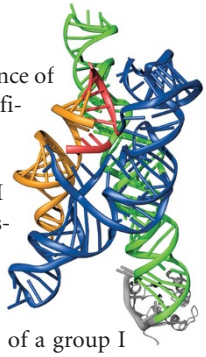
Piecing exons together



Splicing of group I introns can occur in the absence of proteins and involves two successive transesterification steps. In the first step, the 3′-OH of a guanosine cofactor acts as a nucleophile attacking the 5′exon-intron junction. Then the 3′-OH of the excised 5′ exon attacks the 3′ junction phosphate in the second step, the two exons are ligated and the intron is released. To provide a structural basis for splice site selection, Strobel and colleagues have solved the crystal structure of a group I intron from the purple bacteria *Azoarcus*. The structure represents an intermediate in the splicing reaction, after the first step but before the second. The authors were able to 'stall' the reaction by replacing four ribose nucleotides with their deoxyribose counterparts, thereby substantially reducing the splicing activity of the molecule. The structure explains a large body of biochemical data on group I introns and provides the first view of the complete active site of the ribozyme. The 5′ splice site is specified by an invariant G•U wobble, which is recognized by a conserved loop rich in adenine nucleotides. Tertiary interactions between the 5′ exon and the intron keep the cleaved fragment in the complex for the second step of the reaction. The 3′-OH of the 5′ exon is positioned close to the junction between the intron and the 3′ exon; this arrangement allows inline nucleophilic attack of the scissile phosphate bond between the intron and the 3′ exon. The authors located two metal ions within the active site that may be involved in activating the nucleophile and coordinating the 2′-OH of the terminal nucleotide (ΩG) of the intron. ΩG is recognized by a pocket formed by four nucleotides in a novel conformation, marking the 3′ splice site. The structure reveals how the 5′ and 3′ exons are positioned to precisely splice out introns. (*Nature* advance online publication, 2 June 2004, doi:10.1038/nature02642) *HPF*

Two pathways to the rescue

It has been thought that homologous recombination (HR) is the main mechanism working in the late S/G2 phase of the cell cycle. HR uses an undamaged template to repair double-strand breaks (DSBs) created during replication. Another mechanism, nonhomologous end-joining (NHEJ) has been thought to work primarily on DSBs during the G1 phase. But this division of labor may not be quite so simple. There is some evidence that HR and NHEJ can work simultaneously on a DSB or even compensate for one another if one pathway is weakened by mutation. Recently, Mills *et al.* and Couëdel *et al.* created mutant mice where components exclusive to either HR or NHEJ were removed. A deletion of Rad54 causes a very mild phenotype compared to mutations in other HR components. However, when it was combined with a moderate NHEJ mutation, in Ku80, the number of mice and the longevity were severely diminished compared to either mutation alone. *Rad54*$^{-}$ *Lig4*$^{-}$ embryonic fibroblasts had greatly increased DSBs and greater genomic defects such as fragmented chromosomes when compared to *Lig4*$^{-}$ cells, which have no NHEJ. These double mutant cells were also severely compromised for growth and seemed to die in G2. These findings reveal a new synergy between the two repair pathways, with Rad54 playing a more prominent role in DSB repair than suspected and NHEJ having an important role outside of G1. (*Genes Dev.* **18**, 1283–1292 and 1293–1304, 2004) *MB*

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A means to the end

Maintenance of telomere length is critically dependent on interactions between telomere-binding proteins, such as TRF1 and TRF2 that bind double-stranded telomere repeats. TRF1 interacts with several proteins including TIN2 and POT1. Although the oligonucleotide-binding (OB) fold of POT1 can directly bind to single-stranded telomeric DNA, a POT1 mutant lacking the OB fold can still be recruited to telomeres. This observation indicates that POT1 could be targeted to DNA ends by an additional factor. Songyang and colleagues now report the identification of a novel human telomere protein, PTOP (POT1- and TIN2-organizing protein), that may be involved in this recruitment. Gel filtration analysis identified a cellular complex consisting of TIN2, TRF1, TRF2, POT1 and PTOP. PTOP co-localizes with telomere proteins and is required for POT1 telomere localization. The PTOP-binding region of POT1 alone was sufficient for POT1 localization to telomeres. Binding of POT1 to PTOP did not require its OB-fold, which may explain the observed recruitment of the OB-fold mutant to telomere ends. The authors observed that expression of either the POT1-interacting region of PTOP, or the POT1 domain that interacts with PTOP, resulted in extended telomeres. These results reveal an important role for PTOP-POT1 interactions in telomere length regulation. The detailed mechanisms underlying POT1 localization, how PTOP itself is recruited to telomeres, and how these interactions modulate telomerase activity remain to be deciphered, but only by identifying all the component parts can telomere length regulation begin to be fully understood. (*Nat. Cell Biol.* **6**, 673–680, 2004) *RC*

Regulating transcription with lipids

The complexity of cell membranes, which are composed of a diverse array of lipids, is regulated primarily by feedback that acts at the level of lipid biosynthesis. Recent insights into the homeostatic mechanisms that maintain the concentrations of different lipids have emerged from the study of membrane-bound transcription factors at the major site of lipid synthesis, the ER. In response to changes in lipid concentrations, portions of lipid sensor complexes are released from the ER membrane and move to the nucleus where they modulate transcription of target genes. How the activation of these transcription factors is coupled to changes in lipid composition of the ER membrane is not known. In yeast, phospholipid metabolism is regulated by three transcription factors (Ino2p, Ino4p and Opi1p) that respond somehow to inositol, the headgroup of phosphatidylinositol (PI) and many different lipids derived from it. Levine and colleagues find that phosphatidic acid (PA) in the ER, a precursor of many different lipids including PI, directly binds to and inactivates Opi1p. This protein has at least two PA-binding sites, one of which is localized to a basic domain in the N-terminal half of the protein and is important for inositol-mediated activation of Opi1p. Yeast are poised to respond to appreciable levels of exogenous inositol, using high affinity transporters that feed inositol to PI synthase, thus triggering the rapid consumption of PA. As the concentration of PA decreases, Opi1p is released from the ER membrane and translocates into the nucleus where it represses the transcription of genes involved in phospholipid metabolism. Although many proteins are known to bind PA, the studies from Levine and colleagues demonstrate a physiological role for a pool of PA and show that it can function as a signaling lipid to couple membrane composition to transcriptional regulation of lipid biosynthesis. (*Science* **304**, 1644–1647, 2004) *EJ*