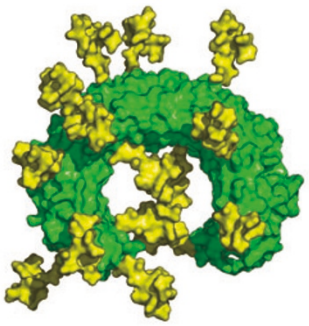
RNA takes a Toll



Toll-like receptors (TLRs) recognize and bind pathogen-associated molecules to initiate innate and adaptive immune responses. They can be found on the surface of cells and subcellular compartments and have a ligand-binding extracellular domain, or ectodomain (ECD), comprised of leucine-rich repeats (LRRs) and an intracellular domain that recruits downstream signaling partners. Human TLR3 can be activated by dsRNA associated with viral infection. Wilson and colleagues have determined the structure of human TLR3 ECD. It has 23 LRRs that form a horseshoe-shaped, right-handed solenoid. Although the ECD has 15 potential glycosylation sites, electron density is observed for only 1 or 2 sugar moieties at 8 of the sites. When oligomannans are modeled into all 15 sites, this reveals that most of the ECD surface, with the exception of one major face, is covered with carbohydrates. Surprisingly, the inner concave surface is glycosylated and has many negatively charged residues, making it an unlikely site for RNA binding. The authors suggest instead that RNA binding occurs at a dense basic patch on the unglycosylated face. The authors propose that dsRNA binds to one face of a TLR3 ECD to form a ternary complex with two TLR3s. Crystal packing interactions indicate that a portion of the glycosylation-free surface may be part of a dimer interface, as it contains a cluster of conserved residues. Similar to growth factor and cytokine receptors, RNA binding may promote signal transduction either by causing a conformational change to a pre-existing receptor dimer or by inducing receptor dimerization. (*Science*, published online 16 June 2005, doi:10.1126/science.1115253) *MM*

RNAPII runs interference

RNA interference (RNAi) has been widely used to knock down expression of genes of interest. In this process, RNAi-associated factors act on long double-stranded RNAs (dsRNAs), generating small interfering RNAs (siRNAs) that target homologous mRNAs for cleavage. In some species, siRNAs induce modification of chromatin as well. However, the mechanism for this action is unclear. Two papers now show that DNA homologous to the siRNA must be transcribed by RNA polymerase II (RNAPII) to induce chromatin modification. Murakami and colleagues have identified a mutation in RNAPII in *Schizosaccharomyces pombe* that results in the loss of heterochromatic histone modifications, the accumulation of centromeric transcripts and the absence of siRNAs. These phenotypes are similar to those in yeast lacking RNAi, so the authors theorize that RNAPII connects transcription near the centromere with siRNA processing. Allshire and colleagues, also working with *S. pombe*, confirm this, showing that RNAi-mediated chromatin modification occurs only if homologous centromeric DNA sequences are transcribed by RNAPII. Furthermore, the authors show that truncation of the regulatory C-terminal domain (CTD) of RNAPII interferes with transcriptional silencing and with the association of Argonaute1, a key component of the RNAi machinery, with centromeric chromatin, although deletion of the CTD does not affect siRNA generation. These data suggest that the CTD of RNAPII may facilitate the conversion of RNAi signals into chromatin modification, perhaps by acting as a scaffold for Argonaute1 and the transcriptional silencing machinery. Although further studies are needed to dissect the exact mechanism behind this conversion, these results show that RNAi-mediated chromatin modification, like other RNA processing events, is coupled to transcription. (*Science*, published online 9 June 2005, doi:10.1126/science.1114955, and *Nature* advance online publication, 19 June 2005, doi:10.1038/nature03652) *DM*

Research highlights written by Evelyn Jabri, Boyana Konforti, Michelle Montoya and Dorothy Moore.

Staying alive

When cells divide, each chromosome must be accurately replicated. During this process the replication machinery can encounter obstacles (either natural or induced) that may cause it to pause or stall. In some systems, these replication fork barriers (RFBs) can induce replication checkpoint proteins that protect stalled forks and prevent disassembly of the replication machinery. In other cases, RFBs have been associated with increased recombination. Because human cancers are often linked to genomic rearrangements caused by aberrant recombination, Carr and colleagues wanted to examine the connections between RFBs, checkpoints and recombination. To do so, they established a system where they could induce fork stalling at a particular site in the *S. pombe* genome. They show that recombination proteins are recruited to stalled replication forks and are required for cell survival. Interestingly, cell viability does not depend on replication checkpoint activity. Instead, in this case, the replication forks are not stabilized and recombination is induced to bypass the RFBs. Further, they show that this RFB-induced recombination results in gross chromosomal rearrangements. Thus, though recombination is necessary to get past the RFB, it comes at a considerable price—that is, genome instability. (*Cell* **121**, 689–702, 2005) *BK*

A remodeling job

In yeast and animal cells, the nuclear envelope expands during the cell cycle to accommodate changes in chromatin content. The molecular mechanism underlying this nuclear membrane growth is not understood but involves an increase in lipid and protein components of the phospholipid bilayer. Two proteins, Nem1 and Spo7, are anchored to the nuclear membrane and are essential for the maintenance of the correct nuclear morphology. Now Siniossoglou and colleagues show that the Nem1–Spo7 complex is a phosphatase that regulates the growth of the nuclear membrane by controlling recruitment of Smp2, the yeast homolog of the adipogenic factor Lipin1, to chromatin. The authors show that Smp2 is phosphorylated in a cell cycle–dependent manner by mitotic cyclin–Cdc28/Cdk1. A pool of Smp2 is recruited to promoters of genes involved in lipid biosynthesis and represses their expression. Interestingly, deletion of the Nem1–Spo7 phosphatase disrupts the interaction of Smp2 with these promoters, indicating that recruitment of Smp2 to DNA is regulated by phosphorylation. The authors also show that the accumulation of dephosphorylated Smp2 inhibits nuclear growth and mitotic division. These data suggest that the primary role of the Nem1–Spo7 phosphatase complex is to repress nuclear growth by dephosphorylating Smp2, and they provide evidence that these proteins are crucial in membrane remodeling during the cell cycle. Since mutations in nuclear envelope proteins have been linked to lipodystrophy and mutations in mouse Lipin1 cause fatty liver dystrophy, these data could further underscore the significance of proper nuclear membrane biogenesis in human physiology. (*EMBO J.* **24**, 1931–1941, 2005) *EJ*