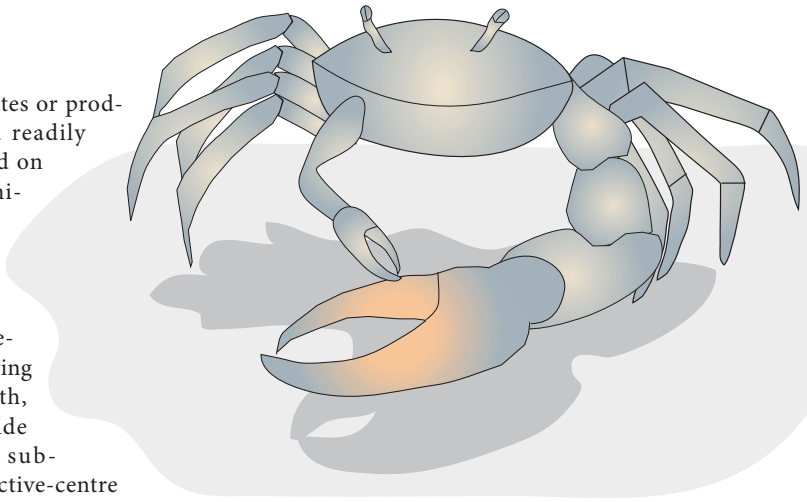
MILESTONE 23

Polymerase pincers



Before 1999, much of what was known about prokaryotic and eukaryotic multisubunit cellular RNA polymerases (RNAPs), their association with DNA and the process of transcription came from biophysical, biochemical and genetic experiments. Such studies defined three stages in the transcription cycle. In the first stage ('initiation'), RNAP locates specific binding sites on the DNA called promoters (see Milestone 5), melts the DNA helix at the transcription start site to yield a transcription bubble and begins synthesis of an RNA chain. In the second stage ('elongation'), RNAP progressively elongates the RNA chain while translocating along the DNA with the transcription bubble and a short RNA–DNA hybrid. Finally, in the third stage ('termination'), RNAP releases the RNA transcript and the DNA in response to specific termination signals. How these distinct stages were related to the structure of RNAP remained unknown.

Despite repeated attempts by different laboratories to obtain three-dimensional crystals of the well-studied *Escherichia coli* RNAP, these efforts had been abandoned (at least for the moment) by the mid-1990s. Against this backdrop, the high-resolution structures of a thermophilic bacterial RNAP published in 1999 and yeast RNAP II published in 2000 were an important breakthrough. Darst and co-workers solved the structure of the bacterial RNAP core from *Thermus aquaticus* at 3.3 Å, and Kornberg and co-workers determined the structure of yeast RNAP II to 3.5 Å. The structures revealed a crab-claw-shaped molecule with two pincers and a central cleft. The active centre was located on the floor of the cleft, where three absolutely conserved aspartic-acid residues chelated a Mg^{2+} ion. Although the structures were solved in the absence of templates, substrates or products, these could readily be modelled based on previous biochemical results. Both groups proposed that the DNA template binds in the active-centre cleft, following a sharply bent path, and that nucleoside 5′-triphosphate substrates enter the active-centre cleft through one tunnel, whereas the RNA product exits the active-centre cleft through another.

The structures were significant for several reasons. First, they were a technical *tour de force* representing the largest high-resolution structures without internal symmetry determined up until then. Second, the structures revealed a remarkable, indeed astonishing, degree of similarity between bacterial RNAP and yeast RNAP II, implying a fundamental similarity in mechanisms of transcription among all living organisms. Third, the availability of the structures transformed the study of transcription. For the first time, it became possible to design structure/function studies targeted to specific structural elements and protein–nucleic-acid interactions. For example, basic questions could be answered, such as how the enzyme pulls apart the two DNA strands to allow transcription to occur, how the transcription bubble and RNA–DNA hybrid remain constant in size yet move along DNA, how termination occurs and how regulation is achieved.

These questions began to be addressed almost immediately. Just a year later, in 2001, Kornberg and co-workers published a structure of yeast RNAP II in the act of transcription elongation. The structure revealed the position of the DNA–RNA hybrid and its key interactions with RNAP. In addition, the structure showed that one pincer of the RNAP crab claw was mobile — apparently swinging out to allow DNA to enter the active-centre cleft and swinging back in to clamp DNA inside it — and implied that specific conformational changes were associated with nucleotide addition.

Since 2001, there has been a veritable flood of RNAP structures, and biophysical, biochemical and genetic results. The Darst and Kornberg papers opened the floodgates.

Boyana Konforti, Editor,
Nature Structural & Molecular Biology

References and links

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