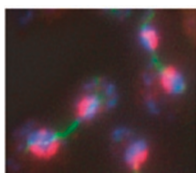
© 2006 Nature Publishing Group http://www.nature.com/nsmb

Clamping down on unequal exchange



The ribosomal DNA (rDNA) locus can consist of >10,000 repeating units, raising the issue of how unequal exchange is prevented during recombination. Although the chromatin-silencing factor Sir2 has been linked to inhibition of recombination at this locus, unique characteristics of rDNA silencing, such as the high rate of transcription, suggest a novel recombination-repression mechanism. Moazed and colleagues have recently defined a series of interaction and recruitment events linking the RENT complex, which inhibits rDNA recombination, to a complex that clamps homologous loci together. In particular, Tof2 is shown to copurify with RENT complex components, but it also interacts with and recruits Lrs4 and Csm1, members of the monopolin complex, which bridges and co-orients sister chromatids. Tof2, Lrs4 and Csm1 are shown to be required for silencing of a reporter, mediated by an rDNA spacer region known to inhibit recombination. In addition, Lrs4 and Csm1 mutants have increased rates of unequal sister-chromatid exchange, as assessed by unequal segregation of a reporter in the rDNA locus, and Csm1 has previously been shown to interact with the cohesin complex. Altogether, this suggests a mechanism for preventing aberrant expansion or contraction of rDNA repeats: Tof2 localizes with RENT to a specific rDNA sequence, where it in turn recruits Lrs4 and Csm1 to clamp chromatids together via interactions with cohesin, preventing lateral slippage and consequent unequal exchange. Intriguingly, Lsm4 and Csm1 are released from the nucleolus during anaphase. The implications of this dynamic cell cycle–dependent localization and the inclusion of Cdc14 in the protein-protein interaction network suggest cell cycle regulation, the mechanism and implications of which await further investigation. (*Genes Dev.* **20**, 2887–2901, 2006) *SL*

Chaperoning nascent polypeptides

In bacteria, transcription and translation are often coupled, and translation speed is faster than in eukaryotes. The bacterial chaperone trigger factor (TF) associates with the ribosome and contacts the nascent polypeptide chain to assist in protein folding. Recent work from the Hartl laboratory provides new insight on how TF functions. By labeling TF simultaneously with two fluorophores (donor and acceptor), the authors were able to monitor by FRET the conformational changes in TF during its interaction with the ribosome and the newly forming protein. When TF associates with purified ribosomes, it adopts an activated form, with a more open conformation that is able to interact with an emerging polypeptide chain. As it dissociates from the ribosome, TF undergoes compaction and returns to its inactive conformation. However, when TF interacts with ribosomes actively engaged in translation, it follows a different course after undocking from the translating ribosome: TF remains in an open conformation, associated with the elongating polypeptide chain. The amount of time TF spends on the nascent chain correlates with the presence of hydrophobic motifs on the polypeptide. As these aggregation-prone, hydrophobic stretches are properly folded and buried inside the polypeptide, TF is finally released. Thus, TF assists folding by associating with hydrophobic stretches in the unfolded nascent polypeptide chain, thereby preventing misfolding and aggregation. These findings provide important details about the interaction cycle of a chaperone with its substrate, highlighting the power of fluorescence spectroscopy to study dynamic processes such as protein folding. (*Nature*, advance online publication 15 October 2006, doi:10.1038/nature05225) *IC*

Mismatch made in heaven

Translesion (Y family) polymerases use different mechanisms to promote mutagenic, damage-tolerant synthesis past DNA lesions. One Y family polymerase, pol κ, is unusual in that it has higher fidelity than other such polymerases and cannot incorporate a nucleotide opposite a lesion; in addition, it can extend from a mismatched or lesion-containing terminal base pair. Washington and colleagues performed pre–steady-state experiments on pol κ–mediated incorporation opposite matched and mismatched primer termini. Surprisingly, in the presence of a matched primer terminus, pol κ is bound in an unproductive complex. On a mismatched primer terminus, the initial burst of synthesis activity is 80-fold slower (as expected, given that there should be a kinetic cost for extending from mismatched base pairs), but the amount of productive complex formed is substantially greater. Nonproductive complexes on matched termini can slowly convert to productive complexes without dissociating, and when this occurs, they are 100-fold more efficient at incorporating the following correct nucleotide. Although it remains to be determined how the nonproductive complex forms (for example, does it occur when pol κ binds DNA or when nucleotide binds the enzyme–DNA complex?), these studies do show that pol κ does not discriminate between matched and mismatched termini at the level of DNA or dNTP binding. Yet somehow the enzyme has evolved so that it forms a nonproductive complex on the 'normal' substrate and now recognizes the mismatched template as its natural substrate. (*Proc. Natl. Acad. Sci. USA* **103**, 15776–15781, 2006) *AKE*

A look at desensitization

Ligand-gated ion channels typically occupy three conformational states: resting, activated and desensitized. Ligand binding by a channel agonist promotes conformational change to the active state, generally leading to channel opening. The channel can then either return to the resting state, with dissociation of ligand, or transition to a desensitized state, in which ligand remains bound but the receptor is insensitive to its activating effects. Extensive structural studies on the extracellular ligand-binding domain (LBD) of ionotropic glutamate receptors (iGluRs) have allowed visualization of the resting (apo) and the active (glutamate-bound) states. Glutamate binds in the crevice between the halves of the clamshell-like LBD. These LBDs are arranged as a dimer of dimers within the full-length tetrameric receptor, with the domains arranged back to back and an extensive interface within each dimer. The degree of LBD closure controls channel activation, with the apo state having the largest clamshell opening and the agonist-bound state the smallest. Information on the mechanism behind desensitization has been limited. Now, data from the Gouaux lab offer unique insight into how desensitization occurs. Using a collection of mutations located at the LBD dimer interface of iGluR2, combined with electrophysiological and structural analysis, the authors show that receptor desensitization involves disruption of the dimer interface. The conformational change that allows these two domains to partially disengage from each other negates the effect of agonist binding on LBD closure, leading to closing of the channel. Further experimentation will elucidate whether this strikingly simple mechanism is used by other ligand-gated ion channels. (*Cell* **127**, 85–97, 2006) *MM*

Research highlights written by Inês Chen, Angela K. Eggleston, Sabbi Lall and Michelle Montoya.