should also remember that telomeres and subtelomeric regions are not the only "reservoir" for these proteins. Sir2p has an incredibly complicated second job inside the nucleolus, interacting with the RENT (*re*pression of

*n*ucleolar silencing and *t*elophase) complex, as reviewed by Garcia and Pillus (1999) in this issue of *Cell*.

There are many interesting findings—and an equal number of new questions—raised by these papers. Who would have thought that Ku proteins would be so critical in maintaining Sir proteins at telomeres? Why should DNA damage be acted upon or sensed differently in S phase from either G1 or G2? What is the state of EcoRI-cut DNA and its associated chromatin that allows a Ku^+ cell to repair all the breaks without making dozens of deletions? What is the relationship between mating type, ploidy, and the importance of *SIR* genes in NHEJ?

And, finally, why should the same proteins that are critical for telomere maintenance—to keep the natural ends of chromosomes from being degraded or fusing or recombining—also be needed to facilitate the joining of other broken DNA ends? Assuming that this is not simply a cosmic joke, we will have to look even more deeply into the processes of DNA repair and chromatin structures to find the answer.

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