Hybrid Bordetella pertussis-Escherichia coli RNA Polymerases: Selectivity of Promoter Activation

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We constructed hybrid Bordetella pertussis-Escherichia coli RNA polymerases and compared productive interactions between transcription activators and cognate RNA polymerase subunits in an in vitro transcription system. Virulence-associated genes of B. pertussis, in the presence of their activator BvgA, are transcribed by all variants of hybrid RNA polymerases, whereas transcription at the E. coli lac promoter regulated by the cyclic AMP-catabolite gene activator protein has an absolute requirement for the E. coli σ subunit. This suggests that activator contact sites involve a high degree of selectivity.

In Bordetella pertussis, the causative agent of whooping cough, the expression of virulence-associated genes including fhaB, cyaA, and ptx, encoding filamentous hemagglutinin, adenylate cyclase-hemolysin, and pertussis toxin, respectively, is controlled by the BvgA-BvgS two-component system (1, 8, 13, 15, 16, 21). BvgA is a global transcriptional regulator that is phosphorylated by the BvgS sensor kinase (22, 26–28). Phosphorylation of BvgA increases its affinity for target promoter sequences and confers a global transcriptional regulator that is phosphorylated by the BvgA-BvgS two-component system (1, 8, 13, 15, 16, 21). BvgA is a master activator that regulates many virulence genes, including those involved in adhesion, invasion, and toxin production (2, 4, 10, 11, 18, 29). In addition, differences in the DNA binding sites for BvgA at various virulence-associated promoters (2, 4, 10, 11, 18, 29) suggest that the specificity of RNA polymerase (RNAP) interactions may control transcriptional activation at these promoters. It has been demonstrated in vitro, with a set of mutated reconstituted Escherichia coli RNAPs, that the C-terminal domain of the α subunit (α-CTD) of E. coli RNA polymerase is required for BvgA-dependent transcription at the fha promoter (4). However, in a recent study we reported that the RNAP σ subunit of B. pertussis confers enhanced expression of fhaB in E. coli (19), indicating that determinants in σ are important for BvgA-dependent transcription in vivo.

The α and major σ subunits of the B. pertussis RNAP have been cloned and characterized (6, 19). These subunits are the most commonly cloned activator targets on the bacterial RNAP (5). To further characterize the BvgA-RNA polymerase interactions responsible for transcriptional activation of B. pertussis virulence genes, we developed an in vitro transcription system using hybrid B. pertussis-E. coli RNAPs containing the α and/or the σ subunits of B. pertussis. We demonstrate the use of this system for the study of RNAP-activator interactions.

Construction of hybrid reconstituted B. pertussis-E. coli RNAPs. We obtained hybrid reconstituted RNAPs by the method described by Tang et al. for the E. coli RNAP (23, 24). Briefly, each single subunit of the RNAP was overexpressed separately in E. coli BL21. Then, 6 M guanidine hydrochloride extracts containing the different RNAP subunits were prepared, and the core enzyme-containing fractions were mixed.

Renaturation of the core enzyme and the sigma-containing fraction was performed by dialysis. The renatured core enzyme and sigma fraction were mixed, and the reconstituted holoenzyme was purified by using Ni2+ affinity chromatography, taking advantage of the fact that the σ subunit was His tagged. For overexpression of the E. coli RNAP subunits, we used the vectors described by Tang et al. (24). Overexpression of the B. pertussis RNAP subunits was performed by using the pET/BL21 system (Novagen) with the vectors pES80 and pES70 for the σ subunits and pE6HNaBP for the α subunit. pES80 coding for the major sigma factor, σ52, of B. pertussis and pES70 coding for an N-terminally truncated form of σ54, named σ72, were described earlier (19). pE6HNaBP is a derivative of pET19 (Novagen) containing the sequence of the B. pertussis rpoA gene (6). This construction allowed the obtention of the B. pertussis σ subunit with an N-terminal extension including a 6-His tag followed by an enterokinase cleavage site. Recreation mixtures containing the α and/or σ52 or σ72 subunit of B. pertussis were performed by using the same protocol as previously described for the E. coli RNAP (23). We constructed and purified the following enzymes: αE6H (E. coli) and σ52 (B. pertussis) (αE6Hσ72)Eco (B. pertussis) (E. coli). For each combination, the amount yielded by the wild-type B. pertussis RNAP, with values of approximately 50 U/mg (18), thus demonstrating that heterologous subunits can be successfully assembled to yield functional hybrid RNAPs.

Transcriptional activities of the reconstituted hybrid RNAPs on the trc and lac promoters. In vitro transcription assays of the strong, artificial trc promoter and of the cyclic AMP-catabolite gene activator protein (cAMP-CAP)-dependent lac promoter, both present on superhelical templates, were performed as described previously (12, 18). As shown in Fig. 2A, the trc promoter is as efficiently transcribed by the hybrid RNAPs as by the wild-type B. pertussis and E. coli enzymes (PhosphorImager measurements indicated that the relative transcriptional activities of trc transcripts varied from 70 to 100% compared to the amount yielded by the B. pertussis wild-type RNAP). This result demonstrates the functionality of the hybrid polymerases in an in vitro transcription system. Next we tested their properties on the cAMP-CAP-activatable lac promoter.

The lac promoter was not recognized by the B. pertussis wild-type RNAP in the presence of cAMP-CAP (Fig. 2B, lane
cAMP-CAP is indicated. The presence (lanes 7 and 3, respectively) of cAMP-CAP, whereas RNA1 and RNA2 transcripts were efficiently produced (Fig. 2B, lanes 1 and 5). This result confirms earlier reports (7, 9) that the RNAP subunit of the E. coli trc promoter is indispensable for transcription of the wild-type RNAP and (αβ′)Ecoσ80B. pertussis RNAP (αβB(β′)Ecoσ80) allowed transcription of the lac promoter, whether in the absence or in the presence of cAMP-CAP, whereas RNA1 and RNA2 transcripts were efficiently produced (Fig. 2B, lanes 1 and 5). This result confirms earlier reports (7, 9) that the α subunit of E. coli RNAP is indispensable for transcription of the cAMP-CAP-dependent lac promoter. It also suggests that different specificities regarding activator contact sites exist between the α subunits of B. pertussis and E. coli, despite their striking sequence homologies (see below).

In vitro transcription of bvg-regulated genes. Previously we found that, compared to the B. pertussis RNAP, the E. coli RNAP was much less, if at all, efficient in the transcription of the fha, cya, and ptx promoters in the presence of phosphorylated BvgA (BvgA-P) (18). We therefore performed in vitro transcription assays similar to those performed earlier (18) on the fha, cya, and ptx promoters with the purified hybrid RNAPs. As shown in Fig. 3, all hybrid polymerases allowed transcription at the fha, cya, and ptx promoters in the presence of BvgA-P (lanes 2, 4, 6, 8, and 10), and virtually no transcripts were observed in the absence of BvgA-P (lanes 1, 3, 5, 7, and 9). The αEcoB(β′)Ecoσ80 enzyme transcribed these promoters almost as efficiently as the wild-type B. pertussis RNAP (Fig. 3, lanes 2 and 12), whereas the σ80-containing hybrid enzymes exhibited lower activities (Fig. 3, lanes 8 and 10). Strikingly, B. pertussis σ80-containing hybrid enzymes were particularly inefficient in transcribing the ptx promoter (lanes 8 and 10). This may be due to specific features in ptx promoter architecture (20) and/or to an inhibiting effect of the N-terminal extension present in the B. pertussis major sigma factor. Indeed, we frequently noted that our wild-type B. pertussis RNAP preparations contained, in part, N-terminally truncated proteolyzed forms of σ80 (18, 19). Nevertheless, when the σ subunit is σ80, stringent ptx promoter interactions might require B. pertussis wild-type RNAP architecture to correctly position the N-terminal extension of σ80. The reduced transcription effect observed with σ80 could be relieved by using hybrid E. coli-B. pertussis RNAPs containing σ80, an N-terminally truncated form of σ80 (αβB(β′)Ecoσ80B). Hybrid RNAPs containing σ80 transcribed all three promoters with efficiencies similar to that of the B. pertussis wild-type RNAP (Fig. 3, lanes 4, 6, and 12). Under similar conditions, the transcription efficiencies obtained with wild-type E. coli RNAP for the fha and ptx promoters were about 10 to 15% and for the fha promoter it was about 80% of that obtained with wild-type B. pertussis RNAP (data not shown). Unexpectedly, hybrid RNAPs containing B. pertussis σ factors efficiently transcribed all three virulence-associated promoters in vitro, which was not

FIG. 2. In vitro transcription of the trc and lac promoters. All reactions were carried out as described earlier (12, 18). Samples were analyzed on denaturing 6% polyacrylamide-6 M urea gels. (A) The trc promoter is activated by all tested RNAPs. The trc transcript is indicated. Lane 1, αEcoα(β′)Ecoσ80; lane 2, αEcoB(β′)Ecoσ80; lane 3, αEcoB(β′)Ecoσ80; lane 4, αEcoB(β′)Ecoσ80; lane 5, αEcoB(β′)Ecoσ80; lane 6, wild-type B. pertussis; lane 7, wild-type E. coli. (B) The α subunit of the E. coli RNAP is necessary for activation of the lac promoter. The lac and RNA1 and RNA2 control transcripts are indicated. The RNAP assignments are shown at the top of the panel. The presence (+) or absence (−) of cAMP-CAP is indicated.

FIG. 3. In vitro transcription of bvg-regulated promoters in the presence or absence of BvgA-P. All reactions were carried out as described earlier (18) with slight modifications: the reactions were carried out in 20-μl volumes, BvgA-P was added prior to RNAP, and the reactions were stopped by the addition of 10 μl of formamide loading buffer (17). A total of 10 μl of each reaction mixture was then analyzed on denaturing 6% polyacrylamide-urea gels. RNAP assignments are shown at the top, and the fha, cya, and ptx transcripts are indicated. The presence (+) or absence (−) of BvgA-P is shown. Relative amounts of transcripts were evaluated by PhosphorImager measurements and normalized for the various RNAPs by the respective activities obtained on the trc promoter. Note that levels of transcripts initiated at different promoters are not comparable because of different compositions of messenger RNAs and different film exposure times.
case when the \textit{B. pertussis} \(\sigma\) factor replaced a thermosensitive \textit{E. coli} \(\sigma\) factor in vivo (19).

In conclusion, all of the tested hybrid RNAPs, \(\sigma_{\text{Bp2}}\) \((\beta^\prime \sigma^\prime)_{\text{Ec}}\alpha_{\text{Bp2}}\beta^\prime \sigma^\prime_{\text{Ec}}\alpha_{\text{Bp2}}\beta^\prime \sigma^\prime_{\text{Ec}}\sigma_{\text{Bp2}}\) and \(\sigma_{\text{Bp}}\) \((\alpha \beta \beta^\prime \sigma^\prime)_{\text{Ec}}\alpha_{\text{Bp}}\) and \((\alpha \beta \beta^\prime \sigma^\prime)_{\text{Ec}}\sigma_{\text{Bp}}\), except for the presence of BvgA-P, to initiate transcription at the \(fha\), \textit{cya}, and \(ptx\) promoters with good efficiencies. This suggests that these poly-

mers contain all the determinants necessary for in vitro BvgA-dependent transcriptional activation. The high levels of transcription activity obtained with hybrid RNAPs compared to that obtained with wild-type \textit{E. coli} RNAP could, in part, be accounted for by more than stoichiometric amounts of different \(\sigma\) factors present in the hybrid RNAP preparations (see Fig. 1). Recently, Boucher et al. (4) used the wild type and a set of truncated as well as mutated reconstituted RNAPs to study BvgA-RNA polymerase interactions at the \(fha\) promoter.

**REFERENCES**


