Shielding Broken DNA for a Quick Fix

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MOLECULAR BIOLOGY

Shielding Broken DNA for a Quick Fix

A fast-acting DNA repair mechanism involves a protein complex that blocks an alternative process that requires a cell to wait for repair.

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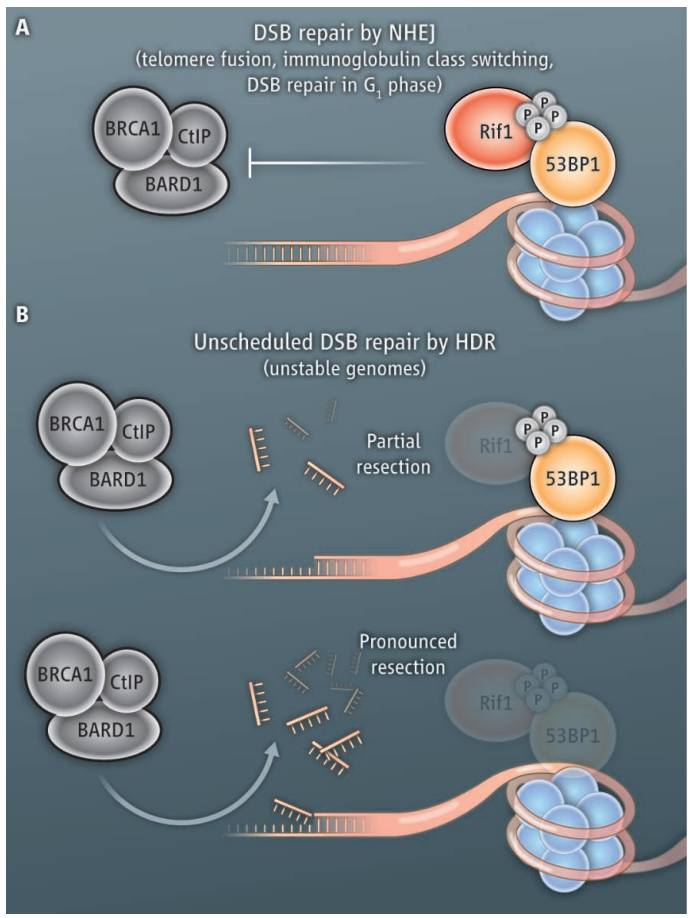
DNA double-strand breaks are constantly generated throughout the cellular life span, making the genome vulnerable to mutation and irreversible damage. Cells are equipped to mend such breaks through two mechanisms, each with positive and negative aspects: homology-directed repair (HDR), which tends not to make mistakes, but requires that a cell wait until DNA replication generates homologous templates; or a faster process called nonhomologous end joining (NHEJ), which, though error-prone, can rapidly "glue" DNA breaks together (*1*). Making the right choice between HDR and NHEJ is vital for genome integrity. Recent studies (*2*, *3*), including those on page 711 and 700 of this issue by Di Virgilio *et al*. (*4*) and Zimmermann *et al*. (*5*), respectively, show how the HDR pathway is blocked so that the alternate repair process can proceed.

In a number of physiological settings such as immunoglobulin diversification in blood cells, the natural erosion of telomeres (protective ends of chromosomes), or DNA breakage during G_1 phase of the cell division cycle, the HDR repair process must be actively suppressed to avoid genomic rearrangements. This is achieved by blocking the action of enzymes that create single-strand DNA at the break sites, in preparation for recombination. The barrier against excessive resection involves 53BP1, a large adaptor protein that interacts with the DNA scaffold made of histones and histone-binding proteins (*6*).

To elucidate how 53BP1 shields DNA ends, Di Virgilio *et al*. and Zimmermann *et al*. took advantage of the fact that for 53BP1 to block DNA-end resection, it must be phosphorylated by the enzyme ataxia telangiectasia mutated (ATM) (*7*). Di Virgilio *et al*. performed a mass spectrometry screen for proteins that interact with phosphorylated 53BP1, whereas Zimmermann *et al*. extended their previous work on proteins that interact with phosphorylated 53BP1 at dysfunctional telomeres. Rewardingly, both groups identified Rap1-interacting factor 1 (Rif1) as a key suppressor of DNA-end resection that acts downstream of 53BP1 (see the figure). To demonstrate the physiological relevance of this finding, Di Virgilio *et al*. show that depletion of Rif1 in activated B lymphocytes triggered excessive 5′ DNA-end resection, impaired immunoglobulin class switch recombination (a DNA rearrangement strictly dependent on NHEJ), and led to genetic instability including translocations at the immunoglobulin H (IgH) locus. Zimmermann *et al*. show that at telomeres deprived of the protective shelterin complex (and thus converted to DNA ends resembling double-strand breaks), depletion of Rif1 also enhanced 5′ DNA-end resection, thereby rendering the DNA ends unsuitable for direct ligation and reducing the frequency of telomere fusions.

Most DNA repair reactions can be regarded as a double-edged sword. Although NHEJ is beneficial under physiological settings, it can turn against the genome under pathological conditions that arise during cellular transformation. One cytological manifestation of this is the formation of radial chromosomes, aberrant structures caused by toxic NHEJ reactions in tumor cells with compromised BRCA1 function (*1*). BRCA1 is a tumor suppressor protein that acts in the HDR repair pathway. Consistent with its role in promoting NHEJ, Zimmermann *et al*. show that Rif1 depletion in cultured mammalian cells lowered the frequency of aberrant chromosomes under these conditions. However, this and all other phenotypes associated with Rif1 depletion were milder than in 53BP1-deficient cells, indicating that although Rif1 substantially contributes to DNA-end protection downstream of 53BP1, it cannot completely explain the entire spectrum of anti-resection cellular activities. To explain this, Zimmermann *et al*. suggest that the enhanced mobility of 53BP1-bound DNA ends (a function of 53BP1 that is Rif1-independent) increases their chance of finding each other,



Protected ends. (**A**) At the site of a DNA double-strand break, Rif1 interacts with chromatin-bound and phosphorylated (P) 53BP1 to protect DNA ends against resection (executed in part by the BRCA1-CtIP complex). This allows DNA repair by the NHEJ mechanism. (**B**) In the absence of Rif1 (alone or with 53BP1), DNA ends undergo excessive resection, which may lead to unscheduled repair by HDR, and consequently to genomic instability. BARD1, BRCA1-associated RING domain protein 1.

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whereas the mass spectrometry screen of Di Virgilio *et al*. reveals additional proteins that interact with phosphorylated 53BP1, raising the possibility that some of them might join Rif1 in shielding DNA ends.

53BP1 has stood out as a molecular shield against 5′ DNA-end resection at double-strand breaks without a clear mechanism attached to it. Adding Rif1 to the picture breaks this deadlock and provides the much-needed impetus to mechanistically explore DNA-end protection by addressing previously unforeseen questions. The recent studies by Escribano-Díaz *et al*. (*2*) and Ross Chapman *et al*. (*3*) provide an important lead by showing that during G_1 phase of the cell division cycle, Rif1 opposes the function of the complex involved in DNA-end resection, composed of BRCA1 and C-terminal binding protein-interacting protein (CtIP) (*1*). Conversely, after entry into S phase and activation of cyclin-dependent kinases, the phosphorylated BRCA1-CtIP complex prevails, unloads Rif1 from chromatin, and initiates DNA-end resection. This neatly explains how periodicity of the cell cycle is linked to repair pathway choice, but additional mechanisms should not be discounted. For instance, Rif1 is a multifunctional protein that organizes higher-order chromatin structure at origins of DNA replication. It is possible that chromatin accessibility contributes to restraining DNA resection at double-strand breaks (*8*, *9*).

Other unresolved issues include how Rif1 binds to phosphorylated 53BP1 and how the activated BRCA1-CtIP complex disrupts this interaction. The absence of a phospho-recognition motif in the Rif1 sequence indicates that enhanced interaction between 53BP1 and Rif1 after DNA damage is mediated by a hitherto unknown molecular linker. This is not merely an academic problem—mechanisms that influence DNA-end resection have a therapeutic potential in cancer (*10*), and if we are to expand the list of targets for more efficient synthetic treatments with modalities that involve DNA breakage (radiotherapy, many forms of chemotherapy), factors that enhance recognition of phosphorylated 53BP1 by Rif1 after DNA damage could be of crucial relevance as targets for therapeutic interventions. Regardless, defining the role of Rif1 in human diseases associated with unstable genomes deserves attention, and the discovery of its function in DNA-end processing is an important milestone toward achieving this goal.

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ECOLOGY

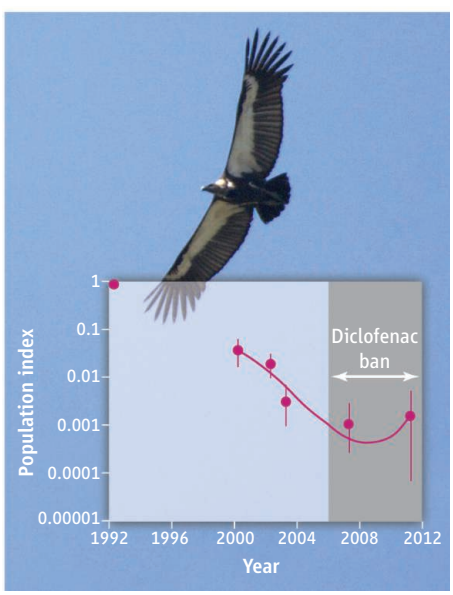
Pollution, Politics, and Vultures

The catastrophic collapse of south Asia's vultures may at last be coming to an end, thanks to a ban on the veterinary drug responsible.

Andrew Balmford

Fifty years after the publication of Rachel Carson's groundbreaking book *Silent Spring*, environmental pollutants, whose impacts are hard to diagnose and harder still to control, continue to cause grave damage to nontarget organisms. The range of substances of concern has expanded since Carson's day from nutrients, pesticides, and heavy metals and now includes pharmaceuticals. Although drug pollution problems have been particularly difficult to address (*1*), recent developments in south Asia offer some positive news on one of the best-known examples. Scientists and politicians are at last making progress in reversing the accidental but catastrophic poisoning of the region's vultures by a widespread veterinary drug.

Two decades ago, vulture populations across the Indian subcontinent began a collapse to just 1% of what they had been (*2*). As well as being a crisis for bird conservation, this was a serious problem for public health, because it ended the free disposal by the birds of the region's vast annual tonnage of cattle carcasses (*3*). Ten years after the decline began, its cause was finally identified: The vultures were being poisoned by widespread use of the out-of-patent nonsteroidal anti-inflammatory drug diclofenac, which causes kidney failure when the birds feed on carcasses of recently treated cattle (*4*).



Turning a corner? Changes in population indices of the oriental white-backed vulture *Gyps bengalensis*, from 6 years of repeat surveys of a large number of road transects in India. Vertical lines show 95% confidence limits derived by bootstrapping; the curve shows the cubic log-linear trend for 2000 to 2011. The *y* axis has a logarithmic scale.

Based on evidence that deaths of vultures by diclofenac were widespread and frequent and that contamination of cattle carcasses was sufficient to account for the rapid decline (*5*), the governments of India, Pakistan, and Nepal banned the veterinary use of diclofenac in 2006. Bangladesh followed suit in 2010, and in May 2012 the four governments reached an unprecedented political agreement to further coordinate and improve actions to prevent adverse effects of veterinary drugs on vultures (*6*).

These responses were a considerable achievement for conservation science, but the diclofenac ban did not solve the vulture problem overnight. Surveillance of diclofenac contamination of cow carcasses in India soon after the ban showed little change (*7*), and test purchases in pharmacies found

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