tion and restoration. Such analyses should be based ideally on state-of-the-science techniques of decision support and benefit-cost analysis that account for the diversity of societal values (including market and nonmarket values) associated with the causes and consequences of salinization. Given that evidence will improve, developing standards and wider governance arrangements will require an iterative approach over time. The Australian Murray-Darling Basin salinity management strategy (*14*) is an example of how planning and consultation can lead to catchment-specific salinity targets. This strategy sits within the overall Murray-Darling Basin Plan, where legislation requires a TBL approach.

MANAGEMENT MEASURES. Several management actions could help meet existing and emerging standards and could prevent or remediate damage associated with freshwater salinization. Essential to all of these options is providing incentives for reducing salinization, such as by market-based instruments, subsidies for technology development and implementation, or load-based charges on saline effluents. Examples of good practices include the following.

(i) *Implement agriculture practices that use less water and thereby reduce salt loading to freshwaters.* For example, the Colorado River Basin Salinity Control Program has reduced salt loading to the river by an estimated 1.3 million tons/year, mostly by improving irrigation practices (*15*).

(ii) *Reduce or eliminate the use of salts as pavement deicers by making more effective application and use of the salts that are applied or by using alternative deicers.*

(iii) *Reduce point-source production and discharge of salts to freshwaters.* For example, innovative methods of resource extraction that sequester soluble minerals away from water sources have potential to reduce effluent discharge to streams.

(iv) *Implement cap-and-trade schemes.* This cost-effective approach is being used in Australia (e.g., the Hunter River Salinity Trading Scheme), where miners and power generators trade permits to discharge salt-rich effluents during moderate to high flow periods.

(v) *Develop specific management options for salt-rich effluents.* Salt-rich urban discharges could be routed to retention basins rather than treatment plants or streams. Although currently prohibitively expensive, water desalinization may become a viable treatment, particularly solar-powered systems, such as are in development in the Middle East. Recovering salts could reduce costs, e.g., using magnesium to recover ammonia and phosphate in the form of struvite, which has commercial value.

(vi) *Promote practices that reduce salinization.* Recognition of water-wise products (e.g., via eco-labels) or support for direct economic incentives to commercialize crops that demand less water (*14*) can be useful tools to alter behaviors.

Fortunately, few large-scale ecological disasters have been caused by salinization of freshwater ecosystems to date, but those that have [e.g., the fisheries collapse in the Aral Sea in Central Asia (*16*)] should be a wake-up call. International cooperation and scientific knowledge-sharing are needed to develop solutions that can be applied globally. Experiences like those near the river Werra in Germany, where a combination of total ion and ion-specific discharge requirements led to ecosystem recovery (*17*), show that rehabilitation of salt-polluted freshwater ecosystems is possible. Prevention of salt damage is much more likely if water managers, stakeholders, and scientists work together to identify social, economic, and ecological costs and the benefits that can accrue from prevention and restoration. ■

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DNA

Breaking DNA

A long-sought protein that helps to break DNA is finally discovered

By **Corentin Claeys Bouuaert** *and* **Scott Keeney**

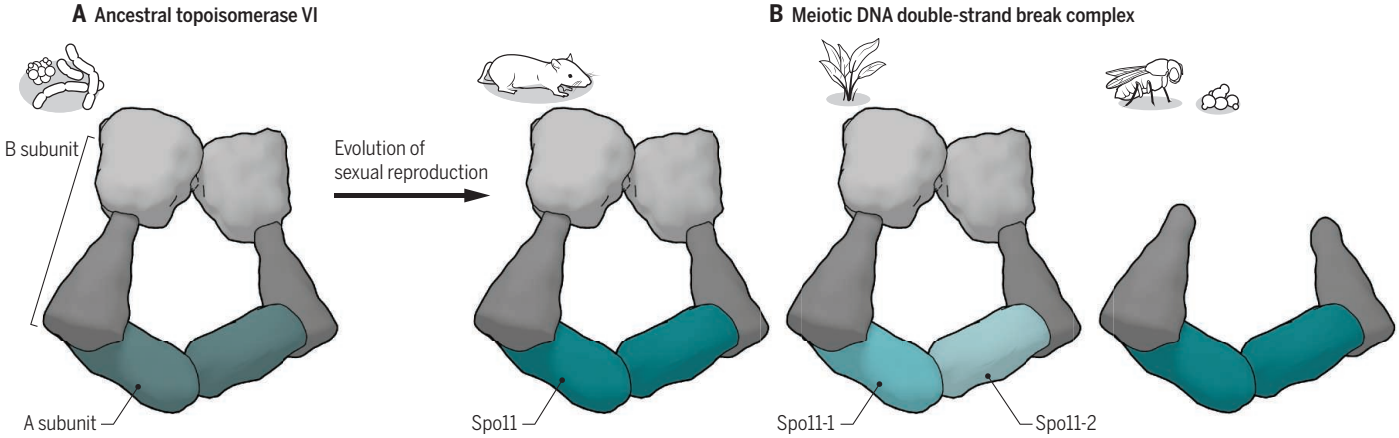
To maintain a constant number of chromosomes from one generation to the next, sexual organisms reduce the genome complement in their gametes through the specialized cellular division of meiosis. Accurate separation of homologous chromosomes during meiosis relies on a dedicated mechanism of DNA recombination that is initiated by DNA double-strand breaks (DSBs) made by a protein called sporulation protein 11 (Spo11) (*1*). Meiotic recombination helps connect homologous chromosomes to promote their accurate segregation, and also shuffles alleles between homologous chromosomes to increase diversity. Spo11 is encoded in nearly all sequenced eukaryotic genomes, and it is likely that most species

"...meiotic cells play the risky game of forming a great deal of...self-inflicted DNA damage..."

that carry out meiotic recombination use Spo11-generated DSBs as the initiators (*2*). Spo11 is thus an ancient and fundamental part of sexual reproduction. On pages 939 and 943 of this issue, Vrielynck *et al.* (*3*) and Robert *et al.* (*4*) report the discovery of a long-sought partner of Spo11 in plants and mice, respectively.

Spo11 evolved from an ancestral type II DNA topoisomerase (Topo VI) that is found today in Archaea and a few eukaryotic lineages, including some plants (*2*). Topoisomerases create transient breaks in DNA to change DNA supercoiling or untangle intertwined DNA duplexes, thereby facilitating processes such as transcription or replication. Topo VI comprises two "A" subunits that cleave DNA and two "B" sub-

Howard Hughes Medical Institute, Molecular Biology Program, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. E-mail: s-keeney@ski.mskcc.org



From topoisomerase to DSB-forming machine. (**A**) Topo VI uses ATP binding and hydrolysis by the B subunit to coordinate the passage of a DNA duplex through a DSB made by the A subunits, thereby altering DNA topology. (**B**) An ancestral Topo VI evolved into a meiotic DSB-forming complex that has different tertiary and quaternary structures in different organisms.

units that use adenosine triphosphate (ATP) binding and hydrolysis to control DNA cutting and to effect cycles of "gate"-closing and -opening that are needed for DNA strand passage (see the figure) (*5*).

Not surprisingly, Spo11 derives from the DNA-cleaving A subunit (*6*, *7*), but the evolutionary fate of the B subunit has been a mystery because no meiotic equivalent to the B subunit had ever been identified. This mystery has been solved with the discovery of a Topo VIB–like partner of Spo11 by Vrielynck *et al*. and Robert *et al*. This is a landmark achievement that has important implications for understanding the mechanism and regulation of meiotic DSB formation.

Vrielynck *et al*. performed a forward genetic screen in the plant *Arabidopsis thaliana*, searching for mutants with reduced fertility. The authors mapped two mutant alleles to a gene that, based on sequence similarity alone, did not reveal homology to any known functional domains. However, structural model–based homology searches revealed a match with archaeal Topo VIB. Indeed, the identified protein—named MTOPVIB for "meiotic"—retains most of the structural features of the ancestral Topo VIB, including the transducer domain that interacts with the A subunit and the ATP-binding fold (*5*). It is not known whether the latter region, known as the GHKL fold (for gyrase, Hsp90, histidine kinase, MutL), retains ATP binding and hydrolysis activity.

As expected for a bona fide partner of Spo11, Vrielynck *et al*. found that MTOPVIB is essential for DSB formation and interacts with both SPO11-1 and SPO11-2, the two Spo11 paralogs that are both required for meiotic DSB formation in *A. thaliana* (*3*). Interestingly, MTOPVIB supports an interaction between SPO11-1 and SPO11-2, which suggests that they may assemble into a heterotetrameric complex containing both SPO11 variants. This solves a long-standing puzzle as to how SPO11-1 and SPO11-2 might work together to make DSBs.

Robert *et al*. set out to identify the mouse (*Mus musculus*) MTOPVIB ortholog. Clever molecular sleuthing involving computational methods to recognize weak homology between highly diverged protein sequences led them to a meiosis-specific mouse gene that they have named *Top6bl*, for *Topo VIB–like*. As for its *A. thaliana* counterpart, mouse TOP6BL protein is predicted to be structurally similar to archaeal Topo VIB and is essential for meiotic DSB formation. TOP6BL interacts physically with mouse SPO11 and, interestingly, does so specifically with the β isoform of SPO11, a splicing variant that is responsible for the majority of DSBs in mice. Remarkably, TOP6BL does not appear to interact with SPO11α, which lacks a 38–amino acid segment in the N-terminal part of the protein that was previously suggested to be equivalent to the B subunit binding interface on archaeal Topo VIA (*5*). When coexpressed in *Escherichia coli*, TOP6BL and SPO11β can be purified as a complex that behaves as a heterotetramer.

Robert *et al*. also searched in other eukaryotes and made the surprising discovery that *Saccharomyces cerevisiae* Rec102, *Schizosaccharomyces pombe* Rec6, and *Drosophila melanogaster* MEI-P22 each have structural similarity to the archaeal B subunit. This is impressive detective work because, although the yeast and fly proteins have been known for years to be required for DSB formation and the yeast proteins had long been connected physically to Spo11 (*1*), the structural similarity to Topo VIB had never been noticed before. However, unlike the mouse and plant proteins, those from yeast and fly share homology only to the Topo VIB transducer domain and lack the GHKL fold. This points to the possibility of intriguing mechanistic variability in the core DSB machinery of these organisms or may suggest that there are as yet unidentified subunits that have taken over the (still hypothetical) ATP binding and hydrolysis activity.

The studies of Vrielynck *et al*. and Robert *et al*. change our view of the initiation of meiotic recombination by showing that the core DSB machinery resembles the ancestral topoisomerase more closely than anticipated. In principle, DNA topology-changing strand passage might no longer have been required once the ancestral topoisomerase VI had been co-opted to catalyze meiotic DSBs during the evolution of sexual reproduction. It was therefore not clear a priori that Spo11 would need to retain a B-type subunit. That it does suggests that there are new regulatory mechanisms within the DSB machinery to be revealed. So far, mechanistic studies of meiotic recombination initiation have been lacking, largely because of the insolubility of recombinant Spo11. The successful coexpression and purification of mouse TOP6BL and SPO11 may thus open new avenues for biochemical investigation.

DSBs are intrinsically dangerous, yet meiotic cells play the risky game of forming a great deal of this self-inflicted DNA damage, numbering in the hundreds of DSBs per meiotic cell in yeast and mouse (*8*). Topo VIB now finally joins Spo11 at center stage in this process. ■

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