A Backup DNA Repair Pathway Moves to the Forefront

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Chromosomal translocations between antigen receptor loci and oncogenes are a hallmark of lymphoid cancers. Several new studies now reveal that programmed DNA breaks created during assembly of antigen receptor genes can be channeled into an alternative DNA end-joining pathway that is implicated in the chromosomal translocations of lymphoid cancers (Corneo et al., 2007; Soulas-Sprauel et al., 2007; Yan et al., 2007).

Antigen receptor genes are assembled in developing B and T cells by a site-specific recombination reaction referred to as V(D)J recombination. Mature B cells further diversify their immunoglobulin (Ig) genes by class-switch recombination (CSR) and somatic hypermutation. To maintain genomic stability during these reactions several double-strand break (DSB) repair pathways protect against aberrant chromosomal rearrangements, including homologous recombination and nonhomologous end-joining (NHEJ). A cluster of recent papers, two published in *Nature* and one in the *Journal of Experimental Medicine*, now show that a backup pathway for NHEJ functions in V(D)J and CSR (Corneo et al., 2007; Soulas-Sprauel et al., 2007; Yan et al., 2007). This alternative end-joining pathway may also contribute to the chromosomal translocations that give rise to lymphoid cancers.

NHEJ joins DNA ends directly with no homology necessary (unlike homologous recombination) and is therefore prone to introduce errors during the processing of noncompatible DNA ends. There appear to be two phases of NHEJ: a rapid phase, when most simple lesions are repaired, and a slower phase that resolves complex DSB lesions that require significant processing (Riballo et al., 2004). Several lines of evidence also suggest that alternative and less well-defined backup end-joining pathways play an important role in physiological and pathological DSB repair. First, substantial end-joining occurs in response to γ-irradiation even in the absence of core NHEJ (C-NHEJ) factors, such as the DNA Ligase LIG4 and Ku (a subunit of the DNA-dependent protein kinase) or proteins that aggregate on damaged chromatin (Riballo et al., 2004; Wang et al., 2006) (Figure 1). Second, spontaneous chromosomal aberrations and oncogenic *c-myc/IgH* translocations are observed even in the absence of Ku or the ligase complex component XRCC4 (Jankovic et al., 2007). Third, rare aberrant V(D)J coding joins are found in lymphocytes lacking Ku and the DNA-dependent protein kinase catalytic subunit (Bogue et al., 1997). Finally, mice lacking either Ku or XRCC4, as well as the p53 tumor suppressor protein, invariably develop pro-B lymphomas that result from translocations between the *IgH* locus

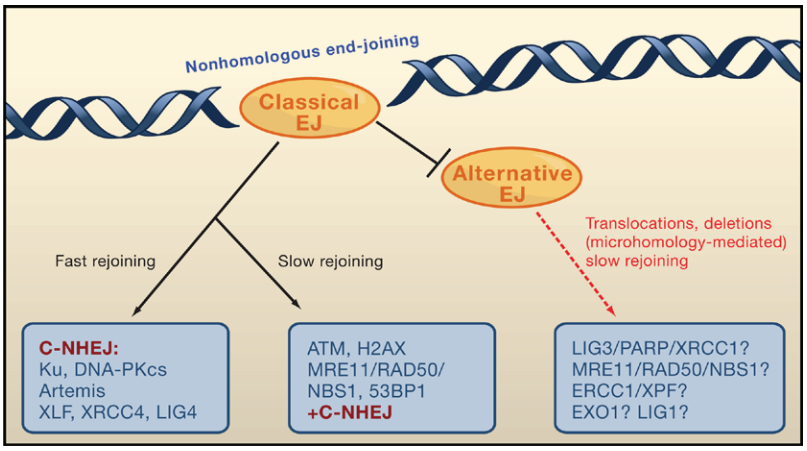

Figure 1. Classical and Alternative End-Joining Pathways
The core pathway for nonhomologous end-joining (C-NHEJ) relies on the DNA-dependent protein kinase subunits (DNA-PKcs and Ku), the Artemis endonuclease, and the XLF-XRCC4-LIG4 ligation complex. Compatible ends are rapidly joined by the C-NHEJ machinery. In addition, a subset of lesions handled by C-NHEJ also requires factors—such as phosphorylated histone H2AX, ATM, 53BP1, and Nbs1—that aggregate into large chromatin domains surrounding the site of the double-strand break. Some ends may require ATM-dependent processing prior to ligation or require changes in chromatin structure that might facilitate access by C-NHEJ. Microhomology-mediated joining uses components of other DNA repair pathways and may act as a backup or alternative pathway to classical end-joining (Corneo et al., 2007; Soulas-Sprauel et al., 2007; Yan et al., 2007). DSB repair assays in irradiated cell lines suggest that this form of repair is relatively slow and inefficient (Wang et al., 2006).

and *c-myc* (Difilippantonio et al., 2002; Zhu et al., 2002). Three hallmark features of the backup end-joining pathway are that the repair junctions are characterized by deletions, insertions, and relatively large regions of microhomology.

Several recent papers from the Alt, Roth, and de Villartay laboratories now document the role of alternative end-joining in intrachromosomal CSR and V(D)J recombination (Corneo et al., 2007; Soulas-Sprauel et al., 2007; Yan et al., 2007). CSR is initiated by activation-induced cytidine deaminase (AID), which introduces U:G mismatches in switch region DNA. In G1 phase of the cell cycle these lesions are processed into DSBs (Petersen et al., 2001) by base excision or mismatch recognition pathways and repaired by NHEJ pathways, which require Ku. Recombination between switch regions deletes intervening DNA and results in a switch from expression of IgM to expression of IgG, IgE, or IgA.

Given the requirement for Ku in CSR and the essential role of Ku in recruiting XRCC4/LIG4 to sites of DSBs, loss of XRCC4/LIG4 should also severely compromise end-joining during CSR. However, this is not what was observed in XRCC4- or LIG4-deficient mice (Soulas-Sprauel et al., 2007; Yan et al., 2007). Both groups report that switching was only reduced to 25%–50% of normal levels (although deletion was incomplete in the work of Soulas-Sprauel et al.). Yan et al. also discovered that 20%–30% of XRCC4-deficient cells harbored *IgH* locus chromosome abnormalities, and that 25% of these abnormalities involved translocations. Thus, as in Ku80-deficient B cells (Ramiro et al., 2006), antigen receptor-associated translocations form in the absence of the XRCC4-LIG4 complex (Yan et al., 2007), suggesting that alternative NHEJ pathways mediate their formation.

Examination of the switch recombination junctions unveiled an additional surprise. Almost all of the junctions in XRCC4-deficient B cells examined by Yan et al. were mediated by regions of microhomology and were biased toward long stretches of homology. Likewise, all of the 33 CSR junctions examined so far in LIG4-deficient mature B cells involve microhomology-mediated joining (C. Boboila, C. Yan, and F.W. Alt., personal communication), and an increase in homology at switch junctions was also found in patients with deficiency in LIG4 activity (Pan-Hammarstrom et al., 2005). Thus, in the absence of XRCC4-LIG4, alternative NHEJ efficiently resolves AID-dependent DSBs both by intrachromosomal CSR or interchromosomal fusion. In striking contrast, CSR-associated DSBs in Ku-deficient cells are not channeled efficiently into intrachromosomal joins, although they are substrates for translocations (Ramiro et al., 2006). One possibility is that absence of Ku80 leads to a hypersensitive DNA damage checkpoint that rapidly eliminates most cells before they have an opportunity to join into an intrachromosomal switch. An alternative possibility is that Ku has an additional function in bringing together distal CSR regions in preparation for end-joining, or that there are multiple alternative end-joining pathways, only some of which are dependent on Ku.

Unlike CSR, all of the known components of the classical NHEJ pathway are essential for V(D)J recombination. This reaction is initiated by RAG proteins, which introduce nicks in recombination signal sequences that flank antigen receptor gene segments. The RAG synaptic complex converts the nicks to DSBs via *trans*-esterification, and recombination results in the deletion or inversion of intervening DNA. In a complementary study on V(D)J recombination, Roth and colleagues showed that the RAG proteins actively guide DNA breaks into the classical NHEJ pathway (Corneo et al., 2007). They discovered mutations in RAG1 and RAG2 that did not impair DSB cleavage of recombination substrates in vitro but resulted in robust microhomology-mediated joining in cell lines. This work indicates that one of the functions of the postcleavage complex is to prevent RAG-dependent breaks from accessing the backup pathway for NHEJ. Alternatively, there may be direct interactions between the postcleavage complex and classical NHEJ factors, or RAG-dependent activities might regulate chromatin structure in a manner that facilitates the recruitment of classical end-joining proteins. Future work examining the in vivo consequences of these aberrant joining events with respect to assembly of antigen receptor genes, translocations, and the formation of lymphomas will be of great interest.

The alternative end-joining mechanisms involved in translocation, CSR, and V(D)J recombination have not been identified, but the process likely includes substantial degradation of the ends by exonucleases to produce single-strand tails, a homology search, and excision of nonhomologous nucleotides before annealing of complementary ends. These would be expected to occur at a markedly slower rate than the joining of blunt ends. It is likely that one of the other two DNA ligases present in mammalian cells (LIG1 or LIG3) should be involved. LIG1 is required for the joining of Okazaki fragments during DNA replication, excision repair, and homologous recombination. LIG3 functions in base-excision repair and single-strand break repair and, together with XRCC1 and Poly(ADP-Ribose) Polymerase, has already been implicated in DSB repair as a backup to the classical NHEJ pathway (Audebert et al., 2004; Wang et al., 2006). An end-joining pathway that is independent of Ku and LIG4, active in S/G2/M phases of the cell cycle, is mediated by microhomology, and relies on components of the homologous recombination and mismatch repair machinery has recently been identified in fission yeast (Decottignies, 2007). All of these alternative pathways might provide a backup for XRCC4/LIG4 during the repair of AID-initiated lesions in CSR. For example, LIG3 and the repair machinery for single-strand breaks might be recruited to single-strand switch regions generated by endonuclease nicking of the DNA backbone and therefore might be present when DSBs are created from apposing single-strand nicks during G1 phase. Switch region nicks might occasionally be converted to DSBs as a result of DNA replication, and homologous recombination and LIG1-dependent joining pathways may seal these ends. Thus, there may be many mechanisms that could hold, process, and

eventually join two DSB ends together. The studies by Alt, de Villartay, and Roth and their colleagues pave the way for determining the genetic requirements for these alternative DNA repair pathways and for establishing their role in the chromosomal translocations that cause cancer.

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When Two Is Better Than One

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Gene duplication and divergence has long been considered an important route to adaptation and phenotypic evolution. Reporting in *Nature*, Hittinger and Carroll (2007) provide the first clear example of adaptations in both regulatory regions and protein-coding regions after gene duplication. This combination of evolutionary changes appears to have resolved an adaptive conflict, leading to increased organismal fitness.

Genes that perform more than one function can get caught in an evolutionary tug of war, when improving one function necessarily compromises another. Gene and genome duplications have long been considered an important means of breaking these adaptive deadlocks. Original models of gene duplication were developed under the neutral theory: following duplication, one gene would be constrained to perform the ancestral function, whereas the other, its paralog, would be free of selective constraints and therefore able to take on a new function (Ohno, 1970). Decades later, it is clear that many genes perform more than one function prior to duplication, often through alternative regulatory inputs, splicing, or post-translational processing. More recent models have focused on these processes as an explanation for the survival of duplicated genes (Hughes, 1994; Hughes, 1999). The duplication, degeneration, and complementation (DDC) model, for instance, posits that paralogs that perform some subset of the ancestral functions can be preserved through complementary loss of regulatory sequences without invoking natural selection (Force et al., 1999). One example of this is in the Hoxb1 paralogs of zebrafish, Hoxb1a and Hoxb1b, which appear to have developed subfunctions after the whole-genome duplication within the bony fishes. Hoxb1a and Hoxb1b expression domains have become subdivided among paralogs due to complementary degenerative mutations in *cis*-regulatory sites, whereas most protein function appears to be largely conserved following duplication (McClintock et al., 2002). This regulatory divergence is consistent with a DDC-like process that occurred in the absence of adaptation. However, the relative roles of selection and degeneration, whether selection works on mutations in regulatory or