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DNA Double-Strand Break Repair: A Relentless Hunt Uncovers New Prey

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A major pathway for repair of DNA double-strand breaks is nonhomologous end-joining (NHEJ). In this issue of *Cell*, Buck et al. (2006a) and Ahnesorg et al. (2006) report the discovery of a new NHEJ factor called Cernunnos-XLF. Both groups report that this protein is mutated in a rare inherited human syndrome characterized by severe immunodeficiency, developmental delay, and hypersensitivity to agents that cause DNA double-strand breaks.

Double-strand breaks (DSBs) are a dangerous form of DNA damage. Unrepaired or misrepaired DNA ends can cause detrimental outcomes for cells and organisms, including cell death, chromosomal instability, and neoplastic transformation (Mills et al., 2003). These catastrophic lesions are generated during normal metabolic processes such as DNA replication or upon exposure to exogenous agents such as ionizing radiation or certain chemotherapeutic compounds. Several pathways exist that recognize and repair these lesions, including the nonhomologous end-joining (NHEJ) pathway, which serves to protect and directly ligate broken ends (Haber, 2000). Remarkably, despite the inherent risks, there are examples throughout nature where organisms have evolved systems to intentionally induce DSBs. These processes usually function to increase diversity of species or somatic cells by initiating the rearrangement of DNA at specific regions of the genome. An incredible example of this is V(D)J recombination, which occurs during B and T lymphocyte development to generate the vast diversity of antigen receptor genes that form the basis of the adaptive arm of our immune system. Although this process is initiated by lymphoid-specific factors, the rearrangements are completed by the ubiquitously expressed NHEJ components (Rooney et al., 2004).

To date, six NHEJ factors have been discovered: Ku70, Ku80, the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), Artemis, XRCC4 and DNA Ligase IV. Ku70 and Ku80 comprise a heterodimer that binds to DNA ends and recruits DNA-PKcs, a serine/threonine protein kinase. DNA-PKcs forms a functional complex with Artemis, which possesses an intrinsic single-strand 5′ to 3′ exonuclease activity. DNA-PKcs phosphorylates and activates the endonuclease activity of Artemis, allowing this protein to cleave DNA hairpins and other structures containing single- to double-stranded transitions. Thus, Artemis provides an important nucleolytic processing activity to prepare DNA ends for ligation. The NHEJ ligation activity is provided by Ligase IV in complex with the XRCC4 cofactor. Together, these six proteins possess the major activities required for NHEJ, which suggested that all members of the pathway had been identified.

Genes encoding two of the six factors, *Artemis* and *Ligase IV*, have been

found mutated in rare inherited syndromes (O'Driscoll et al., 2004). The individuals suffer from whole-body and cellular hypersensitivity to ionizing radiation and DNA-damaging chemotherapeutic compounds and are immunocompromised due to defective V(D)J recombination. Additionally, several of the Ligase IV-deficient patients have severe developmental delay including microcephaly, which likely reflects the need for this ligase in all end-joining reactions whereas only a subset require Artemis. The critical role for Ligase IV in NHEJ is highlighted by the embryonic lethality of Ligase IV-deficient mice in contrast to the viability and normal size of Artemis-deficient mice (Rooney et al., 2004). Recently, intriguing findings were reported from studies of a cell line derived from a severely immunocompromised child (Dai et al., 2003). The line (termed 2BN) was found to exhibit DNA DSB repair and V(D)J recombination defects that could not be complemented by any of the known NHEJ genes, thus raising the exciting possibility that an NHEJ factor remained undiscovered.

The hunt for this new NHEJ factor has now ended with two groups reporting its identification in this issue of *Cell*. de Villartay and colleagues (Buck et al., 2006a) have given it the name Cernunnos (an enigmatic Celtic god of the hunt, the underworld, fertility, and possibly more), whereas Jackson and colleagues (Ahnesorg et al., 2006) have chosen the descriptive name XLF for XRCC4-like factor. We shall refer to it as Cernunnos-XLF by fusing both names in alphabetical order.

The two studies used different approaches to discover Cernunnos-XLF. de Villartay and colleagues (Buck et al., 2006a) identified a group of patients with phenotypes consistent with an NHEJ defect. Chief among these was an immunodeficiency syndrome featuring low numbers of B and T lymphocytes and functional mature NK cells (NK cells derive from a common precursor but do not undergo V(D)J recombination). In addition, they identified individuals with developmental anomalies noted in other DNA-repair syndromes, including growth retardation, microcephaly, and mental retardation, which likely result from general inability to repair spontaneous DNA damage throughout the body. Having identified a small cohort of patients, they then determined that their cells exhibited increased radiosensitivity, DNA DSB repair defects, and impaired V(D)J recombination. After excluding the six known NHEJ factors, they used an elegant strategy to clone the gene by cDNA complementation of cellular sensitivity to a DNA DSB-inducing agent. One common complementing cDNA was identified, which encoded a new gene, *Cernunnos*, with no known motifs. Buck et al. (2006a) then confirmed *Cernunnos* was mutated in each of the patients.

Jackson and colleagues (Ahnesorg et al., 2006) undertook a different strategy, beginning with a yeast two-hybrid screen to identify XRCC4-interacting proteins. One positive clone of interest was found to be an uncharacterized human open reading frame encoding a 33 kDa protein. Although standard sequence analysis did not reveal conserved domains suggesting a DNA-repair role, computer algorithms predicted structural similarity to XRCC4, which is comprised of a globular N-terminal "head" domain and a C-terminal coiled-coil structure. Thus, the authors named the new gene *XRCC4-like factor* (*XLF*). Evidence for a role in NHEJ was provided by increased radiosensitivity and defective DNA DSB repair in cells where Cernunnos-XLF activity was blocked by RNA interference (RNAi). Interaction between Cernunnos-XLF and XRCC4, as well as with Ligase IV, was confirmed both in vivo and in vitro. Its role in human disease was identified rapidly thereafter with the finding that the Cernunnos-XLF protein could not be detected in the 2BN cultured cell line and its cDNA rescued the cellular defects.

Clues to the function of Cernunnos-XLF can be gleaned from the initial analyses of the molecular phenotypes of Cernunnos-XLF-deficient cells. Cells lacking DNA-PKcs or Artemis exhibit less sensitivity to ionizing radiation and a milder DSB-joining defect in comparison to Ku70, Ku80, XRCC4, and Ligase IV deficiencies, supporting

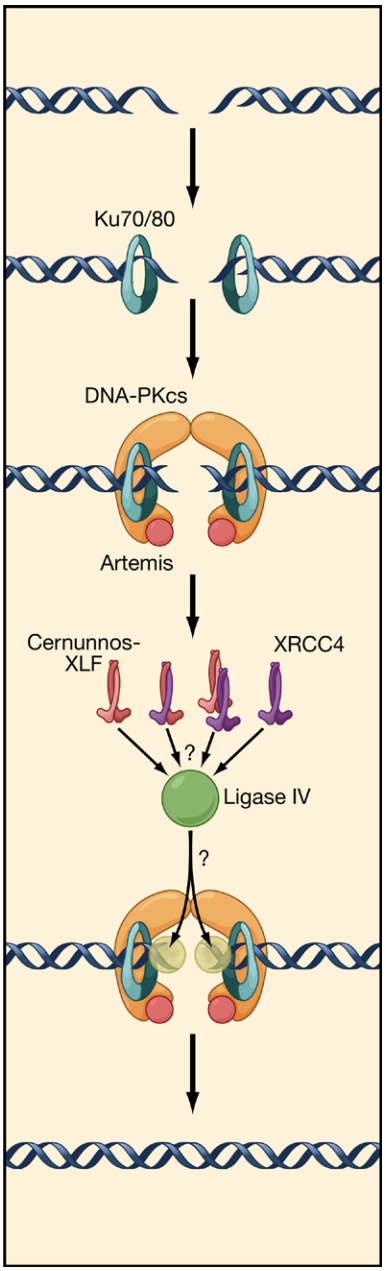

Figure 1. Nonhomologous End-Joining in Mammalian Cells

The Ku70/Ku80 heterodimer forms a hollow ring that preferentially binds to DNA ends. Ku70/Ku80 bound to DNA ends recruits DNA-PKcs, which forms a complex with the Artemis nuclease. DNA-PKcs may tether the ends, while Artemis nucleolytically processes DNA ends prior to joining. The Cernunnos-XLF protein forms complexes with XRCC4, Ligase IV, or XRCC4 and Ligase IV simultaneously. The exact nature of the active complex is currently undefined, but could involve the formation of heteromultimers with XRCC4 or the XRCC4-Ligase IV complex. The final stage of NHEJ is the ligation of DNA ends catalyzed by XRCC4-Ligase IV. Cernunnos-XLF promotes this process in an unknown way.

the notion that DNA-PKcs and Artemis play specialized roles in repairing DNA ends, whereas the other factors function during all NHEJ events (Rooney et al., 2004). The direct interaction of Cernunnos-XLF with the XRCC4-Ligase IV complex and similar levels of ionizing radiation sensitivities and DSB-repair defects exhibited by Cernunnos-XLF- and XRCC4- or Ligase IV-deficient cells suggests that this newly discovered factor may function in all NHEJ events (see Figure 1). Clearly, however, definitive conclusions await more comprehensive examination of the phenotypes of human patients and future knockout mouse models.

The NHEJ factors play unique roles in processing and ligating DNA ends generated during V(D)J recombination. These recombination events are initiated by the lymphoid-specific RAG1/2 endonuclease (RAG1/2), which recognizes specific recombination signal (RS) sequences flanking V, D, and J coding segments (Fugmann et al., 2000). Cleavage by RAG1/2 generates two different end structures: 5′ phosphorylated blunt RS ends and covalently closed hairpin coding ends. Unlike RS ends, hairpins at coding ends must be nicked open prior to ligation. The core components of the NHEJ machinery, Ku70, Ku80, XRCC4, and Ligase IV, are required for both coding and RS joint formation. The significant V(D)J recombination defects in both coding and RS end joining in Cernunnos-XLF-deficient cells further supports the notion that this a general factor for end joining (Buck et al., 2006a; Dai et al., 2003). More detailed analyses of coding and RS joining in additional Cernunnos-XLF-deficient human cell lines and mutant mouse models will further reveal the functions of this new factor in V(D)J recombination.

What is the exact role of Cernunnos-XLF in DNA DSB repair and V(D)J recombination? Ahnesorg et al. (2006) suggest that Cernunnos-XLF may serve as a bridge between XRCC4-Ligase IV and the other NHEJ factors located at DNA ends, may facilitate recruitment of other factors to sites of repair, or may regulate XRCC4-Ligase IV activity via modulation of active and inactive multimeric states of XRCC4 (Figure 1). Alternatively, Cernunnos-XLF may participate in reconfiguration of the end bound NHEJ factors to allow XRCC4-Ligase IV access to the DNA termini. It will be interesting to determine how Cernunnos-XLF interaction with XRCC4-Ligase IV modulates the NHEJ ligation activity and whether its functions are influenced by phosphorylation mediated by DNA-PKcs. Clearly, careful biochemical analyses of interactions between the seven known NHEJ factors are essential for our understanding of end joining in mammalian cells.

The human syndrome associated with *Cernunnos-XLF* mutations typifies the features expected from loss of the NHEJ pathway. However, there is variability among the individuals that may derive from differing mutations. Interestingly, human *Ligase IV* and *Artemis* deficiencies also display significant differences among affected individuals with distinct mutations (Buck et al., 2006b; Ege et al., 2005; O'Driscoll et al., 2004). Detailed biochemical and genetic analyses of the disease-associated variants promise to shed light on the reasons for such variability.

Although the characterized NHEJ syndromes are rare, the variability in alleles and disease symptoms raises the possibility that only a small fraction of mutations that confer obvious effects while allowing viability have been identified. Subtle mutations that minimally affect the immune system and have little impact in childhood would be more difficult to detect and could therefore be prevalent in the human population. Given the implications of this possibility, the hunt for new NHEJ factors and the elucidation of detailed mechanisms of this pathway will remain fertile areas of research in the future.

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