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Cloning and Purification of Bacteriophage K11 RNA Polymerase

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Bacteriophage T7 is the prototype of a group of bacterial viruses that each encode a single subunit DNA-dependent RNA polymerase (RNAP). Other members of this class include the coliphages T3 and BA14, the *Salmonella* phage SP6, the *Pseudomonas* phage gh-1 and the *Klebsiella* phage K11 (9,16). The phage RNA polymerases have proven to be useful in a variety of applications, including large-scale RNA synthesis, nucleic acid amplification and as the basis for efficient expression systems in both prokaryotic and eukaryotic cells (5,7,8,15,23,24, 26). To facilitate these applications, the T7, T3 and SP6 RNA polymerases have been cloned (2,11,12,17,24) and more recently have been expressed as histidine-tagged fusion proteins, which greatly simplifies their purification (1,6,10).

Figure 1. Purification of His-tagged phage RNAPs. Cultures of pBH161, pBH118, pBH176 and pMR105, which express his-tagged T7, T3, SP6 and K11 RNAPs, respectively (10) were induced with IPTG, and the RNAPs were purified by affinity chromatography on Ni⁺⁺-agarose beads. An aliquot containing 0.5 µg of each RNAP was analyzed by electrophoresis in 10% polyacrylamide gels. We estimate that the samples are more than 90% pure, as judged by the absence of significant extraneous bands after staining with Coomassie® Blue (10,21). Lane 1, T7 RNAP; lane 2, T3 RNAP; lane 3, K11 RNAP; lane 4, SP6 RNAP; lane 5, molecular-weight markers (low range; sizes are given in the margin in kDa; Bio-Rad, Hercules, CA, USA).

In this work, we describe the expression and purification of a histidine-tagged form of the RNA polymerase encoded by bacteriophage K11. The availability of K11 RNAP furnishes opportunities for additional expression and transcription systems and also provides a useful molecular reagent for comparative analysis of this class of enzymes.

Bacteriophage K11 and the host bacterial strain *Klebsiella pneumoniae* were obtained from the laboratory of Rudolph Hausmann (Freiburg, Germany) via Ian Molineux (University of

Texas, Austin, TX, USA). The phages were propagated and harvested by differential centrifugation as previously described for bacteriophage T7 (22), and DNA was isolated from the phage particles by phenol extraction (21). The K11 RNAP gene was amplified from phage DNA by polymerase chain reaction (PCR) using *Pfu* DNA Polymerase (Stratagene, La Jolla, CA, USA) and the primers MR113 (CCGCTCGA-GATGAACGCATTAAACATTGG) and MR114 (CGGAATTCTTACG-CAAACGCGAAGTCAGA). These

Figure 2. Specificity of K11 RNA polymerase. (Top panel) Promoter sequences for the T7, T3, SP6 and K11 RNAPs are aligned; positions are numbered relative to the start site for transcription (+1). A consensus phage promoter sequence is indicated (CON); note that the region from -8 to -12 is not well-conserved (13). (Bottom panel) Plasmids carrying phage promoters were transcribed by the RNAP indicated, and the products were resolved by electrophoresis (10). The plasmid template utilized, the restriction enzyme used to linearize the plasmid, and the expected sizes of the runoff product from each promoter are as follows: T7: pBluescript II® SK+ (Stratagene), *Afl*III and *Bsm*FI, 520 nt; T3: pBluescript II SK+, *Afl*II and *Bsm*FI, 323 nt; K11: pMR104, *Nde*I, 250 nt; SP6: pDL56, *Nru*I, 200 nt. The reactions shown in lanes 1–4 contained only the template indicated (1 µg); reactions shown in lanes 5–8 (M) contained an equal mixture of all 4 templates (0.25 µg each). Transcription was carried out for 15 min at 37°C in a volume of 20 µL containing 20 mM Tris-HCl, pH 7.9, 10 mM magnesium acetate, 1 mM dithiothreitol (DTT), 0.05% Tween® 20, 0.5 mM ATP, GTP, CTP and UTP, 0.1 µCi [α -³²P]ATP, 4–20 ng RNAP and 1 µg template. pDL56 contains the SP6 promoter from the pGEM® series (Promega, Madison, WI, USA) in a pBR322-derived background (D. Lyakhov and W.T. McAllister, unpublished).

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primers are complementary to the first 7 and last 7 codons of the K11 RNAP gene (underlined). For the complete sequence of the K11 RNAP gene, see Reference 4. The primers also introduce an upstream *Xho*I site and a downstream *Eco*RI site (italics). The amplification products were digested with *Xho*I and *Eco*RI and cloned into the corresponding sites of pBH161 (10),

replacing the T7 RNAP gene in that vector. In the resulting plasmid (pMR105), the K11 RNAP gene is fused to a hexameric histidine leader sequence and is positioned downstream from an inducible *lac* promoter. The predicted N-terminal amino acid sequence of the fusion protein is: MGSSHHHHHSSGLVPRGSHMLE-MNALNIG, in which the underlined

residues correspond to the first 7 amino acids of K11 RNAP (4). This same arrangement was previously utilized to express and purify His-tagged forms of the T7, T3 and SP6 RNAPs (10).

Cultures containing pMR105 were propagated and induced with isopropyl-1-thio- β -D-galactoside (IPTG), and the histidine-tagged RNAP was purified by affinity chromatography using Ni⁺⁺-agarose beads as previously described in He et al. (10) and Temiakov et al. (25). The yield and purity of K11 RNAP were similar to those previously obtained with other histidine-tagged RNAPs (Figure 1).

The phage RNAPs are highly specific for their own promoters. Each of these promoters is related to a conserved 23-bp sequence that extends from -17 to +6 (where +1 is the start site for transcription; see Figure 2). To characterize the activity and specificity of the K11 RNA polymerase, a plasmid template that carries the K11 promoter was constructed. The synthetic oligomers MR119 (CCGAAGCTTAA-**TTAGGGCACACTATAGGGAGAT-CTAGAGC**) and MR120 (GCTCTA-**GATCTCCCTATAGTGTGCCCTA-ATTAAGCTTCGG**) contain the K11 promoter sequence (bold; see Reference 3) flanked by *Hind*III and *Xba*I sites (underlined). These oligomers were annealed together, digested with *Hind*III and *Xba*I, and the fragment was cloned into the corresponding sites of pUC19 to generate pMR104.

As shown in Figure 2, digestion of pMR104 with *Nde*I and transcription by K11 RNAP resulted in the efficient synthesis of a transcript of the expected size (250 nucleotide [nt], lane 3). Like the T7, T3 and SP6 RNAPs, the K11 RNAP is highly specific for its own promoter, as demonstrated by the observation that it utilized only this promoter in the presence of a mixture of templates that also contained promoters for the other 3 RNAPs (Figure 2, lane 7).

Due to their structural simplicity, the phage RNAPs have provided an attractive model system to study the mechanisms of transcription. Of particular interest has been the manner in which these enzymes recognize their specific promoters. For the T7 and T3 RNAPs, it has been demonstrated that a specificity loop (amino acid residues

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742–773) that projects into the DNA-binding cleft is required for specific recognition of base pairs in the region from -8 to -11 (14,18,20). A comparison of promoter sequences for the T7, T3 and SP6 K11 RNAPs indicates that, whereas most positions of the promoter are conserved, this region is divergent (Figure 2). To determine whether we could switch the specificity of the T7 or K11 RNA polymerases, we attempted to swap the region of the T7 RNAP gene that encodes the specificity loop with the corresponding region of the K11 RNAP gene (19). While the resulting enzyme retained nonspecific catalytic activity [as assessed by the synthesis of poly(rG) on a poly(dC) template] it was not active in promoter-dependent transcription from either a T7 or a K11 promoter (19). This result suggests that swapping of the loop perturbs the structure of the RNAP in a manner that prevents promoter recognition in a general manner, or that other regions of the RNAP may also be involved in promoter specificity.

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