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A transcriptional specialist resolved

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Abstract

Three structures of the enzyme RNA polymerase III, which is responsible for the synthesis of abundant short RNAs, reveal the specializations that make it an adept terminator and reinitiator of transcription.

RNA polymerase enzymes have no small job. Present in every cell, they are essential for transcribing DNA into RNA. Bacteria and archaea each use a single RNA polymerase (Pol), whereas all other organisms (called eukaryotes) have three specialized Pols¹ — Pol I and Pol II, which synthesize different types of long RNA, and Pol III, which makes short RNAs. In a paper online in *Nature*, Hoffmann *et al.*² report three structures of Pol III, all at near-atomic resolution. These structures allow the authors to make comparisons with existing Pol I and Pol II structures, and to suggest how Pol III terminates and reinitiates transcription. Moreover, their work completes the set of five Pol structures.

Pol III produces a huge supply of short structural RNAs that collectively outnumber all other RNAs in the cell, and the enzyme must therefore initiate and terminate transcription more frequently than Pols I or II (ref. 3). Pol III is adapted for this role — it is the largest of the three eukaryotic Pol enzymes, with 17 subunits, some of which are Pol III-specific relatives of transcription factors that transiently associate with Pol II during initiation. The stable association of these subunits with Pol III enables efficient initiation of transcription and enzyme recycling⁴. The Pol III-specific subunits are organized into two subcomplexes, a C82–C34–C31 heterotrimer and a C53–C37 heterodimer, the latter of which is also involved in transcription termination⁵. But precisely how these subunits are positioned so as to contribute to the specialized functions of Pol III has remained unclear.

During transcriptional elongation of the nascent RNA strand, Pol III acts in a closed-clamp conformation, in which a cleft in the enzyme exerts a tight grip on the DNA awaiting transcription. Hoffmann *et al.* used cryo-electron microscopy to determine the structures of apoenzyme Pol III (the enzyme minus the DNA and RNA) from the brewer's yeast *Saccharomyces cerevisiae* in both closed- and open-clamp conformations, achieving resolutions of 4.6 and 4.7 Å, respectively. They also resolved the structure of the enzyme complexed with RNA and DNA during active elongation, to 3.9 Å.

Hoffmann and colleagues' structures reveal that the cleft of Pol III grips DNA more tightly than that of other Pols, in part because the clamp-head domain in subunit C160 is larger than in the other enzymes. The C82–C34–C31 heterotrimer packs onto this clamp head through C82–C160 interactions, and extensions of C82 wedge the DNA in the cleft at around 15 and

7 base pairs away from the active centre, where transcription occurs. Several sections on the other side of the heterotrimer face and are close to a stalk structure, which is common in Pols and is involved in initiation in Pol III. The stalk adopts different orientations in the two apoenzymes, possibly reflecting different stages of transcription.

The structures provide several clues to how Pol III is adapted for termination and initiation. The genes transcribed by Pol III end with a short string of thymine (T) bases called the terminator sequence⁶; its transcription produces an unstable RNA–DNA hybrid that causes Pol III to dissociate from DNA⁶. This process is aided by terminator T sequences in the complementary, untranscribed DNA strand. Termination and subsequent reinitiation are known⁵ to involve the collective activities of the C53–C37 heterodimer and another subunit required for efficient termination and reinitiation cycles, C11.

The authors find that C53–C37 is connected to C11 and attached to lobe and jaw domains (named after their shape), which lie across the cleft from the clamp head. C37 connects to DNA in the cleft through a helical extension, then continues to the active centre and makes contact with untranscribed sequences from the complementary DNA. This structure suggests a model in which C37 transmits the terminator signal to the cleft through a mechanism involving C11(refs 7, 8), to trigger opening of the clamp. Such a set-up might ensure that the downstream DNA is released in sync with termination (Fig. 1).

The C11 subunit contains two terminal domains, which are related to proteins called Rpb9 and TFIIS (ref. 9) that interact with Pol II. The TFIIS-related carboxy-terminal domain of C11 mediates cleavage of the end of the RNA in the active centre when the enzyme pauses (a prerequisite for termination). Hoffmann *et al.* report that, in the apoenzyme, this region is stored close to an entry gate to the active centre called the funnel pore. It is tethered by a linker to C11's amino-terminal domain, which is anchored by multiple contacts to C37, the lobe and the jaw, in agreement with an earlier study¹⁰. This set-up provides a firm anchor from which the C-terminal domain can swing on the linker, accessing the active centre during pausing, termination and, perhaps, reinitiation. Indeed, the authors could not map the C-terminal domain in the complexed structure, presumably because of its dynamic interactions with the active centre.

Of particular interest is the architecture of the active centre. Hoffmann and colleagues found that this centre grips the RNA–DNA hybrid only loosely, and they suggest that the unusually tight downstream cleft compensates for this during elongation. Because ‘melting’ of the intrinsically weak hybrid into single strands is a key determinant of termination¹¹, this loose grip probably allows Pol III to readily release the RNA from the active centre on cue.

These structures complete the triad of Pols that evolved from the bacterial enzyme, and provide explanations for the specialized properties that enable division of labour among the eukaryotic Pols. The three structures of Pol III give us much more information than one can. However, each represents only one state of a dynamic process. There is more to be learnt — for instance, large parts of the heterotrimer subunits C34 and C31 are yet to be resolved. Much of the heterodimer subunit C53 also remains unresolved, as do the interactions between the complementary DNA strand and C37, the lobe and the jaw. A better

understanding of these subunits should provide further insights into the mechanism by which termination occurs. Moreover, they might provide clues to how Pol III is reset for initiation.

Hoffmann and colleagues' structures are in agreement with much existing physical, biochemical and genetic data^{5–9,12,13}. They are also excellent substrates for mutational and mechanistic studies that could cast light on how cancers that depend on Pol III for growth might be attacked¹⁴.

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Figure 1. Function encoded in structure

Hoffmann *et al.*² resolved three structures of the enzyme RNA polymerase (Pol) III, which transcribes short RNAs. Pol III contains specialized subunits — the C82–C34–C31 heterotrimer and the C53–C37 heterodimer — that enable it to terminate and reinitiate transcription. The authors find that the heterotrimer packs onto the enzyme's clamp-head domain, and extends helices that help to hold the DNA awaiting transcription in a tight grip in the structure's cleft. The heterodimer connects to the lobe and jaw domains on the lower side of the cleft. By contrast, DNA in the active centre (where transcription occurs) is held loosely. During termination, the untranscribed DNA strand, which contains terminator signal sequences, makes contact with C37. C37 might transmit termination signals to the lower side of the cleft, releasing the downstream DNA. The authors propose that the loose grip in the active centre then enables the DNA to easily separate from the enzyme. For simplicity, not all Pol III subunits are shown.