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A Fully Recombinant System for Activator-dependent Archaeal Transcription*

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The core components of the archaeal transcription apparatus closely resemble those of eukaryotic RNA polymerase II, while the DNA-binding transcriptional regulators are predominantly of bacterial type. Here we report the construction of an entirely recombinant system for positively regulated archaeal transcription. By omitting individual subunits, or sets of subunits, from the in vitro assembly of the 12-subunit RNA polymerase from the hyperthermophile Methanocaldococcus jannaschii, we describe a functional dissection of this RNA polymerase II-like enzyme, and its interactions with the general transcription factor TFE, as well as with the transcriptional activator Ptr2.

Transcription in archaea is notable for its essentially mosaic character: the core transcription apparatus, RNA polymerase (RNAP) and the essential factors for promoter recognition, initiation, and elongation of transcripts, is of eukaryal type, while the DNA-binding transcriptional regulators are predominantly (although not exclusively) of bacterial type (reviewed in Ref. 1). This mixed nature of the archaeal transcription machinery does not pose conceptual difficulties for the understanding of archaeal transcriptional repressors, which perform their inhibitory roles by physically blocking the assembly of the basal machinery at promoters (reviewed in Ref. 2). It is, however, much less clear how bacterial type transcription factors can activate transcription in such systems.

The 12-subunit RNA polymerase from the hyperthermophile Methanocaldococcus jannaschii has been assembled from recombinant subunits in vitro. In conjunction with the likewise recombinant basal transcription factors TFB and TBP, the recombinant RNAP is capable of promoter-specific transcription (3). M. jannaschii also encodes two putative transcription regulators, Ptr1 and Ptr2, that are members of the Lrp/AsnC family of bacterial transcription regulators (4). Ptr2 is a potent activator of transcription in vitro and conveys its stimulatory effects on its cognate transcription machinery from two upstream Ptr2-binding sites. Activation is achieved, at least in part, by Ptr2-mediated recruitment of TBP to the promoter (5).

Prior work on this system was complicated by the fact that it employed conventionally purified archaeal RNAP fractions. It therefore could not be ruled out that these might contain small amounts of other, not yet characterized (e.g. RNAP-associated), factors that influenced the transcription-stimulating properties of the preparation. The availability of a completely defined recombinant enzyme puts this critique to the test and allows a precise dissection of the functional contributions of all the components present in the transcription reaction. Here we report the construction and characteristics of an entirely recombinant system for positively regulated archaeal transcription and define fundamental parameters of transcription stimulation.

MATERIALS AND METHODS

Protein Purification—The recombinant RNA polymerase from Methanocaldococcus jannaschii was assembled and purified essentially as described (3). The overproduction and purification of the recombinant M. jannaschii transcription factors TBP and TFB have also been described (5). M. jannaschii TFE was overproduced in E. coli Rosetta (pLyS8) (Stratagene) harboring plasmid pMO-194. This plasmid expresses a fusion gene encoding an N-terminal His6 tag, followed by the product of the M. jannaschii MJ0777 open reading frame. The His-tagged factor was affinity-purified on nickel-nitrilotriacetic-agarose and was eluted with 250 mM imidazole. Protein concentrations were measured by using the Micro BCA assay (Pierce), with bovine serum albumin as the standard.

DNA Templates—DNA fragments encompassing the M. jannaschii rb2 promoter region were generated by PCR, using oligonucleotides ON116 (5′-GGGAAAATCGAAAAAGGATCTGC-3′) and ON117 (5′-CGGCTCACCTTTGTCTTCATCATAC-3′), and were purified by native PAGE.

In Vitro Transcription—Reaction mixtures for Fig. 1 were assembled in 50 μL (final volume) of transcription buffer (20 mM Na-Hepes, pH 7.8, 10 mM MgCl2, 500 mM NaCl, 1 mM dithiothreitol, 2.5% (w/v) glycerol, 0.1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride). Single rounds of transcription were then initiated by the addition of nucleoside triphosphates, 0.5 μM [γ-32P]UTP (3,000 Ci/mmol), 0.1 mM [α-32P]ATP, 0.1 mM [α-32P]CTP, and 0.1 mM [α-32P]GTP (3,000 Ci/mmol), poly(dG-dC)·poly(dG-dC) to 80 μM. The incubation was terminated by the addition of rifampicin (100 μg/ml) and S1 nuclease (20 U/ml), and the reaction was stopped by the addition of 10 μl of formamide loading buffer. The reaction products were then separated by electrophoresis on 2%/10% polyacrylamide gels, and the radioactivity was detected by autoradiography.

RESULTS AND DISCUSSION

We examined the ability of the fully recombinant M. jannaschii in vitro transcription system to sustain Ptr2-dependent transcriptional activation at the previously analyzed rb2 promoter (Fig. 1A). Ptr2 strongly stimulates transcription at this very weak promoter (Fig. 1B, lanes 2–4) to levels similar to those previously reported for native M. jannaschii RNAP (5). These results thus prove that transcriptional activation in archaea does not depend on any yet uncharacterized activities, such as coactivators, and can be fully and accurately reproduced in a completely defined in vitro system.
Recombinant System for Activator-dependent Archaeal Transcription

The ability to omit individual subunits, or sets of subunits, from the polymerase assembly opens up an opportunity for a functional dissection of this archaeal enzyme and of its interactions with the initiation factors as well as with transcriptional effectors/regulators. As already shown in the context of other promoters (3), subunits H, K, and F/E (homologous to eukaryotic TFIIE and has been found in nearly all sequenced archaeal genomes. A recently determined crystal structure of the N-terminal domain of Rpb7) to a pocket formed by subunits Rpb1, Rpb4/Rpb7, respectively) are not essential for basal rb2 transcription. Indeed, the corresponding subunit drop-out enzymes also support Ptr2-activation (Fig. 2). The effects of recombinant M. jannaschii TFE on transcription by the recombinant M. jannaschii RNAP are shown in Fig. 2A. As expected, TFE stimulates basal levels of transcription at the weak rb2 promoter (−2-fold; lane 2) and to a somewhat greater extent at the more diverged (cryptic) promoter, P' (−3-fold; lane 2). TFE also stimulates transcription by recombinant enzyme lacking subunit H (Fig. 2A, lane 4). Enzyme preparations lacking either subunit K or F/E failed, however, to respond to TFE (Fig. 2A, lanes 6 and 8). Both of these mutant phenotypes are due to the absence of F/E, as incorporation of the F/E heterodimer into RNAP depends on K.2

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DNA (8). Since Ptr2 also exerts its stimulatory effect by stabilizing a weak TBP/TATA-box complex, we wondered whether the actions of Ptr2 and TFE might synergize.

The recently solved structure of the complete 12-subunit yeast RNAPII shows Rpb4/Rpb7 binding (through the N-terminal domain of Rpb7) to a pocket formed by subunits Rpb1,
Rpb2, and Rpb6 (Fig. 2B) (9, 10), with most of the Rpb4/Rpb7 surface exposed and accessible for interactions with general transcription factors and, possibly, nucleic acids. This topology is likely to be preserved in RNAPI, RNAPII, and archaeal RNAPs (11, 12); the F/E dimer, like Rpb4/Rpb7, could serve as a bridge to initiation factors.

In the RNAPII transcription cycle, TFIIE functions along with TFIH primarily in steps that follow RNAPII entry into the promoter complex. TFIIE binds directly to RNAPII (13) and may interact with double-stranded as well as single-stranded DNA in the promoter complex. Electron crystallographic analysis shows TFIIE binding near the RNAPII cleft (14), consistent with DNA interaction in the vicinity of the transcription start site (15). Our present work uncovers a role for the archaeal homologue of the eukaryotic Rpb4/Rpb7 complex in TFE-dependent enhancement of archaeal transcription. It is not clear, however, whether this role is direct, through intimate protein-protein contacts between TFE and the F/E complex during preinitiation complex assembly and initiation, or indirect, through structural constraints imposed by the F/E complex on the conformational state of the RNAP clamp and its mobility relative to the other structural domains of the enzyme. The available information about TFIIE does not provide a clear distinction between these alternatives. Indeed, TFIIE may contact widely separated parts of the RNAPII surface (15). Alternatively, bubble opening may be hampered by the increased flexibility of the clamp that results from the loss of Rpb4/Rpb7.

Having gained further insight into the functional contribution of TFE to basal transcription, we next asked whether TFE would have a similar effect on Ptr2-activated transcription. The results shown in Fig. 2C demonstrate that this is not the case; the presence of TFE does not “superactivate” transcription (lanes 6–8).

In summary, our findings specify that the stimulatory effects of a transcriptional activator and the basal factor TFE are exerted through non-overlapping mechanisms involving distinctive protein-protein interactions. The ability to assemble bacterial RNAP from its subunits in vitro has uniquely provided access to manipulations of structure that have generated important insights into transcription mechanisms (16–18). The obvious desideratum of an in vitro assembled eukaryotic RNAP has not yet been achieved, but the assembly of the archaeal enzyme may point the way, while also opening new approaches to the dissection of the peculiarly mosaic archaeal transcription machinery.

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