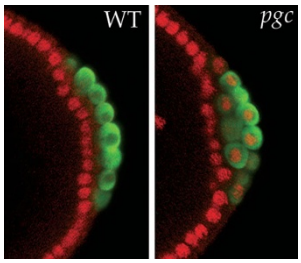
Noncoding RNAs repress transcription



Control of gene expression is essential for proper development of an organism. For example, transcription within germ cells—undifferentiated cells that will eventually form the egg or sperm of the organism—is shut down during a critical stage in embryo development. This repression is thought to specify the developmental fate of the cells and prevents their differentiation while the surrounding cells mature. The Lehmann group now shows that a noncoding RNA, *polar granule component* (*pgc*) is required for transcriptional repression in *Drosophila* germ cells. *In vivo*, this repression is alleviated when two residues, Ser2 and Ser5, in the C-terminal domain (CTD) of RNA polymerase II (Pol II) are phosphorylated. In wild-type germ cells (green, left panel), Pol II is phosphorylated at Ser5 but not Ser2, so the enzyme is transcriptionally inactive. In germ cells lacking *pgc* RNA (green, right panel) Pol II is phosphorylated at both Ser5 and Ser2 (orange dots indicate phosphorylated Ser2 in the CTD). The addition of this second modification leads to transcription activation in these mutant cells. Martinho and colleagues propose that *pgc* RNA blocks the transition from pre-initiation to transcription elongation in germ cells, perhaps by sequestering components required for CTD phosphorylation. (*Curr. Biol.* **14**, 159–165, 2004). *EJ*

Completing the elongation cycle

The first step in expressing genetic information is the synthesis of RNA based on a DNA template. This reaction is catalyzed by RNA polymerases, and the reaction consists of two stages: initiation and elongation. In the first stage, the polymerase initiates transcription *de novo*, but the enzyme is not very processive; after initiation, the polymerase converts to a highly processive complex. During the elongation cycle, the enzyme binds nucleoside triphosphates and incorporates a nucleoside monophosphate moiety into the nascent transcript, releases the pyrophosphates and translocates along the DNA. Understanding how nucleotide addition is coupled to forward movement on the DNA requires a detailed description of the conformational changes in the polymerase during the enzymatic cycle. Two crystallographic studies of a single-subunit RNA polymerase—that of bacteriophage T7—now provide new structural information that goes some way toward completing the cycle.

The overall architecture of the catalytic domain of T7 RNA polymerase is like a cupped right hand, where the palm domain contains the catalytic residues, and the finger and thumb domains are involved in positioning the substrates for catalysis. In one study, Temiakov and co-workers report the structure of the C-terminal domain of T7 RNA polymerase in complex with a nucleic acid substrate and an ATP analog. They found that the incoming ATP analog forms base-specific interactions with the templating nucleotide, and that a tyrosine residue specifically recognizes the 2′ OH group of the ATP analog. However, the β- and γ-phosphates of the ATP analog are distant from the catalytic residues, and the authors suggest that the structure corresponds to the 'pre-insertion' state of the polymerase. These observations suggest that substrate selection by T7 RNA polymerase occurs before the enzyme becomes capable of incorporating nucleotide.

The two structures of the T7 RNA polymerase reported by Yin and Steitz represent the substrate and the product complexes, respectively. In the substrate complex (with nucleic acid and an incoming ATP analog), part of the finger domain undergoes a conformational change, closing over the incoming nucleotide binding site and bringing the β- and γ-phosphates into close proximity with the catalytic residues. This structure corresponds to the 'insertion' state of the polymerase. The overall structure of the product complex (with nucleic acid and bound pyrophosphate) is very similar to that of the substrate complex and is in a 'pre-translocation' state. A comparison of this structure with that of a T7 RNA polymerase in the 'post-translocation' state (without bound pyrophosphate) shows a rotation of a part of the finger domain that is associated with both polymerase translocation and pyrophosphate dissociation. Thus, pyrophosphate release appears to induce the conformational changes that drive the forward movement of the polymerase on the DNA. These conformational changes are also associated with strand separation of the downstream DNA duplex and displacement of the RNA. (*Cell*, 116, 393–404 and 381–391, 2004) *HPF*

The SAGA of an activator complex

When yeast cells are asked to live on galactose as their sole source of sugar, an intricate transcriptional program culminating in the formation of galactose-utilization enzymes is rapidly put into place. One key player, Gal4p is required for transcriptional activation at the promoter upstream of the galactose-utilization genes. It has been unclear how Gal4p recruits the transcriptional machinery needed for proper gene expression. Sukesh Bhaumik and colleagues now implicate a protein within one of the multiprotein complexes that Gal4p is thought to recruit. Using a FRET-based two-hybrid approach, they found that, when cells were placed in galactose, Gal4p interacts with Tra1p, one of the 14 members of the SAGA transcriptional initiation complex. Their results suggest a stepwise assembly where Gal4p first recruits Tra1p, in the context of an intact SAGA complex. Next, SAGA recruits a second complex called Mediator which is finally responsible for recruiting the core transcriptional machinery and RNA polymerase II. Curiously, Tra1p and its human equivalent, TRRAP are also involved in separate complexes distinct from SAGA, suggesting that they may be generally involved in orchestrating the recruitment of other collections of proteins including those containing the oncogene products c-myc and E2F. (*Genes Dev.* **18**, 333–343, 2004) *MB*

Delaying death

Cell death and cell survival are two sides of the same coin. In keeping with this, Olivier Donzé and colleagues find that a single protein, the proapoptotic protein kinase PKR, also activates a survival pathway that protects cells against death. By studying the activation kinetics of two PKR targets—NF-κB and eukaryotic translation initiation factor-2α (eIF-2α)—and the PKR-induced gene expression profile, the researchers find that PKR carries out these two separate functions in a chronological manner. It initiates, in a kinase-independent fashion, a survival response mediated by NF-κB. The activation of this pathway delays apoptosis induced by PKR's phosporylation of eIF-2α. The authors propose that, through these kinase-dependent and -independent mechanisms, PKR acts as a 'molecular clock' to regulate the timing of the survival and cell death responses. (*EMBO J.*, published online 29 January 2004; doi:10.1038/sj.emboj.7600078) *DL*

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