activate a hydroxide ion nucleophile that would attack the scissile phosphate, facilitate gathering negative charge on a nonbridging oxygen of that phosphate, or stabilize a 3′ oxyanion on the cleaved phosphodiester bond. But in the *Aquifex* structure, no Mg^{2+} is seen near a second group of acidic residues proposed to catalyze cleavage of the other dsRNA strand. Perhaps the missing Mg^{2+} is only present when the dsRNA substrate is bound to the enzyme. The present *Aquifex* structure does not include dsRNA; a more detailed description of the catalytic mechanism of RNase III awaits the determination of the enzyme bound to dsRNA, perhaps to a dsRNA analog with cleavage-resistant scissile bonds. Meanwhile, new biological functions will surely continue to accrue to this remarkable enzyme family.

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NHEJ Deficiency and Disease

In mouse and human, diseases associated with deficiency of DNA ligase IV, a protein involved in DNA double-strand break repair, have been identified. Manifestation of some of these disease phenotypes, namely tumorigenesis, may require additional checkpoint deficiencies.

Nonhomologous end-joining (NHEJ) is the process by which nonligatable DNA ends become ligatable through the action of DNA end-processing proteins and alignment factors. The cohort of NHEJ proteins (i.e., Ku70, Ku80, and DNA-PKcs, which collectively form the DNA-dependent protein kinase, and the XRCC4/DNA ligase IV complex), which are crucial to the repair of the programmed double-strand breaks (DSBs) formed during immunoglobulin and T cell receptor rearrangement, has been implicated more broadly in the general repair of chromosomal DSBs, suggestive of a "genomic caretaker" role for these proteins. Such a role would predict that deficiencies in these proteins would lead to disease phenotypes in both lymphoid and nonlymphoid tissues. Two reports in this issue of *Molecular Cell* assess this in vivo role for DNA ligase IV. Sharpless et al. (2001) examine the effects of DNA ligase IV haploinsufficiency in a cancer-prone mouse model, while O'Driscoll et al. (2001) have identified mutations in the DNA ligase IV gene in patients presenting with a novel syndrome. In both reports, a link is established between deficiencies of this protein and disease, although the resulting disease phenotypes are diverse.

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To begin with the murine results, Sharpless et al. demonstrate that in a tumor-prone *Ink4a/Arf*$^{-/-}$ strain, loss of one allele of *Lig4* leads to the frequent development of soft-tissue sarcomas and accelerated morbidity. That this effect is due to haploinsufficiency, rather than loss of activity from the remaining wild-type *Lig4* allele, is supported by several lines of evidence including retention of the wild-type allele in tumors. At the cellular level, DNA ligase IV haploinsufficiency leads to increased radiosensitivity and translocations and other chromosomal aberrations associated with clonal expansion of the tumor cells, as assayed by both spectral karyotyping and array-based comparative genomic hybridization. Additionally, *Lig4*$^{+/-}$ *Ink4a/Arf*$^{-/-}$ embryonic fibroblasts show increased focus formation in the *Myc/Ras* cotransformation assay. Notably, once mutated, reexpression of *Lig4* is insufficient to reduce focus formation, consistent with a caretaker role for *Lig4* in suppressing the accumulation of mutations in growth control genes, rather than having a direct role in growth control itself.

Accelerated tumor development caused by deficiency in NHEJ components has been previously demonstrated in a *p53*$^{-/-}$ background for XRCC4, DNA ligase IV, Ku80, and DNA-PKcs. However, in these cases the tumors are primarily aggressive pro-B-cell lymphomas (for review see Pierce et al., 2001) and are likely to be associated with misrepair of DSBs generated during antigen receptor rearrangement since Rag recombinase deficiency can suppress their formation (Vanasse et al., 1999). Loss of p53 in these previous studies may have masked the more global genomic caretaker role for DNA ligase IV in suppressing tumor formation outside of the immune system that is now observed using the *Ink4a/Arf*$^{-/-}$ background. Other evidence for a more global caretaker role for NHEJ in suppressing tumorigenesis is found in Ku80 mutant mice, since a *Ku80*$^{-/-}$ background com-

bined with *p53* heterozygosity accelerates the development of the sarcomas, as well as the lymphomas, that are found with *p53* heterozygosity alone (Lim et al., 2000).

The analysis of human patients by O'Driscoll et al. (2001) began with the supposition that since DNA ligase IV is a core component of the NHEJ machinery, reduced-function mutants should manifest an immunodeficient, radiosensitive phenotype. As these are also hallmarks of Nijmegen breakage syndrome (NBS), which is caused by defects in the function of the nibrin protein, the authors screened a number of NBS patients that manifested symptoms of the disease but who showed no nibrin coding-sequence defects by DNA sequencing. Likewise, cell lines derived from patients with uncharacterized immunodeficiency were screened for potentially inactivating *LIG4* mutations. Three patients with NBS-like symptoms had mutations in *LIG4,* as did an immunocompromised patient. These mutations lead to impaired ligase activity either directly, by mutation in the active site of the enzyme (which has previously been found in one other radiosensitive patient with leukemia [Riballo et al., 1999]), or indirectly, by disrupting interaction of the protein with XRCC4. Notable amongst these patients was the presence of NBS-like features such as microcephaly and growth retardation, although unlike NBS, cancers were not observed in these four new patients carrying *LIG4* mutations. O'Driscoll et al. appear therefore to have identified a distinct syndrome arising from DNA ligase IV deficiency, although further analysis will be necessary with larger patient populations to more precisely define its characteristics.

A cell line derived from one of the DNA ligase IV-deficient patients demonstrates the expected increase in sensitivity to ionizing radiation, and in a plasmid-based assay V(D)J signal joint fidelity is impaired even though signal and coding joint formation frequency is largely indistinguishable from wild-type. Unlike nibrin mutant cells (Petrini, 2000), however, DNA ligase IV mutant cells show intact cell cycle checkpoints and correspondingly display no radioresistant DNA synthesis.

The analysis of *Lig4* in the mouse indicates that genome-destabilizing lesions arising from DNA ligase IV deficiency are not tumor-promoting unless an *Ink4a/Arf*$^{-/-}$ (or *p53*$^{-/-}$) background is provided, suggesting that Ink4a/Arf is involved in clearing this type of damage. Importantly, Arf stabilizes p53 while Ink4a inhibits the action of the cyclin-dependent kinases (Sherr, 2001), making it reasonable to suppose that lesions that would otherwise be repaired by DNA ligase IV trigger cell-cycle arrest, and possibly p53-dependent apoptosis, in the absence of repair. Applying this mechanistic information to the human study, the observation that cells from DNA ligase IV-deficient patients possess intact cell cycle checkpoints is consistent with the lack of a cancer-prone phenotype.

Taking these two new manuscripts together, what can we conclude about the genomic caretaker role for DNA ligase IV in the repair of DSBs? While defects in other types of DNA repair lead to obvious cancer-prone syndromes, such as malignant melanoma in xeroderma pigmentosa patients with defective nucleotide excision repair and colorectal carcinoma in hereditary nonpolyposis colorectal cancer patients with defective DNA mismatch repair (Hoeijmakers, 2001), cancer syndromes arising solely from defects in DSB repair have been much less forthcoming. An explanation may be that, unlike persistent pyrimidine dimers or transient DNA mismatches, DSBs are sufficiently obvious to the cellular genome stability surveillance systems so that cells bearing these lesions are effectively cleared even when repair is deficient. That the mammalian cell takes this extra care to deal with DSBs can be comforting to the readers of these two papers. It will be interesting to determine if DSB repair cancer syndromes with unaccompanied checkpoint deficiencies can be unearthed.

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