A new partner for telomerase protein

Cancerous cells can rapidly divide and have the potential to live indefinitely. The survival of such cells through cycles of cell division critically depends on their ability to maintain chromosome ends—or telomeres—by a multisubunit enzyme called telomerase. In a new study, Lin and Blackburn show that, in *Saccharomyces cerevisiae*, one protein called yPinX1p may help to regulate telomerase function. When yPinX1p, a nucleolar protein, was overexpressed in the cell, they observed shortened telomeres. In contrast, overexpression of the reverse transcriptase component of telomerase, Est2p, slightly lengthened the telomeres. If both proteins were overexpressed, telomeres returned to the wild-type length. The observed regulatory effect of Est2p on telomerase activity is a result of direct interaction between yPinX1p and Est2p *in vivo*. Interestingly, the region of Est2p that binds yPinX1p overlaps with the region of Est2p that binds the essential telomerase RNA component TLC1. So, either yPinX1p or TLC1—but not both—can bind Est2p at any one time. Indeed, the authors found distinct telomerase complexes containing Est2p: an active complex contains Est2p and TLC1 RNA and another inactive complex contains Est2p and yPinX1p. This competition for Est2p binding suggests that yPinX1p can presumably sequester Est2p in the nucleolus, thereby regulating the activity of the telomerase. (*Genes Dev.* **18**, 387–396, 2004) *MB*

RAG-mediated translocations

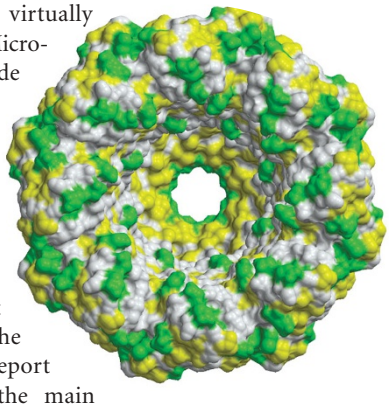
Reciprocal translocation occurs when two chromosomes are broken, the broken ends swapped and the DNA rejoined to a new partner. If such an event disrupts a gene, it can be deleterious to the cell. Many human lymphoma cells are found to contain reciprocal translocations between chromosomes 14 and 18. The sites of breakage on chromosome 14 are in the vicinity of the immunoglobulin heavy-chain locus. The chromosome 18 breakpoints lie within a well-defined, 150-bp major breakpoint region (Mbr) in the *Bcl-2* gene, the protein product of which has both pro- and anti-apoptotic functions. Lieber and colleagues have now reproduced the breakage event leading to this translocation and identified how it is initiated. It has been previously shown that the lymphoid-specific RAG complex promotes DNA cleavage during somatic recombination at the V(D)J locus near the chromosome 14 breakpoints. They therefore constructed a system in human cells to test whether overexpressed RAG complex nicked a cloned Mbr sequence propagated on an extrachromosomal element. They detected RAG-dependent nicking *in vivo* and *in vitro*, even though the Mbr lacks the specific sequences thought to be required for RAG cleavage activity. This observation is explained by the propensity of the Mbr to form an unusual, stable non-B-form structure with significant single-stranded character and possibly some DNA bending. These results implicate the RAG complex in lymphoid cell translocation events and demonstrate that its presumed sequence requirements can be overridden if a substrate with appropriate structural characteristics is provided. They also show that the RAG complex, which has a central role in generating antibody diversity, can have a detrimental effect if its activity becomes dysregulated. (*Nature* 428, 88–93, 2004) ***AKE***

Research Notes written by Mirella Bucci, Boyana Konforti, Angela K Eggleston and Deirdre Lockwood.

Getting into the nucleus

The compartmentalization of proteins within the nucleus or cytoplasm of a eukaryotic cell can be used to control gene expression and cell cycle progression. But for this to work well, transport between the two compartments has to be rapid, selective and highly regulated. All macromolecules move in and out of the nucleus through nuclear pore complexes, large channels that are embedded in the nuclear envelope. The best-characterized nuclear transport process involves recognition of a nuclear localization signal (NLS) on the cargo protein by factors such as importin-α and -β. Importin-α recognizes and binds to the NLS and importin-β mediates transport of the importin-α–cargo complex through the nuclear pore. In many cases transport of an NLS-containing protein into the nucleus has been shown to be regulated by phosphorylation of the cargo protein. Phosphorylation could regulate protein import by causing a conformational change in the protein which reveals or masks an NLS; causing release or binding of an NLS masking protein; or directly modulating the affinity between the NLS and importin-α. Now, Corbett and co-workers directly test the latter idea. They show that mimicking phosphorylation of an NLS can decrease the affinity of an NLS for importin-α *in vitro*. This decrease in affinity correlates with a decrease in nuclear accumulation of the NLS cargo protein *in vivo*. These studies define one way to regulate nuclear import by the NLS-mediated pathway. (*J. Biol. Chem.* published online ahead of print, 3 March 2004, doi:10.1074/jbc.M401720200) *BK*

A goblet in the outer membrane



Mycobacteria have thick, virtually impermeable membranes. Microbes in this genus include pathogens that cause tuberculosis and leprosy, and their tough outer membranes allow them to evade most antibiotics. Protein channels embedded in the outer membrane called porins permit select nutrients to enter the cell. Schulz and colleagues report the structure of MspA, the main porin from *Mycobacterium smegmatis*. The structure reveals that MspA consists of eight identical subunits. Its overall shape is like a goblet and this architecture does not resemble any other known bacterial porins. MspA contains a circular central channel that tapers to a narrow 'eyelet.' Each subunit is divided into two parts: a globular domain, which forms the rim of the goblet, and a loop region, which forms its stem and base. The narrowest point of the channel is within the base, and has a diameter of ~10 Å. The thickness of the presumed membrane-inserted region of MspA is ~37 Å. This is much smaller than the estimated thickness of the mycobacterial outer membrane, ~90 Å. Thus, future studies will be necessary to resolve this discrepancy. This is the first reported structure of a mycobacterial outer-membrane protein, and the authors suggest that this structure could aid studies of other porins in the mycobacterium that cause tuberculosis. (*Science* **303**, 1189–1192, 2004) *DL*