

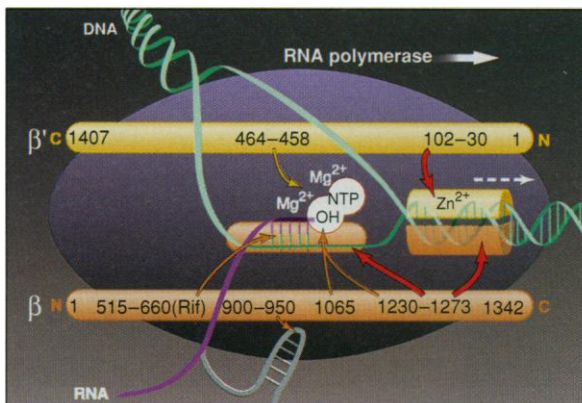
The Shrewd Grasp of RNA Polymerase

Robert Landick and Jeffrey W. Roberts

RNA polymerase synthesizes messenger RNA from the gene. As promised by the early visionaries of molecular biology, regulatory factors that act on RNA polymerase mediate key events in biological development and (when dysfunctional) in disease. How does this complex molecular machine maintain its grip on RNA and DNA as it traverses thousands or even millions (in higher eukaryotes) of base pairs (bp) and still respond to signals that instruct it where to start and stop and how rapidly to transcribe? This question remains one of the field's great unsolved mysteries. A report in this issue scores a major advance by defining the basis of polymerase association with DNA (1).

Two highly conserved large subunits (termed β and β') carry the basic polymerization activity and combine with a varied set of other subunits to form a core enzyme of nearly a half-million daltons or more ($\beta\beta'\alpha_2$ in *Escherichia coli*). Such complexity must reflect the disparate demands of tight DNA and RNA binding, long-distance stability, and extensive regulation, since one-subunit polymerases suffice for some viruses. What protein-nucleic acid interactions accommodate these requirements? A popular but controversial idea has been that RNA:DNA base pairing (8 to 12 bp) stabilizes the transcription complex (2, 3). An alternative model emphasizes the role of multiple protein-nucleic acid interactions (4, 5). Nudler *et al.* (1) show that stability requires contacts of ~ 10 bp of duplex DNA at the leading edge of the transcription complex to the COOH-terminal portion of β and an NH₂-terminal zinc-finger-like domain in β' (see figure).

These results are especially significant for RNA chain elongation, which enjoys varied modes of regulation. Unlike DNA polymerase, which can begin at any priming-strand terminus and only stops when the growing chain abuts an existing end, the $\beta\beta'\alpha_2$ core of RNA polymerase cannot restart chains once they are released; furthermore, its elongation progress is modulated by pause and termination signals that include both DNA and RNA (especially hairpin) elements (6, 7).



The transcription complex: Map of key contacts by RNA polymerase's large subunits (β and β') to RNA and DNA. β and β' are shown antiparallel to reflect contacts to the catalytic center and downstream DNA, although this scheme must greatly oversimplify their true three-dimensional paths. Contacts are depicted by arrows to positions in the subunits, with the duplex DNA clamp and salt-sensitive template strand contact (1) in red. Aspartates in the highly conserved β' 458 to 464 region probably chelate two Mg^{2+} ions (17), as in DNA polymerases and T7 RNA polymerase (18). Other aspects of the figure are from (19). Pausing and termination may be caused by the combined effects of hairpin formation (in gray) and forward movement of the downstream clamp (dotted line).

External factors further modify elongation, including gene-specific regulators that counter or promote termination, pausing, or arrest. In bacteria these include the phage λ N and Q transcription antiterminators, the Nun termination protein and *put* antitermination RNA of phage HK022, and the RNAs of attenuation control sites (7-9). In eukaryotes, factors like HIV Tat promote an antitermination modification that may be generally required for RNA pol II to remain stably attached to DNA over millions of base pairs, and even to escape from some initiation regions (10). Aberrant activity of other elongation factors (Elongin and Ell) is tied to two different hereditary cancers (11).

Recent discoveries suggest that these regulatory events depend on changes in the way domains of polymerase contact RNA and DNA (5, 6, 12). As polymerase approaches pause and termination sites, the downstream edge of polymerase stops moving along the DNA, and RNA stops exiting even though its synthesis continues. At these sites, the downstream edge jumps forward ~ 10 bp. These rearrangements, seen only in halted complexes (but which must reflect changes of some type in moving polymerase), are thought to act with an RNA hairpin that

either stabilizes a paused intermediate by interacting with polymerase or triggers RNA release at a terminator (see figure). Antitermination may occur by inhibiting one or more of these steps.

Nudler *et al.* began by serendipitously resolving a curiosity of RNA polymerase activity in vitro: it can make transcripts longer than the linear DNA templates it copies. Using an RNA polymerase containing a hexahistidine tag that can be fixed on nickel-containing beads and "walked" to specific sites by successive additions of nucleoside triphosphate precursors, Nudler *et al.* show that the explanation is template switching: successive linear templates adjoin on the enzyme. By characterizing interactions of RNA polymerase with different regions of the DNA, they dissected how the complex is held together.

When polymerase switches to a short synthetic DNA, it forms a complex stable to high salt, a hallmark of elongation complexes, but only if the second DNA has a duplex region of about 9 bp located a few base pairs ahead of the growing site (see figure). Salt-stable binding to DNA might be explained by hydrophobic protein-protein interactions that encircle the DNA duplex, reminiscent of the separate trimeric processivity factors that lock DNA polymerases onto the template (13). However, a single template strand alone also serves as the second DNA. The resulting complex lacks stability to high salt, thus mapping an ionic interaction of polymerase to about six nucleotides, from -4 to +2 relative to the template base being decoded. Using second templates containing photoactivatable cross-linkers, Nudler *et al.* then located the portions of RNA polymerase that make these contacts.

An intriguing speculation is that closing and opening of the duplex DNA "clamp" may reflect entry to (initiation) and exit from (termination) the stable mode of transcript elongation, which corresponds to a difference in the low-resolution structures of elongation and initiation forms of the enzyme (14). Nudler *et al.* suggest that RNA occupancy of the RNA exit channel closes the clamp. This could be one of the two clamps postulated to explain discontinuous movements of RNA polymerase as well as its ability to retain its grasp during prolonged elongation and, remarkably, during passage of a replication complex through the very DNA it is holding (15). These findings might also

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explain why mutations that abrogate anti-termination mediated by the HK022 *put* RNA structure occur in the β' zinc finger (9) and why the β half-clamp in eukaryotes contains a second zinc finger that (genetically) interacts with the β' zinc finger (16): to stabilize megabase transcription.

The results of Nudler *et al.* do not explain how polymerase rotates about the DNA helix. Does the clamp rotate (and lock) in the DNA major groove, and how much flexibility exists between it and other parts of polymerase? They also do not resolve whether an RNA:DNA hybrid exists; RNA:DNA disruption could be required for termination (for example, by inducing clamp opening), but it is clear that an RNA:DNA hybrid is not sufficient for stability. The ability of DNA polymerase to pass through a transcription complex from either direction appears to require that neither the clamp nor a hybrid is the sole determinant of stability under all conditions; each must be disrupted at different points to allow DNA polymerase

passage. How four known or postulated interactions—the downstream DNA duplex clamp, the RNA:DNA hybrid, single-stranded RNA in the exit channel, and RNA hairpin-polymerase contact—conspire to modulate the switches between rapid and stable elongation, pausing, and dissociation remains a challenge for clever experimentalists.

References

1. E. Nudler, E. Avetisova, V. Markovstov, A. Goldfarb, *Science* **273**, 211 (1996).
2. T. D. Yager and P. H. von Hippel, in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, F. C. Neidhardt *et al.*, Eds. (American Society for Microbiology, Washington, DC, 1987), vol. 2, pp. 1241–1275.
3. —, *Biochemistry* **30**, 1097 (1991).
4. T. Johnson and M. Chamberlin, *Cell* **77**, 217 (1995); B. Krummel and M. J. Chamberlin, *J. Mol. Biol.* **225**, 239 (1992).
5. M. J. Chamberlin, in *The Harvey Lectures* (Wiley-Liss, New York, 1995), vol. 88, pp. 1–21.
6. C. L. Chan and R. Landick, in *Transcription: Mechanisms and Regulation*, R. C. Conaway and J. W. Conaway, Eds. (Raven, New York, 1994), vol. 3, pp. 297–321.
7. J. Richardson and J. Greenblatt, in [(2), ed. 2,

- 1996], vol. 1, pp. 822–848.
8. R. Landick *et al.*, *ibid.*, p. 1263; J. W. Roberts, in *Regulation of Gene Expression in E. coli*, E. C. C. Lin and A. S. Lynch, Eds. (Landes, Austin, TX, 1996), pp. 27–44.
9. M. Clerget *et al.*, *J. Mol. Biol.* **248**, 768 (1995).
10. A. Krumm *et al.*, *Bioessays* **15**, 659 (1993); A. Rougvie and J. Lis, *Mol. Cell. Biol.* **10**, 6041 (1990).
11. A. Shilatifard *et al.*, *Science* **271**, 1873 (1996); T. Aso *et al.*, *ibid.* **269**, 1439 (1995).
12. D. Wang *et al.*, *Cell* **81**, 341 (1995); E. Nudler *et al.*, *ibid.*, p. 351.
13. T. Krishna *et al.*, *ibid.* **79**, 1233 (1994); X.-P. Kong *et al.*, *ibid.* **69**, 425 (1992).
14. A. Polyakov *et al.*, *ibid.* **83**, 365 (1995).
15. B. Liu *et al.*, *Nature* **366**, 33 (1993); B. Liu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 10660 (1994); B. Liu and B. M. Alberts, *Science* **267**, 1131 (1995).
16. R. Yano and M. Nomura, *Mol. Cell. Biol.* **11**, 754 (1991).
17. E. Zaychikov *et al.*, *Science* **273**, 107 (1996); V. Markovstov *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3221 (1996); G. Dieci *et al.*, *EMBO J.* **14**, 3766 (1995).
18. T. A. Steitz *et al.*, *Science* **266**, 2022 (1994).
19. D. Wang, K. Severinov, R. Landick, unpublished observations; R. Weilbaecher *et al.*, *Genes Dev.* **8**, 2913 (1994); K. Severinov *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4591 (1995); A. Mustaev *et al.*, *J. Biol. Chem.* **268**, 19185 (1993); A. Mustaev *et al.*, *ibid.* **266**, 23927 (1991); D. J. Jin and C. Gross, *J. Mol. Biol.* **202**, 45 (1988); K. Severinov *et al.*, *J. Biol. Chem.* **268**, 14820 (1993); A. Mustaev *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12036 (1994).

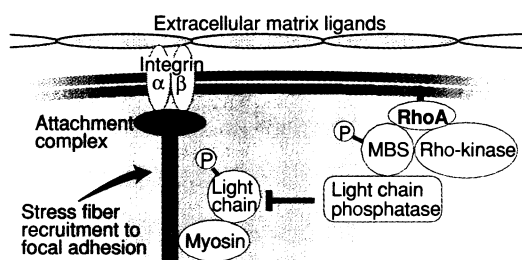
UPDATE

Rho Returns: Its Targets in Focal Adhesions

Howard Bussey

Two recent papers in *Science* (1) revealed why a yeast looks like a yeast. A small Ras-like guanine nucleotide-binding protein, Rho1p, acts as a morphogenetic coordinator to activate $\beta(1\rightarrow3)$ glucan synthase, which makes the glucans of cell walls. A Rho1p homolog in humans, RhoA, also has a morphogenetic role—the regulation of actin stress fibers, which emanate from small patches of the membrane called focal adhesions and allow the cytoskeleton to pull against the extracellular matrix and alter cell morphology. A report in this issue (2) reveals the detailed biochemistry of how mammalian RhoA controls stress fibers.

When activated (by growth factors or phospholipids, for example), RhoA is in its guanosine triphosphate-bound form and can bind to and activate protein kinases, including Rho-kinase. The new results show that an additional role of RhoA is to activate myosin, albeit indirectly. The direct Rho target is a regulatory subunit of a phosphatase that inactivates myosin by light chain dephosphorylation. This phosphatase regulatory subunit, called myosin-binding subunit (MBS), is inactive when phosphorylated. RhoA binds to MBS and also activates Rho-kinase to phosphorylate MBS. The result of this flurry of interactions is to inhibit myosin phosphatase activity, leading to activation of myosin through a net increase in myosin light chain



RhoA recruits actin stress fibers at focal adhesions.

phosphorylation by other protein kinases. Such activated myosin polarizes actin-myosin bundles, allowing the formation of stress fibers.

In yeast, does Rho1p also mobilize actin-myosin at the bud tip, thereby orchestrating both glucan synthesis and the underlying cytoskeleton? There are strong hints that Rho1p does just that. Cortical actin is highly organized at the bud

tip; Rho1p colocalizes with it in yeast cells, as does an unconventional yeast myosin, Myo2p (3). Myo2p also binds yeast calmodulin as a light chain, an interaction required for polarized growth (4). Rho1p may activate Myo2p, much as RhoA activates human myosin, through the inhibition of a myosin phosphatase that acts on phosphorylated calmodulin, the Myo2p light chain. Yeast Rho1p interfaces between protein kinase regulation of polarized glucan synthesis and (possibly) activation of the cytoskeleton, whereas its human homolog RhoA polarizes regions of the actin cytoskeleton to plasma membrane adhesion sites. Thus, this versatile multifunctional switch is an important, conserved component in determining cellular architecture in both yeast and mammals.

References

1. J. Drgonová *et al.*, *Science* **272**, 277 (1996); H. Qadota *et al.*, *ibid.*, p. 279.
2. K. Kimura *et al.*, *ibid.* **273**, 245 (1996).
3. W. Yamochi *et al.*, *J. Cell Biol.* **125**, 1077 (1994); J. Mulholland *et al.*, *ibid.*, p. 381; S. H. Lillie and S. S. Brown, *ibid.*, p. 825.
4. S. E. Brockerhoff, R. C. Stevens, T. N. Davis, *ibid.* **124**, 315 (1994).

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