Research Article

An RNA Polymerase-Binding Protein That Is Required for Communication Between an Enhancer and a Promoter

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Although bacteriophage T4 late promoters are selectively recognized by *Escherichia coli* RNA polymerase bearing a single protein encoded by T4 gene 55 (gp55), efficient transcription at these promoters requires enhancement by the three T4 DNA polymerase accessory proteins, bound to distal "mobile enhancer" sites. Two principles are shown to govern this transcriptional enhancement: (i) Promoter recognition and communication between the enhancer and the promoter require separate phage-coded proteins. Only RNA polymerase that has the T4 gene 33 protein (gp33) bound to it is subject to enhancement by the three DNA replication proteins. (ii) Transcriptional enhancement in this prokaryotic system is promoter-specific. Promoter specificity is generated by a direct competition of phage T4 gp33 and gp55 with the *E. coli* promoter recognition protein, σ^{70}, for binding to the *E. coli* RNA polymerase core. Thus, polymerase that contains σ^{70} is competent to transcribe T4 early and middle genes, but lacks the ability to be enhanced by the DNA replication proteins, while polymerase that contains gp55 and gp33 is capable of enhancement via gp33, but its activity is restricted to T4 late promoters by gp55.

WHEN VIRUSES MULTIPLY IN THEIR HOSTS, THEY FREQUENTLY withhold the expression of certain genes, particularly those that code for proteins of the mature virus particle, until a late stage of the multiplication cycle. In certain cases, expression of such genes depends at least in some measure on viral genome replication. These relationships were first uncovered through studies of the bacteriophage T4 (*1*), but other examples of replication-dependent gene expression are known and involve not only bacterial viruses but also such diverse animal viruses as SV40, adenovirus, and herpes simplex virus 1 (*2*).

Our analysis of a mechanism for connecting DNA replication with regulated gene expression focuses on the late genes of bacteriophage T4. These genes, which cover approximately 40 percent of the viral genome, are turned on just after the start of viral DNA replication, and their expression diminishes if ongoing DNA replication is shut down (*3, 4*). In a recently devised in vitro system for analyzing this connection between replication and late gene expression, three T4 DNA polymerase accessory proteins that are encoded by T4 genes 44, 62, and 45 (gp44, gp62, and gp45) have been shown to stimulate initiation of transcription at a T4 late promoter (*5*). These three replication proteins bind to DNA primer-template junctions (*6*) and have a DNA-dependent adenosine triphosphatase (ATPase) activity (*7*). They serve as the sliding clamp of the DNA polymerase, and greatly increase the processivity of DNA chain elongation by that enzyme (*8*). Transcriptional activation by the three DNA polymerase accessory proteins requires a DNA-binding site that acts like an enhancer, since it can be located at considerable distance upstream or downstream from a promoter. The enhancer and the proteins that bind to it activate T4 late transcription by increasing the rate of promoter opening (*5*). In our experiments, the enhancer is a simple break in the DNA, to which the activating proteins bind; in other words, it is a structure rather than a specific sequence. We have proposed that, in vivo, it is the viral DNA replication fork that acts as the "mobile enhancer" of T4 late gene transcription and that the three DNA polymerase accessory proteins are part of the replisome when they activate transcription (*5*).

In contrast to the complexity of transcriptional enhancement, late promoter recognition can be executed in vitro by *Escherichia coli* RNA polymerase core supplemented only with the RNA polymerase–binding protein encoded by T4 gene 55 (gp55) (*9, 10*), a member of the σ family of proteins (*11*). Late promoters are extremely simple, consisting only of TATAAATA located approximately 10 base pairs (bp) upstream of transcriptional start sites. The recognition sequences that are located 16 to 18 bp further upstream in most *E. coli* and other prokaryotic promoters are lacking in T4 late transcription units (*12, 13*).

There are indications for the existence of one further level of complexity in this system of transcriptional activation. RNA polymerase from T4-infected cells supplemented with the T4 late promoter recognition-specific gp55 can be transcriptionally enhanced in vitro, but RNA polymerase core from uninfected *E. coli*, also supplemented with gp55, cannot (*5*). The host's RNA polymerase undergoes multiple covalent and noncovalent modifications after infection by phage T4 (*14*); the above-cited evidence implies that one of these modifications is required for interaction with the transcription-enhancing proteins. We have now identified the small RNA polymerase-binding protein encoded by T4 gene 33 (gp33) as the required component: gp33 is the link between replication proteins bound at the enhancer and the transcription initiation

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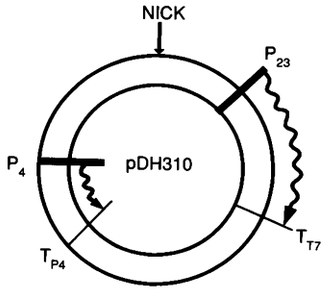

Fig. 1. Principal features of plasmid pDH310. The T4 late (gp55-dependent) $P_{23} \rightarrow T_{T7}$ transcription unit yields a 422-nucleotide (nt) RNA molecule. The σ^{70}-dependent $P_4 \rightarrow T_{P4}$ transcription unit is located near the plasmid replication origin and yields a 108-nt transcript. The nick introduced by the filamentous phage gpII endonuclease is located 222 bp upstream of the start site of P_{23}-initiated transcription. The construction of pDH310 has been described (*5*). The other plasmids used were similar to pDH310 except for the disposition of the 116-bp phage M13 fragment containing the gpII nick site; in pTE110 (*13*) it is absent, and in pDH410 (*5*) it is inverted so that gpII nicks the opposite strand, 232 bp upstream of P_{23}.

complex at the promoter, and it is absolutely required for transcriptional enhancement. We also demonstrate that, in this prokaryotic system, transcriptional enhancement is promoter-specific, and we show how that specificity is engendered.

The role of gp33 in transcriptional enhancement. Expression of T4 late genes is under the control of phage genes 55, 45, and 33 (*1, 4*). Therefore, when *E. coli* RNA polymerase was found to require some additional T4-induced modification in order for its gp55-dependent promoter complex to be subject to gp45-dependent transcriptional enhancement, we tested for the involvement of gp33 by comparing late transcription in vitro with RNA polymerase isolated from *E. coli* infected with T4 wild-type phage and with phage bearing a double amber mutation in gene 33. The DNA template of this experiment was the previously described (*5*) pDH310 (Fig. 1), which bears (i) a principal T4 late transcription unit composed of the gene 23 (major capsid protein) promoter, P_{23}, upstream of a transcriptional terminator, T_{T7}, and (ii) a site for nicking the nontranscribed strand of this transcription unit upstream of P_{23}. The nick thus introduced serves as the binding site for the transcription-enhancing proteins T4 gp44/62 and gp45 (*15*). Nicking the DNA also relaxes it and makes it a relatively poor template for unenhanced initiation of transcription by RNA polymerase containing gp55. Plasmid pDH310 also contains additional late transcription units defined by variant T4 late promoters (*5*). (These transcription units are omitted from Fig. 1 because their consideration is not required for the analysis that follows.)

Transcriptional activation of the T4 late promoter was dependent on the presence of gp33 (Fig. 2). With T4 DNA polymerase accessory proteins (*15*) present, opening of the T4 late promoter P_{23} was enhanced in reactions with RNA polymerase (*16*) from bacteria infected with wild-type phage (Fig. 2, lanes a and b), but not with RNA polymerase from bacteria infected with gene 33–mutant phage (Fig. 2, lanes e and f). Enhancement could be conferred by adding purified gp33 (*17*) to the 33^- T4 RNA polymerase (Fig. 2, lanes g and h), but gp33 did not enhance synthesis of $P_{23} \rightarrow T_{T7}$ RNA in the absence of the replication proteins (lanes c and a and g and e). We noted that gp33 also stimulated enhanced transcription by RNA polymerase prepared from wild-type phage-infected cells (lanes d and b). This probably reflects the presence of a substoichiometric level of gp33 in this enzyme, due either to a partial loss of gp33 during RNA polymerase purification, or to the enzyme being only partly saturated with gp33 in vivo.

In order to ascertain whether gp33 suffices for replication protein-dependent transcriptional enhancement and whether any other T4-induced modifications of RNA polymerase (Table 1) contribute to enhancement, we also purified RNA polymerase (*16*) from (i) uninfected *E. coli*, (ii) *E. coli* infected with T4 phage (*rpbA*$^-$) that do not make the *rpbA* protein, (iii) uninfected *E. coli* overproducing the T4 *rpbA* protein, and (iv) *E. coli* infected with T4 phage (*alt*$^-$ *mod*$^-$) that are incapable of modifying the α subunits of RNA polymerase core by ADP-ribosylation (ADP, adenosine diphosphate). These enzymes were stripped of *E. coli* σ^{70} (that is, they were converted to core enzymes) (*18*) and supplemented with T4 gp55 (*19*) and gp33, as required, in experiments similar to Fig. 2. In surveying transcription initiating at P_{23} with these enzymes, we found that the extent of unenhanced transcription under the standard reaction conditions of Fig. 2 and previous work (*5*) varied considerably, generating backgrounds in our assays of enhancement that complicated analysis and interpretation. In looking for a way to circumvent this problem, we examined the dependence of transcription on electrolyte concentration and found, surprisingly, that the enhanced levels of transcription mediated by gp44/62 and gp45 were maintained at high electrolyte concentrations. In an experiment with T4-modified RNA polymerase from wild-type phage-infected cells (Fig. 3), transcription complexes were first formed by incubating all proteins and DNA in the presence of dATP (deoxyadenosine triphosphate) and then allowed to undergo a single round of transcription. Thus,

Table 1. *Escherichia coli* RNA polymerse and its T4-specific modifications.

Origin*	Modification		
	RpbA†	ADP ribosylation‡	gp33
Uninfected	−	−	−
T4 wild type	+	+	+
T4 *rpbA*$^-$	−	+	−§
T4 alt$^-$/mod$^-$	+	−	−§
T4 33^-	+	+	−
Uninfected, RpbA overproduced	+	−	−

*These enzymes were purified as described (*16*). †The RpbA protein binds tightly to RNA polymerase core throughout transcription (*19*). No role in phage T4 development or gene regulation has been found for RpbA. ‡Each α subunit of RNA polymerase is covalently linked to ADP-ribose through the action of the T4 Alt and Mod proteins. §Removed during the more rigorous purification applied to these enzymes.

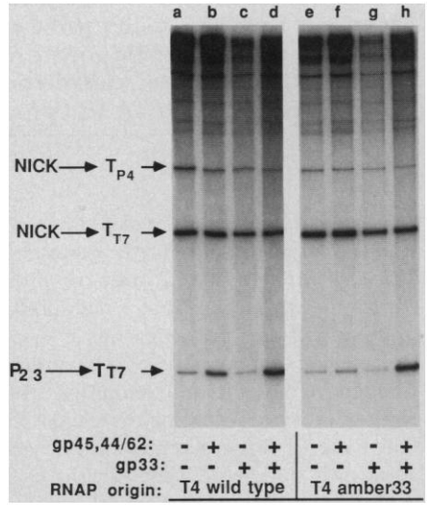

Fig. 2. Involvement of T4 gene 33 protein in enhancement of late transcription by the DNA polymerase accessory proteins. RNA polymerases (1 pmol) and other proteins as indicated were incubated with gp55 (5 pmol) and nicked pDH310 DNA in a reaction buffer containing 66 m*M* potassium acetate and 1 m*M* dATP (deoxyadenosine triphosphate) for 30 minutes at 37°C. RNA synthesis was started by adding four ribonucleoside triphosphates and heparin, allowing a single round of transcription to start at open promoters. Transcription was stopped after 12 minutes. (Lanes a to d) RNA polymerase from *E. coli* infected with wild-type T4; (lanes e to h) RNA polymerase from *E. coli* infected with phage bearing a double amber mutation in gene 33. Transcripts originating at P_{23} and at the nick are indicated at the side. The larger (molecular weight) transcripts at the top of each lane are principally due to readthrough of the transcriptional terminators T_{P4} and T_{T7}, and to the utilization of variant T4 late promoters in pDH310 (*5*). The details were as specified in (*34*).

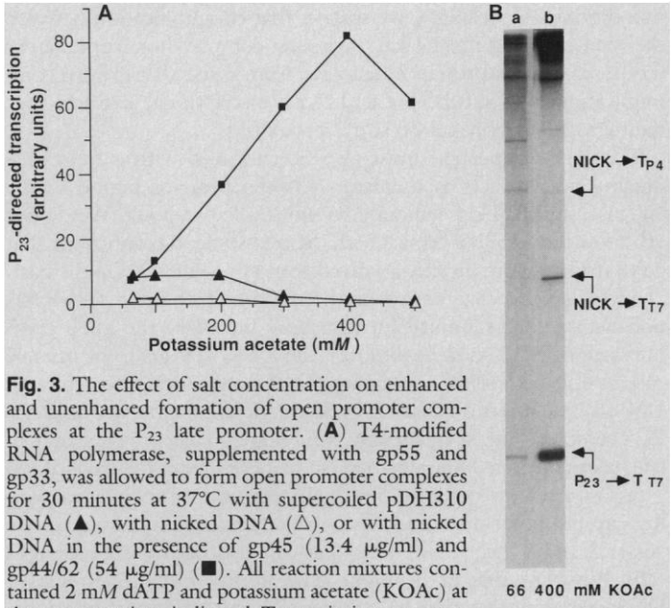

Fig. 3. The effect of salt concentration on enhanced and unenhanced formation of open promoter complexes at the P_{23} late promoter. (**A**) T4-modified RNA polymerase, supplemented with gp55 and gp33, was allowed to form open promoter complexes for 30 minutes at 37°C with supercoiled pDH310 DNA (▲), with nicked DNA (△), or with nicked DNA in the presence of gp45 (13.4 μg/ml) and gp44/62 (54 μg/ml) (■). All reaction mixtures contained 2 mM dATP and potassium acetate (KOAc) at the concentrations indicated. Transcription was started by the addition of an equal volume of a mixture containing all four ribonucleoside triphosphates and heparin, but no KOAc, and was stopped 10 minutes later. In this way, a single round of transcription from promoters that had formed open complexes with RNA polymerase at the indicated concentration of KOAc was allowed to take place at half the indicated concentration of KOAc. Other details of the reaction, and the quantitation of the $P_{23} \rightarrow T_{T7}$ transcript (ordinate) were as specified in (*34*). (**B**) Two examples of autoradiograms from which the numerical data in (A) are derived: nicked DNA in the presence of gp45 and gp44/62 [■ in (A)] at 66 mM KOAc (lane a) and 400 mM KOAc (lane b). Transcripts are identified as in Fig. 2.

the assay measured the formation of stable open promoter complexes. The ability to form these complexes on nicked DNA in the absence of replication proteins was low and relatively sensitive to electrolyte. The formation of open promoter complexes was also suppressed in supercoiled DNA at higher ionic strengths so that transcription in 400 and 500 mM potassium acetate was negligible. In contrast, enhanced late transcription of nicked DNA was optimal in 400 mM potassium acetate and relatively active even in 500 mM potassium acetate. At these salt concentrations, which are within the broad range of ionic conditions that can occur in the *E. coli* cytoplasm (although the use of acetate instead of glutamate and the absence of polyamines makes the mimicry rather crude) (*20*), late transcription was almost absolutely enhancer-dependent (*21*).

It was now possible to show unequivocally that gp33 alone suffices to make *E. coli* RNA polymerase core subject to transcriptional enhancement (Fig. 4). When *E. coli* RNA polymerase core was supplemented only with gp55, it initiated transcription at P_{23} in nicked plasmid DNA more effectively and in a more salt-resistant manner than did T4-modified RNA polymerase (compare Fig. 4A, open squares with Fig. 3, open triangles). Addition of gp33 inhibited transcription in a salt-dependent manner in the absence of the DNA polymerase accessory proteins (Fig. 4A). However, in the presence of accessory proteins and gp33, transcription was greatly stimulated at high ionic strengths (Fig. 4A). The transcriptional enhancement in 400 mM potassium acetate was dependent on the concentrations of the polymerase accessory proteins and on the presence of gp33 (Fig. 4B). Enhancement was absolutely dependent on gp33 and almost maximal at the concentrations of gp44/62 and gp45 chosen for the experiment in Fig. 4A. This experiment therefore revealed that: (i) gp33 is absolutely required for enhancement of T4 late transcription by the three replication proteins (Fig. 4B); and (ii) it increases enhancer dependence by inhibiting unenhanced transcription in an electrolyte concentration-dependent way (Fig. 4A). Similar experiments with the other RNA polymerases (Table 1) showed that neither the *rpb*A subunit nor the ADP-ribosylation of RNA polymerase α subunits can substitute for gp33 in transcriptional enhancement (*22*).

Promoter specificity. The gene 33 protein, like gp55 (*19, 23*), is present in RNA polymerase–DNA binary complexes in the absence of the DNA polymerase accessory proteins. This is shown (Fig. 5) by its co-elution with RNA polymerase bound to supercoiled plasmid DNA in the void volume of a Sepharose CL2B gel filtration column. In the absence of RNA polymerase or plasmid DNA, gp33 eluted near the total volume of the column.

The following indirect, but strong, argument supports the proposition that gp33-dependent enhancement of transcription must be confined to T4 late promoters: (i) gp33 is an RNA polymerase–binding protein and can be present in late promoter complexes, but does not, by itself, bind to DNA (Fig. 5); thus, its effect on transcription is exerted as a ligand of RNA polymerase core; (ii) gp33 and gp55 can bind concurrently to RNA polymerase core; (iii) however, gp33, like gp55 (*19, 23*), competes with σ^{70} for binding to RNA polymerase (*17*) and consequently gp33 cannot participate in σ^{70}-requiring transcriptional initiation; (iv) moreover, all T4 promoters except the late promoters require σ^{70} (Table 2). We tested this argument by examining the ability of gp33 and the DNA polymerase accessory proteins to enhance σ^{70}-dependent transcription of relaxed and nicked pDH310 DNA at high and low concentrations of potassium acetate (Fig. 6). For the experiments with nicked DNA, we also used the closely related plasmid pDH410 (*5*), which is otherwise identical with pDH310 but provides the nick in the other DNA strand (*24*). There are several σ^{70} transcription units in these two pUC-based plasmids. The one that is examined in this figure ($P_4 \rightarrow T_{P4}$) is located in the region of the replicative origin and generates a 108 nucleotide (nt) transcript (*25*).

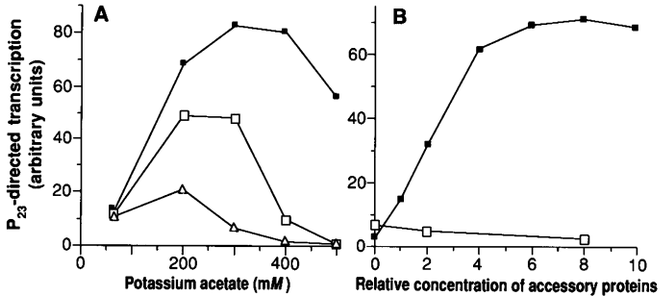

Fig. 4. Dependence of transcriptional enhancement on gp33. (**A**) Dependence on salt concentration. Core RNA polymerase from uninfected *E. coli* was supplemented with gp55 and incubated for 25 minutes at 37°C with nicked pDH310 and dATP (500 μM), in the presence of gp33 (△); gp33, gp45 (26.8 μg/ml), and gp44/62 (108 μg/ml) (■); or no additional proteins (□). Transcription was started by addition of 0.25 volume of a mixture containing four ribonucleoside triphosphates and heparin in transcription buffer (*34*) with 300 mM potassium acetate, and was stopped 12 minutes later. Other reaction conditions are specified in (*34*). (**B**) Dependence on the concentration of DNA polymerase accessory proteins. *E. coli* RNA polymerase core (100 nM) and gp55 were incubated for 30 minutes at 37°C with nicked pDH310 and DNA polymerase accessory proteins, in the presence (■) or absence (□) of gp33 transcription buffer with 400 mM KOAc and 1 mM dATP. The concentrations of the accessory proteins varied together (one unit = 6.7 μg/ml for gp45, and 27 μg/ml for gp44/62), as indicated on the abscissa. A single round of transcription was started by addition of a mixture containing UTP, CTP, GTP, ATP-γ-S (in place of ATP), and rifampicin (final concentration, 25 μg/ml) (*35*). Transcription was stopped after 16 minutes. Other details of the reaction and quantitation of RNA are described (*34*).

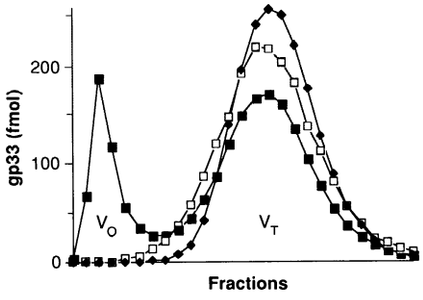

Fig. 5. RNA polymerase-dependent binding of gp33 to DNA. *E. coli* RNA polymerase core (2 pmol), gp55 (8 pmol), [^{35}S]gp33 (2 pmol; 13.7 cpm/fmol), and supercoiled pTE110 DNA (2 pmol) were mixed in buffer, incubated, and then separated by gel filtration. For each fraction, the radioactivity was measured and used to determine its content of gp33. In parallel reactions, the sample either contained all reagents (■), RNA polymerase was omitted (◆), or pTE110 DNA was omitted (□). Any gene 33 protein in complexes with DNA eluted in the void volume (V_0); otherwise it eluted in or near the total volume (V_T). Details of procedure were as specified (*36*).

Table 2. Promoters of bacteriophage T4.

Class	Promoter element		Recognition protein
	Upstream†	−10	
Early (*38*)*	GTTTAC	TA$^{TA}_{CT}$ATA	σ^{70}
Middle (*39*)	$^{AA}_{TT}$TGCTT$^{T}_{C}$A	TATAAT	σ^{70} + T4 MotA
Late (*12*)	None	TATAAATA	gp55

*T4 early promoters have considerable additional sequence information content (*38*).
†Centered at approximately −35 for early promoters and at approximately −30 for middle promoters.

The activity of the P_4 promoter was much greater in supercoiled (S) than in relaxed (R) or nicked (N) DNA, especially at higher ionic strength, but gp33 and the DNA polymerase accessory proteins generated no enhancement of $P_4 \rightarrow T_{P4}$ transcription (Fig. 6), or of the other relatively minor σ^{70}-dependent transcripts that were resolved on the gels from which the data shown in Fig. 6 have been abstracted.

Two principles of the transcriptional enhancement. The above experiments establish two principles that govern the transcriptional enhancement of T4 late promoters. The first principle is that enhancement requires two distinct functions, promoter recognition and communication between the distal enhancer and the promoter. In T4 late transcription, the two corresponding functional elements, gp55 and gp33, are serendipitously separated, so that their mode of action can be examined directly without, at least initially, requiring the kind of molecular genetic dissection that is now assiduously applied to eukaryotic enhancer-binding proteins (*26*). Whether this separation has some functional significance for transcriptional initiation or for coupling T4 late transcription to replication remains to be determined. While gp55 shows traces of its common ancestry with the σ-family proteins (*11*), gp33 is not detectably related to them.

The regulation of the activities of genes whose products are involved in nitrogen fixation and nitrogen metabolism by a wide range of Gram-negative bacteria is also exerted from promoter-distal sites (*27, 28*). The proteins that bind to these distant sites enhance transcription by RNA polymerase containing the special σ^{54} initiation subunit. The single σ^{54} polypeptide confers both promoter-finding and enhancer-responding functions (*27, 28*). It has been proposed that a highly conserved glutamine-rich NH_2-terminal domain of σ^{54} is required for transcription enhancement by the regulatory proteins NtrC and NifA, but not for promoter recognition (*28*). We have searched for a recognizable sequence similarity between gp33 and the known σ^{54}-family proteins (*29*) but have found none. Accordingly, we suspect that communication between the replisome and the T4 late promoter complex involves different modes of protein-protein interaction from those that govern communication between the Nif and Ntr transcriptional activator proteins and RNA polymerase at σ^{54} promoters.

The gene 33 protein must also be endowed with a functional duality, since it acts as mediator between proteins bound to the enhancer and RNA polymerase bound to the promoter. We do not yet know how gp33 interacts with the replisome, but anticipate that gp45 may be more directly involved than gp44 and 62. On the basis of prior genetic analysis, we also anticipate that gp55 and RNA polymerase core subunits are somehow linked to the gp33-gp45 interaction (*30*). On the promoter side, gp33 appears to be capable of affecting how gp55-containing RNA polymerase interacts with DNA. When it acts on its own, gp33 can depress transcription at the P_{23} promoter (Fig. 4A), thereby enforcing enhancer dependency of late transcription. When it acts in concert with the replication accessory proteins, gp33 allows RNA polymerase to interact with the late promoter at remarkably high concentrations of electrolyte (Figs. 3 and 4), concentrations that can also occur in *E. coli* cells (*20*). We think that gp33 might generate this electrolyte-resistant DNA-protein interaction by helping to bring additional non-ionic interactions between RNA polymerase and the T4 late promoter into play. The importance of these functions of gp33 for late transcription in vivo can be gauged from the fact that the defective phenotype of gene 33 mutants is similar to, and only slightly less absolute than, that of gene 55 mutants (*31*).

The second principle is that transcriptional enhancement from the replisome is promoter-specific (Fig. 7). The enhancing proteins are unable to interact productively with RNA polymerase containing σ^{70} (Fig. 6). Since T4 early and middle promoter recognition depends on σ^{70} (Table 2), which directly competes with gp33 (*17*) and gp55 (*19, 23*) in binding to RNA polymerase core, enhancement is confined to the T4 late genes. Evidence that transcriptional enhancement of σ^{54}-dependent RNA synthesis is also promoter-specific in the sense of being ineffective for two σ^{70} promoters, has been presented (*32, 33*). There are clear contrasts between these prokaryotic systems and enhancement or upstream activation of eukaryotic transcription (*26*). That enhancers and upstream elements can be so freely mixed and matched with promoters of eukaryotic RNA polymerase II transcription units probably reflects the fact that the proteins binding to enhancers and upstream activating sites interact with generally required components of the RNA polymerase II transcription apparatus. A second contrast

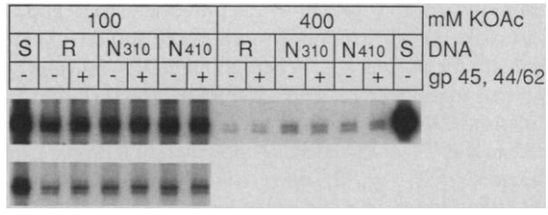

Fig. 6. Inability of σ^{70}-directed transcription to be enhanced by T4 DNA polymerase accessory proteins. Relaxed (R), supercoiled (S), or gpII-nicked pDH310 (N310), or gpII-nicked pDH410 (N410) were transcribed in buffer containing 100 m*M* or 400 m*M* KOAc with σ^{70}-saturated *E. coli* RNA polymerase, gp33, and dATP, with or without the DNA polymerase accessory proteins. Only the band representing the major σ^{70}-directed transcript of these DNA's ($P_4 \rightarrow T_{P4}$ in Fig. 1) is shown; the lower panel at the left is a lighter autoradiographic exposure of the same gel. Transcription from weaker σ^{70}-dependent promoters, represented by bands in portions of the autoradiogram that are not displayed here, similarly showed substantial supercoiling-dependence but no significant effect of gp45 and gp44/62. Details of procedure were as specified (*37*).

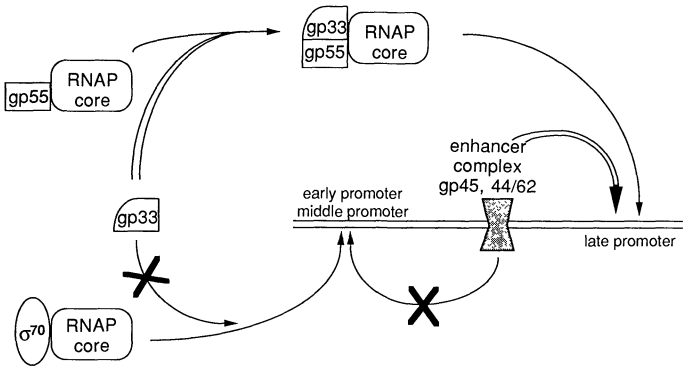

Fig. 7. Promoter-specific enhancement mediated by gp33. Gene 33 protein is required for enhancer complex recognition by RNA polymerase core σ^{55} (upper pathway), yet its binding to RNA polymerase core is not compatible with binding of σ^{70} (lower pathway). As a consequence, only T4 late promoters are subject to enhancement; T4 early and middle promoters, which depend on σ^{70}, are not.

concerns the mechanism by which promoters are selected for enhancement. Despite the relatively large distances over which eukaryotic enhancers can act, they nevertheless exert promoter specificity because of their position within a DNA domain that is shared with their target promoter (or promoters). With T4, transcriptional enhancement is rendered promoter-specific at the level of the RNA polymerase. This alternative strategy fits the mobile nature of the T4 enhancer complex: gp45 and gp44/62 traverse the entire genome as part of the replisome, and gp33-σ^{70} incompatibility provides selectivity to a potentially general system of transcriptional activation.

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15. Gene 44, 62, and 45 proteins were overproduced in *E. coli* HB101 containing the expression vector pTL151WX [J. Rush *et al.*, *J. Biol. Chem.* **264**, 10943 (1989)], provided by W. H. Konigsberg. The gp44 and 62 form a tightly associated complex and are always used together. We designate this material as gp44/62. The successful overproduction of gp44/62 and gp45 allowed for fewer chromatographic steps than used for their purification from T4-infected cells. Proteins were prepared by a shortened version [A. Sussman (UCSF), personal communication] of the previously described method [C. F. Morris, H. Hama-Inaba, D. Mace, N. K. Sinha, B. M. Alberts, *J. Biol. Chem.* **254**, 6787 (1979)]; gp44/62 was purified by chromatography on DEAE cellulose and phosphocellulose, while gp45 was chromatographed on DEAE cellulose and BioGel HTP hydroxyapatite. Some initial experiments were also done with gp44/62 and gp45 provided by J. Barry and B. M. Alberts.
16. RNA polymerase core enzymes were purified from uninfected *E. coli* DG156 ribonuclease (RNase I$^-$), or from the same bacteria infected for 18 minutes at 30°C at multiplicities of about 5 with T4 mutants 33$^-$ (amN134-amC18), *alt*$^-$*mod*$^-$ [C. G. Goff and J. Setzer, *J. Virol.* **33**, 547 (1980)] or *rpbA*$^-$ (C36-am39) [K. P. Williams *et al.*, in preparation], or from uninfected *E. coli* DH5 (pTH4558) [T. Hsu, R. Wei, M. Dawson, J. D. Karam, *J. Virol.* **61**, 366 (1987)] that had been grown for 3 hours at 43°C to induce substantial overproduction of the T4 *rpbA* protein. Cells were disrupted as described [M. Chamberlin, R. Kingston, M. Gilman, J. Wiggs, A. DeVera, *Methods Enzymol.* **101**, 540 (1983)], nucleic acids were removed by precipitation with Polymin P, and the extract was concentrated with ammonium sulfate as described [R. R. Burgess and J. J. Jendrisak, *Biochemistry* **14**, 4634 (1975)]. Heparin-agarose chromatography [M. Chamberlin, R. Kingston, M. Gilman, J. Wiggs, A. DeVera, *Methods Enzymol.* **101**, 540 (1983)] was followed by gel filtration and BioRex 70 chromatography [R. R. Burgess and J. J. Jendrisak, *Biochemistry* **14**, 4634 (1975)]. These stringently prepared enzymes were virtually depleted of gp55, gp33, and σ^{70} activity. The σ^{70}-saturated holoenzyme preparation has been described (*19*), as was the less stringently prepared wild-type T4-infected RNA polymerase (*10*), which contained substantial gp55 and gp33 activity. The content of gp33 in these preparations was determined by their reactivity with antisera to gp33.
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21. In similar experiments linearized DNA was transcribed in order to confirm that the increased production of $P_{23} \rightarrow T_{T7}$ RNA at high ionic strength was due to increased initiation rather than more complete termination of transcription; the efficiency of termination at T_{T7} declined slightly at higher concentrations of potassium acetate.
22. Even the T4-modified polymerase lacking gp33 was electrolyte concentration-sensitive in its initiation of unenhanced transcription at P_{23}. Electrolyte sensitivity was not conferred on *E. coli* RNA polymerase by *rpbA* protein alone. These experiments permitted the tentative conclusion that ADP-ribosylation of the α subunit also confers electrolyte sensitivity on unenhanced transcription at the late promoter P_{23}; S. Malik and A. Goldfarb [*J. Biol. Chem.* **259**, 13292 (1984)] have previously shown that ADP-ribosylation makes σ^{70}-dependent transcription more electrolyte sensitive.
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24. Experiments on enhancement of late transcription by T4 DNA replication proteins bound at a nick in DNA have shown that placing this nick in the nontranscribed strand generates more enhancement of transcription than placing it in the transcribed strand (*5*). Not knowing in advance whether strand placement might be relevant for σ^{70}-dependent transcription, we tried both alternatives.
25. This is the RNA I transcript of pBR322, which functions in regulating plasmid DNA replication. The interaction of *E. coli* RNA polymerase (σ^{70}) holoenzyme with the P_4 promoter in supercoiled and relaxed DNA has been analyzed [D. C. Wood and J. Lebowitz, *J. Biol. Chem.* **259**, 11184 (1984)].
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33. The σ^{70} promoters of *E. coli* clearly are subject to direct positive regulation by other proteins, such as *E. coli* OmpR and CRP, phage λ cII, and T4 MotA. B. C. Hoopes and W. R. McClure, in *Escherichia coli and Salmonella Typhimurium*, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, H. E. Umbarger, Eds. (American Society for Microbiology, Washington, DC, 1987), vol. 2, chap. 75, p. 1231; Y. S. Ho and M. Rosenberg, in *The Bacteriophages*, R. Calendar, Ed. (Plenum, New York, 1988), vol. 2, p. 725; M. Uzan, Y. d'Aubenton-Carafa, R. Favre, V. deFranciscis, E. Brody, *J. Biol. Chem.* **260**, 633 (1985); S. Maeda, Y. Ozawa, T. Mizuno, S. Mizushima, *J. Mol. Biol.* **202**, 433 (1988).
34. For the gp55-dependent synthesis of T4 late RNA in vitro, reaction mixtures contained, in a 20-μl volume, 33 m*M* tris acetate, *p*H 7.8, 10 m*M* magnesium acetate, 0.5 m*M* dithiothreitol (DTT), acetylated bovine serum albumin (BSA) at 150 μg/ml, 5 units of human placental ribonuclease inhibitor (Amersham), pDH310 at 11 μg/ml (5 n*M*), and potassium acetate (from 66 to 500 m*M*, as specified in each figure legend). RNA polymerase was present at 50 n*M*. The gp55 and, when present, gp33 were at ten and four molar equivalents relative to RNA polymerase, respectively, unless otherwise specified. When appropriate, DNA was nicked with gpII endonuclease (*5*). The concentration of DNA polymerase accessory protein was 6.7 μg/ml for gp45 (90 n*M* of trimer) and 27 μg/ml for gp44/62 (165 n*M* of their complex) (Fig. 2), or some specified multiple of these concentrations (Figs. 3 and 4). When proteins were omitted, the appropriate

protein storage buffers were added in their place. Reaction mixtures were first incubated at 37°C with dATP (a substrate for the DNA-dependent ATPase activity of the accessory proteins) in the absence of ribonucleoside triphosphates to form open promoter complexes as specified in the legends to Figs. 2, 3, and 4. The ribonucleotides ATP, GTP, CTP, and UTP were then added to 1 m*M*, 1 m*M*, 100 μ*M*, and 100 μ*M*, respectively. RNA was labeled with [α-^{32}P]UTP or -CTP (NEN; 2000 to 5000 cpm/pmol). The time interval of RNA synthesis is specified in each figure legend. When present, heparin (Calbiochem) was added with the nucleotides to 200 μg/ml. Transcription was stopped with 3.5 to 7 volumes of a solution containing 4 m*M* EDTA, 0.2 percent SDS, and 10 m*M* tris-HCl, *p*H 8. Nucleic acids were purified from reaction mixtures by standard methods and were electrophoretically separated on 4 percent polyacrylamide, 7*M* urea gels. The $P_{23} \rightarrow T_{T7}$ transcript was identified on autoradiograms by its size in relation to markers. Transcripts were quantified by scanning autoradiograms prepared without intensifying screens on a laser densitometer (when necessary, accurate measurements of relative band intensity were obtained by comparing data from different film exposures in the linear range of the film for ^{32}P). Software written by AMBIS (San Diego) aided in the densitometric analysis.

35. We observed that heparin became less effective at inactivating RNA polymerase at KOAc concentrations greater than about 250 m*M*. For this reason, rifampicin was used in single-round transcription assays in 400 m*M* potassium acetate. The ATP analog ATP-γ-S, a competitive inhibitor of the accessory protein ATPase activity, was used as a substrate for RNA synthesis in order to further negate any effects of accessory proteins after the addition of nucleotides.
36. Binding reaction mixtures contained, in a 30-μl volume, 100 m*M* potassium acetate, 10 m*M* magnesium acetate, 10 m*M* potassium-Hepes, *p*H 7.8, 5 percent (v/v) glycerol, 0.1 percent Brij 58, 0.1 m*M* EDTA, 0.1 m*M* DTT. Samples were incubated for 30 minutes at 37°C, then placed on Sepharose CL-2B columns (3 ml) and eluted in the same buffer at 20°C. Seven-drop fractions (about 100 μl) were collected, and 80 μl from each fraction was spotted onto glass fiber filters for liquid scintillation counting to determine its content of ^{35}S-labeled gp33 (*17*). Plasmid pTE110 (*13*) is the parent of pDH310 (Fig. 1), lacking the 116-bp fragment of pDH310 that contains the gpII nick-site.
37. The buffer contained 30 m*M* potassium-Hepes, *p*H 7.8, 10 m*M* magnesium acetate, 0.5 m*M* DTT, acetylated BSA at 100 μg/ml, 0.1 m*M* EDTA, supplemented with either 100 m*M* or 400 m*M* KOAc. Relaxed pDH310 DNA was separately prepared with topoisomerase I (BRL) in each of the above buffers at 37°C, and was transcribed in the buffer in which it was relaxed. In this way, effects of temperature and electrolyte on the partition of topological linkage between twisting and writhing could be avoided. One picomole of σ^{70}-saturated *E. coli* RNA polymerase, 0.1 pmol of plasmid DNA, and 5 pmol of gp33, with or without gp45 (5.4 μg/ml) and gp44/62 (21.7 μg/ml), were incubated for 15 minutes at 37°C in the presence of 400 μ*M* each of ATP, CTP, and GTP, 50 μ*M* [α-^{32}P]UTP (2000 cpm/pmol), and 1 m*M* dATP.
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40. We thank J. Barry and B. M. Alberts for gifts of replication proteins, A. Sussman for advice on protein purification, B. M. Alberts for continued interest and frequent discussions, and S. Kustu, A. Wedel, and B. M. Alberts for helpful comments on the manuscript. Supported by an NIGMS grant and an NIH training grant in Cell and Molecular Biology (D.R.H. and K.P.W.).

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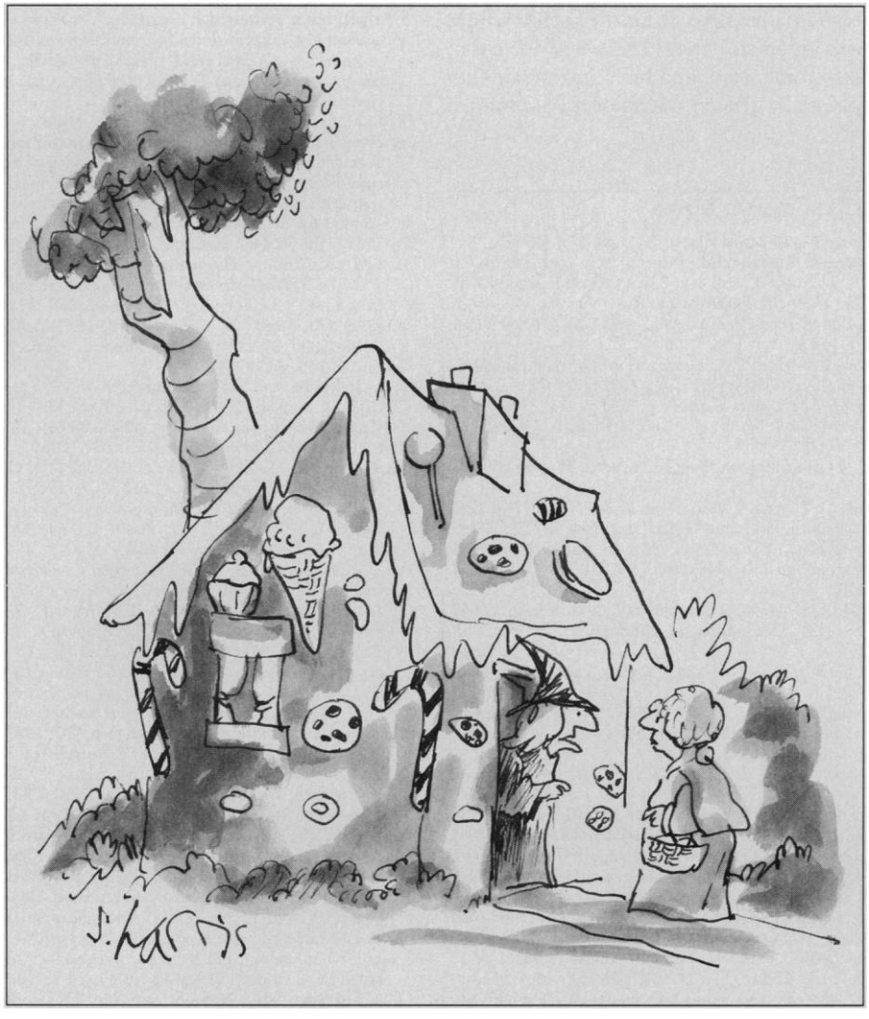

"It's a good thing I built this place when I did. The new zoning law prevents any house from going over 75,000 calories."