Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7525–7529, November 1985
Biochemistry

Products of nitrogen regulatory genes *ntrA* and *ntrC* of enteric bacteria activate *glnA* transcription *in vitro*: Evidence that the *ntrA* product is a σ factor

(RNA polymerase/transcription/DNA binding protein/glutamine synthetase/nitrogen fixation)

J. HIRSCHMAN, P.-K. WONG, K. SEI, J. KEENER, AND S. KUSTU

Department of Bacteriology, University of California, Davis, CA 95616

Communicated by E. Peter Geiduschek, July 16, 1985

ABSTRACT **In enteric bacteria the products of two nitrogen regulatory genes, *ntrA* and *ntrC*, activate transcription of *glnA*, the structural gene encoding glutamine synthetase, both *in vivo* and *in vitro*. The *ntrC* product (gp*ntrC*) is a DNA-binding protein, which binds to five sites in the *glnA* promoter–regulatory region and appears to activate transcription initiation. Using as an assay the stimulation of *glnA* transcription in a coupled *in vitro* transcription–translation system, we have partially purified the *ntrA* gene product (gp*ntrA*). The following evidence is consistent with the view that gp*ntrA* is a σ subunit for RNA polymerase: (*i*) The gp*ntrA* activity copurifies with the σ^{70} holoenzyme ($E\sigma^{70}$) and core (E) forms of RNA polymerase through several steps but can be separated from them by chromatography on heparin agarose. (*ii*) After further purification by molecular sieve chromatography, the partially purified gp*ntrA* fraction allows transcription of *glnA* from the same startpoint used *in vivo*; transcription is dependent on gp*ntrC* and on added E. The gp*ntrA* fraction does not allow transcription from promoters that we have used as controls, including *lac*UV5. $E\sigma^{70}$ has the reverse specificity.**

A number of bacterial proteins including glutamine synthetase, amino acid transport components and degradative enzymes, and nitrogenase with its associated factors are synthesized in large amounts only when combined nitrogen is limiting in the growth medium. Genetic analysis in enteric bacteria (*Salmonella, Escherichia, Klebsiella*) has indicated that two positive factors, products of genes *ntrA* (*glnF*) and *ntrC* (*glnG*), are required for synthesis of these nitrogen-regulated proteins and that they act at the level of transcription (reviewed in refs. 1–5).

The product of gene *ntrC* (gp*ntrC*) was purified from *Escherichia coli* by Reitzer and Magasanik (6), who demonstrated that it repressed transcription from the *ntrBC* (*glnL*) promoter *in vitro* as it did *in vivo*. (Genetic studies indicated that the *ntrC* product could act as a repressor of transcription at the *ntrBC* promoter and a *glnA* promoter; see legend to Fig. 1.) The gp*ntrC* interfered with initiation of transcription at the *ntrBC* promoter by the σ^{70} holoenzyme form of RNA polymerase ($E\sigma^{70}$) (6).

In enteric bacteria the *glnA* gene, which encodes glutamine synthetase, is transcribed from two promoters *in vivo* (refs. 7–9; unpublished observations). The major nitrogen-regulated promoter lies closest to structural information. Both the *ntrA* and *ntrC* products are required to activate transcription from this promoter. A secondary promoter(s) lies $\approx$100 base pairs upstream of the nitrogen-regulated promoter. Transcription from the upstream promoter is activated by the cyclic AMP receptor protein bound to its ligand and does not require either the *ntrA* or the *ntrC* product (ref. 8; unpublished results). In fact, the *ntrC* product represses transcription from the upstream *glnA* promoter, as it does from the *ntrBC* promoter.

We demonstrate here that purified gp*ntrC* from *Salmonella*, which binds to five sites in the *glnA* promoter–regulatory region, activates transcription from the downstream *glnA* promoter in the presence of functional gp*ntrA*. We present several lines of evidence that are consistent with the view that gp*ntrA* is a σ subunit for RNA polymerase—i.e., that it confers a different promoter specificity on the core form of RNA polymerase (E) than does the most abundant σ subunit, σ^{70}. D. Ow (personal communication) and de Bruijn and Ausubel (10) were the first to note this possibility.

METHODS

S1 Nuclease Mapping (11) of Transcription Startpoints for *glnA*. Preparation of mRNA from cells was as described (12) except that nucleic acids were treated with 200 units of DNase I (Worthington Code DPRF). After synthesis of mRNA *in vitro*, transcription mixtures (legends to Fig. 4 and Table 1) were incubated with 200 units of DNase I at 4°C; 50 μg of yeast tRNA was added and RNA was purified as described (12). Hybridization of mRNA to DNA (initially double-stranded) and digestion of hybrids with nuclease S1 were as described (13) except (*i*) heating was to 90°C for 5 min; (*ii*) after gradual cooling to 37°C, incubation was continued for 16 hr; and (*iii*) the reaction mixture was treated with 3000 units of S1 nuclease for 30 min.

"Footprinting" (14) of gp*ntrC* in the *glnA* Promoter–Regulatory Region. The gp*ntrC* was incubated with DNA probe for 10 min at 23°C (50-μl vol; see legend to Fig. 2). DNase I (5 ng) was added and incubation was continued for 1 min. The reaction was stopped as described (14).

Assay for gp*ntrA* Activity. The gp*ntrA* activity was assayed in a coupled transcription–translation system developed by Artz and Broach (15). S30 extracts were prepared from *ntrA*$^+$ strain SK416 [Δ(*glnA–ntrB*)*60 zig205*::Tn*10 relA1 hisT1504 his*Δ*2253*) and from a congenic *ntrA*$^-$ strain SK419 (carrying *ntrA76*). Because they carry Δ(*glnA–ntrB*)*60*, both strains lack gp*ntrC* and lack other proteins whose synthesis depends on the *ntr* system (refs. 5 and 16; unpublished observations); therefore, they presumably differ only by the presence or absence of gp*ntrA*. Standard conditions for coupled transcription–translation in a final vol of 50 μl (15) were modified as described (17), except that the ATP concentration was 2 mM as in ref. 15. The gp*ntrC* was added as noted. The *glnA* template, plasmid pJES40, carries an in-frame protein fusion of the 42nd codon of *glnA* to the 9th codon of *lacZ* in a derivative of pMLB1034 (18) and places β-galactosidase expression under control of the *glnA* promoter–regulatory region (unpublished data). The *lacUV5* template, plasmid pRS229, was kindly provided by R. Simons (19). Activity of β-galactosidase synthesized *in vitro* (2- to

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

40-μl aliquots) was measured essentially as described (20) in a final vol of 60 μl. The nitrophenyl-β-D-galactopyranoside, *o-phenyl*-3,5-^{3}H-labeled substrate (28.7 Ci/mmol; 1 Ci = 37 GBq) was obtained from New England Nuclear and was used at a final concentration of 0.4 mM (60 μCi/μmol). Most activities are expressed as pmol of [^{3}H]orthonitrophenol formed per min at 30°C. For purification of gp*ntrA*, units of activity from the *glnA* template (μmol/min) were determined after subtracting activity of an *ntrA*$^-$ S30 in the presence of gp*ntrC* but in the absence of added gp*ntrA*.

Assay for RNA Polymerase (Eσ^{70} + E) Activity. During purification, RNA polymerase was assayed in a final vol of 100 μl essentially as described (22), except that the radioactive nucleotide was [5,6-^{3}H]UTP.

Transcription by gp*ntrA*. Transcriptional activity of partially purified gp*ntrA* fractions was assessed as described in the legend to Fig. 4 with plasmid pJES86 as template. In this plasmid, a 1.8-kilobase pair (kbp) *Eco*RI fragment carrying the *glnA* promoter–regulatory region and the first 42 codons of *glnA* is inserted at the *Eco*RI site of plasmid pTE102 (kindly provided by T. Elliott and G. Kassavetis) and is followed by a strong T7 terminator (ref. 23; unpublished results).

Purification of gp*ntrA* and gp*ntrC*. The gp*ntrA* (and Eσ^{70} + E) was purified from strain SK35 [Δ(*glnA–ntrB*)*60*], which lacks gp*ntrC*, or from a derivative of strain SK35 that carries plasmid pJES80 (pBR322 carrying a 5-kbp *ntrA*$^+$ insert between the *Hin*dIII and *Eco*RV sites) and overproduces gp*ntrA* ≈10-fold. Enzyme was partially purified from 100 g of cells by using steps previously described for purification of Eσ^{70} and E (refs. 22, 24, and 25; see also *Results* and legend to Fig. 3).

The gp*ntrC* was prepared from strains that overproduced it under control of the leftward promoter of phage λ in plasmid pPLc28 (26). After removal of nucleic acids with streptomycin sulfate (0.1 g per 100 ml), gp*ntrC* was purified to >95% homogeneity by precipitation with ammonium sulfate (24.5 g per 100 ml), heparin agarose chromatography (in TGED, see legend to Fig. 3; elution with a linear KCl gradient from 0 to 0.6 M) and molecular size fractionation on Sephacryl 200 (Pharmacia; in TGED) (unpublished results).

RESULTS

Startpoint for *glnA* Transcription *in Vivo*. The major startpoint for *glnA* transcription *in vivo* (diagrammed in Fig. 1) was determined by high-resolution S1 nuclease mapping (Fig. 2, lanes 9–11). The transcript apparently began with one to five uracil residues and was not synthesized by mutant strains that lacked function of either gp*ntrA* or gp*ntrC*. Secondary transcriptional startpoints for *glnA*, whose utilization did not depend on *ntr* function, were located at least 100 base pairs upstream of this major one (unpublished data).

The gp*ntrC* Is a DNA-Binding Protein. Upstream (5′) of the major transcriptional startpoint for *glnA* are five closely spaced binding sites (within 110 base pairs) for gp*ntrC* (Figs. 1 and 2). These sites were identified by protection of the *glnA* promoter–regulatory region from cleavage by DNase I and by protection of guanine residues on both strands from methylation (Figs. 1 and 2; unpublished data). Each binding site for gp*ntrC* (Fig. 1) appears to be composed of two arms of dyad symmetry (working consensus 5′-GGTGC-3′).

Effects of gp*ntrC* and gp*ntrA* on Expression from the *glnA* Promoter *in Vitro*. We have studied the function of gp*ntrC* and gp*ntrA* in a coupled *in vitro* transcription–translation system from *Salmonella* (see *Methods*). The gp*ntrC* stimulated expression from the *glnA* promoter in an S30 extract from an *ntrA*$^+$ strain (80-fold) but not in one from a congenic *ntrA*$^-$ strain (2-fold inhibition) (Table 1). Addition of gp*ntrC* had no effect on expression from a control promoter *lacUV5*, which was expressed at high levels in both S30 extracts.

Partial Purification of gp*ntrA*. Having noted unusual features of the *glnA* promoter–regulatory region near the major startpoint of transcription (see *Discussion*), we hypothesized that gp*ntrA* might be a new σ subunit for RNA polymerase. Using as an assay its ability to stimulate expression from the *glnA* promoter in an *ntrA*$^-$ S30, we attempted to purify gp*ntrA* by methods known to be effective in purification of Eσ^{70} and E forms of RNA polymerase (22, 24, 25). The gp*ntrA* activity copurified with Eσ^{70} and E on polyethyleneimine precipitation [Miles; 1% (vol/vol) final concentration], extraction from the precipitate with 1.0 M but not 0.5 M NaCl, precipitation with ammonium sulfate (35 g per 100 ml) (60–80% recovery of activity and 3-fold purification to this point) and DEAE-cellulose chromatography (Fig. 3*A*). The gp*ntrA* activity eluted from heparin agarose at a lower salt concentration than did Eσ^{70} + E and was well separated from them (Fig. 3*B*). The gp*ntrA* fraction was further purified by molecular sieve chromatography on Bio-Gel A 1.5 m in the presence of 0.5 M NaCl (22). Recovery of gp*ntrA* activity from a strain with a single chromosomal *ntrA* gene was 5–10%. Based on its mobility on $NaDodSO_4$/polyacrylamide gels the gp*ntrA*, which we have identified as a protein of apparent molecular size 73 kDa (unpublished observations), constituted ≈5% of the final gp*ntrA* fraction; this fraction did not contain detectable amounts of the β and β′ subunits of RNA polymerase core (assessed by staining with Coomassie blue; data not shown). (After heparin agarose chromatography a portion of the gp*ntrA* fraction was purified by high-pressure liquid chromatography on a TSK250 gel filtration column. Consistent with its identification as a product of 73 kDa and with the fact that it was not associated with E at this point in the purification, the gp*ntrA* activity eluted at a

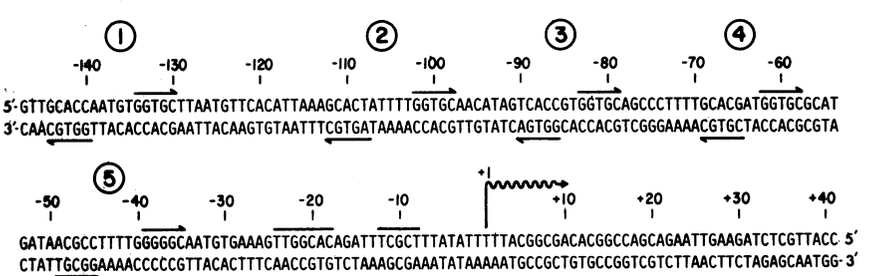

FIG. 1. The *glnA* promoter–regulatory region from *Salmonella typhimurium*. The sequence was determined by Hanau *et al.* (27). The major transcriptional startpoint for *glnA*, as determined both *in vivo* and *in vitro* (Figs. 2 and 4), is designated +1 and positions of all other sites are given with reference to this. Binding sites for gp*ntrC* (see Fig. 2; unpublished data) are designated ← → to indicate dyad symmetry of the half-sites. Sequences similar to ones identified as a nitrogen fixation consensus promoter (refs. 7, 28, and 29; see *Discussion*) are overlined. The start of *glnA* coding information lies at +80. The order of genes in the *glnA* operon is *glnA ntrB ntrC*, with transcription proceeding from left to right as written; the *ntrBC* (*glnL*) promoter lies between *glnA* and *ntrB* (16, 30).

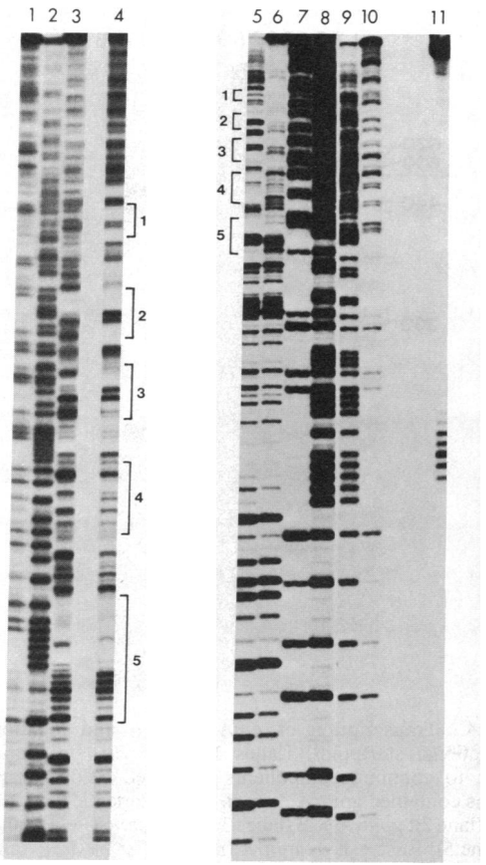

FIG. 2. Binding sites for gp*ntrC* and startpoint for *glnA* transcription *in vivo*. Lanes 3–6, DNase I digestion of a *glnA* promoter fragment (see below; 50,000 cpm, 0.03 pmol) in the presence (lanes 4 and 5) or absence (lanes 3 and 6) of gp*ntrC* (1 μg; purified through heparin agarose chromatography). Buffer contained 10 mM Tris·HCl (pH 7.4), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mM Na_2 EDTA, and 1 mM $CaCl_2$. Brackets indicate the dyad symmetries labeled in Fig. 1. Lane 11, high resolution S1 nuclease mapping of the downstream startpoint for *glnA* transcription *in vivo*. Messenger RNA was isolated from strain SK214 (*ntrB128*) grown in Luria broth; this strain, which has high levels of glutamine synthetase under all conditions of nitrogen availability (31), also has high levels of the downstream *glnA* transcript (unpublished results). RNA (100 μg) was hybridized with the probe described below (≈0.03 pmol, 50,000 cpm). After a 1.5-base correction, the strongest central band in lane 11 corresponds to position +1 in Fig. 1. Some regions of rU:dA are susceptible to cleavage by nuclease S1 and it was therefore possible that the downstream signal was a cleavage artifact rather than a transcriptional startsite. This does not appear to be the case because mutant strains such as SK907 (*glnAp359 ntrB137*::Tn*10*; ref. 9), which greatly overproduce the upstream transcript but cannot synthesize the downstream transcript since they are $NtrC^-$ (9), show no signal at the downstream position (unpublished results). Lanes 1, 2, and 7–10, Maxam and Gilbert sequencing ladders for G (1, 7, 10) or G + A (2, 8, 9) of the bottom strand of Fig. 1. The DNA probe of 310 bp was 5′ labeled at the *Bgl* II site (+31, Fig. 1) and extended to a *Dde* I site upstream of *glnA*. Gels were 8% (lanes 1–4) or 20% (lanes 5–11) sequencing gels (33). The bottom portion is not shown.

position corresponding to 80–100 kDa; highly purified E from *E. coli* eluted at a position corresponding to >400 kDa.) The $E\sigma^{70}$ and E fraction from heparin agarose was estimated to be >80% pure on $NaDodSO_4$/polyacrylamide gels by comparison to highly purified standards (kindly provided by C. Meares and R. Burgess).

The gp*ntrA* Fraction Has Transcriptional Activity That Is Dependent on E, the Core Form of RNA Polymerase. To assess transcriptional activity of the final gp*ntrA* fraction, we monitored the transcript produced from the *glnA* promoter to the strong T7 terminator in plasmid pJES86, a transcript of ≈509 nucleotides. Using this assay, we demonstrated the following (Fig. 4): (*i*) the gp*ntrA* fraction could transcribe from the *glnA* promoter in the presence of gp*ntrC* but not in its absence (lanes 1 and 3); (*ii*) the amount of transcript in the presence of gp*ntrC* increased with time between 1.5 and 6 min (lanes 9–11); (*iii*) transcriptional activity of the gp*ntrA* fraction from the *glnA* promoter was dependent on E (lanes 1 and 2); (*iv*) addition of σ^{70} (kindly provided by J. Erickson and D. Straus) to the gp*ntrA* fraction and gp*ntrC* and E decreased transcription from the *glnA* promoter (data not shown), indicating that σ^{70} was not required; (*v*) $E\sigma^{70}$ could not transcribe from the downstream *glnA* promoter (lanes 5–7; confirmed by S1 nuclease mapping as described below—data not shown) but could transcribe from other promoters on the plasmid; (*vi*) the gp*ntrA* fraction ± E and gp*ntrC* could not transcribe from plasmid promoters used by $E\sigma^{70}$ (lanes 1–3), indicating that it was not detectably contaminated by σ^{70} or $E\sigma^{70}$.

We used S1 nuclease mapping to locate the startpoint of *glnA* transcription in the presence of gp*ntrA*, E, and gp*ntrC* and found that it was identical to the startpoint used *in vivo* (Fig. 4, lanes 13–15). There was no detectable S1 nuclease signal when gp*ntrC* was omitted from the transcription reaction. This was the case whether the portion of the transcript being monitored was only 32 bases (data not shown) or whether it was 207 bases and extended into *glnA* coding information (lane 15). These findings are consistent

Table 1. Expression from the *glnA* and *lacUV5* promoters in $ntrA^+$ and $ntrA^-$ S30 extracts* and transcription by purified gp*ntrA* or $E\sigma^{70}$ + E†

	$ntrA^+$ S30		$ntrA^-$ S30	
	glnA template	*lacUV5* template	*glnA* template	*lacUV5* template
Addition to S30*				
gp*ntrC*	31,795	24,105	121	17,418
None	394	23,775	285	16,380
Transcription components†				
gp*ntrA* + E‡			1220	≤25
$E\sigma^{70}$ + E§			40	559

Results are expressed as pmol of β-galactosidase activity per min. The limit of reliable detection is 25. The *glnA* and *lacUV5* templates were pJES40 and pRS229, respectively. The gp*ntrC* was a mutant form that activates expression of *glnA* and other nitrogen-controlled genes to high levels *in vivo* even under conditions of nitrogen excess (unpublished). The gp*ntrC* was purified through the heparin agarose step and was estimated to be ≈50% pure on $NaDodSO_4$/polyacrylamide gels. Similar results were obtained with highly purified preparations of both mutant and parent (wild-type) forms of gp*ntrC*.

*Standard coupled assay (see *Methods*): concentrations of template and gp*ntrC* (when present) were 100 μg/ml and 17 μg of protein per ml, respectively.

†Modified assay: Transcription (in a vol of 25 μl) was carried out for 30 sec in the presence of a gp*ntrA* fraction or $E\sigma^{70}$ + E, as indicated, and in the presence of DNA template (280 μg/ml) and nucleotides; both gp*ntrC* (34 μg of protein per ml) and E were also present. The mRNA synthesized was then translated (final total vol, 50 μl) in the $ntrA^-$ S30 extract in the presence of other components needed for protein synthesis (see *Methods*) and rifampicin (4 μg/ml) to inhibit further transcription initiations.

‡The gp*ntrA* fraction (3 μl, which gave 104,890 pmol/min in a standard coupled assay) was purified through the sieving step from a strain that overproduced gp*ntrA*. Highly purified E from *E. coli* (120 μg/ml) was added.

§The $E\sigma^{70}$ + E fraction (240 μg of protein per ml) was purified through heparin agarose chromatography from strain SK35.

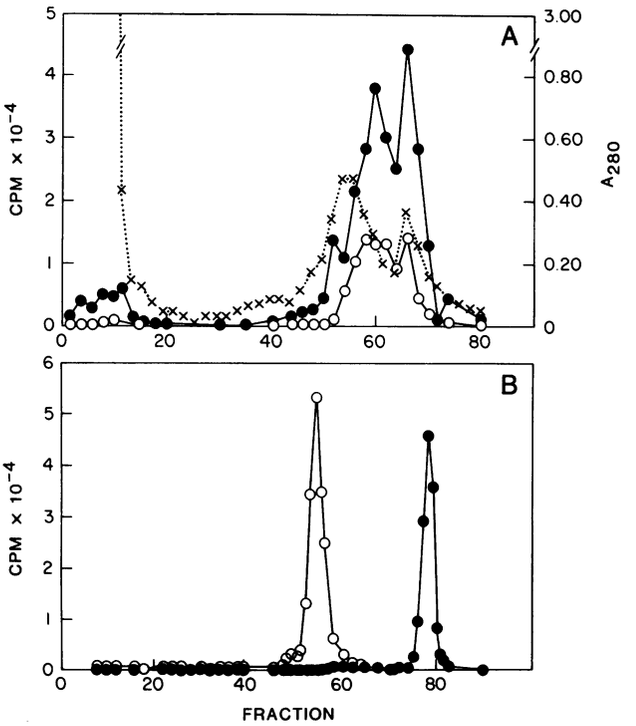

FIG. 3. Profiles of gp*ntrA* and Eσ^{70} plus E activities after chromatography on DEAE cellulose (*A*) or heparin agarose (*B*). The gp*ntrA* activity (○) was measured as described in *Methods* and is expressed in cpm of [^{3}H]orthonitrophenol formed per min. Different S30 extracts were used to obtain profiles in *A* and *B*. Recoveries were assessed with the same S30. The Eσ^{70} and E activities (●) were measured using poly(dA-dT)·poly(dA-dT) (10 μg/ml; P-L Biochemicals) as template and are expressed in cpm of [^{3}H]UMP incorporated into trichloroacetic acid-insoluble material per 10 min. For protein determinations (×), fractions were diluted 1:5 and A_{280} was measured. (*A*) The gp*ntrA* fraction (1177 mg of protein, 3873 units of gp*ntrA* activity) was applied to a DE52 column (50-ml bed vol) in TGED buffer [10 mM Tris·HCl, pH 8.0/1 mM EDTA/1 mM dithiothreitol/5% (vol/vol) glycerol (22)] and was eluted with a linear NaCl gradient from 0 to 0.5 M. The gp*ntrA* and Eσ^{70} + E coeluted approximately halfway through the gradient. Fractions 56–68 were pooled and concentrated by precipitation with 55% ammonium sulfate. Recovery of gp*ntrA* activity was ≈60% and purification was ≈3.5-fold. (*B*) The gp*ntrA* fraction (156 mg of protein, 1864 units of gp*ntrA* activity) was applied to a heparin agarose (from Bethesda Research Laboratories) column (100-ml bed vol) in TGED buffer with 10 mM $MgCl_2$ and 50 mM NaCl (25). The column was eluted with a linear NaCl gradient from 0.05 to 0.75 M. The gp*ntrA* eluted at ≈0.25 M whereas Eσ^{70} + E eluted at ≈0.5 M NaCl. Recovery of gp*ntrA* was ≈30% and purification was ≈7-fold.

with the view that gp*ntrC* activates the initiation phase of transcription.

To further assess transcriptional activity of the final gp*ntrA* fraction, we separated the transcription and translation phases of the standard coupled assay used during gp*ntrA* purification (see legend to Table 1). We ascertained (Table 1) that (*i*) whereas the gp*ntrA* fraction could transcribe from the *glnA* promoter in the presence of gp*ntrC* and E, it could not transcribe from the *lacUV5* promoter (line 3), providing further evidence that it was not contaminated with σ^{70} or Eσ^{70}; and (*ii*) Eσ^{70} had the reverse specificity (line 4). S1 nuclease mapping after the transcription phase of the reaction indicated that the startpoint of *glnA* transcription in the presence of gp*ntrA*, E and gp*ntrC* was identical to the startpoint used *in vivo* (data not shown).

DISCUSSION

Evidence That gp*ntrA* Is a σ Factor. The following evidence is consistent with the view that gp*ntrA* is a new σ subunit for RNA polymerase. (*i*) A partially purified gp*ntrA* fraction, which is not detectably contaminated with σ^{70} or Eσ^{70}, allows transcription of *glnA* from the major nitrogen-regulated promoter; transcription is dependent on gp*ntrC* as it is *in vivo*. (*ii*) Transcription of *glnA* by the gp*ntrA* fraction is dependent on addition of highly purified E, suggesting that gp*ntrA* separates from E during late purification steps (see

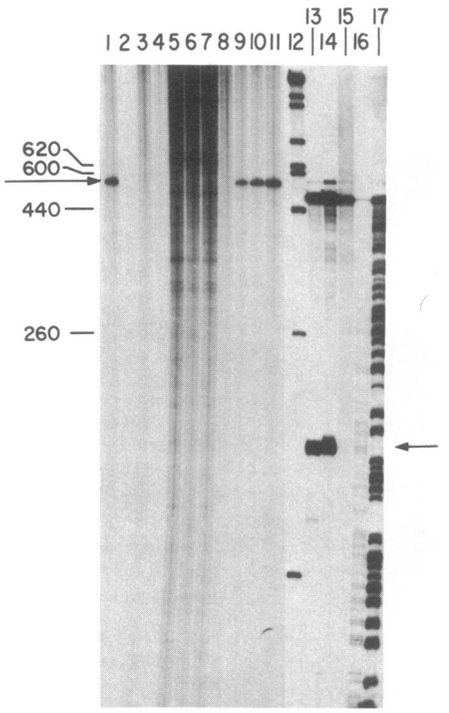

FIG. 4. Transcription of *glnA in vitro* and location of the transcriptional startpoint. Lanes 1–12, *in vitro* transcription. In addition to common components described below, transcription reactions contained gp*ntrA* + gp*ntrC* + E (lanes 1, 9–11); gp*ntrA* + gp*ntrC* (lane 2); gp*ntrA* + E (lanes 3 and 8); gp*ntrC* + E (lane 4); Eσ^{70} + E (lane 5); Eσ^{70} + E + gp*ntrA* (lane 6); Eσ^{70} + E + gp*ntrC* (lane 7). The gp*ntrA* (2 μl per reaction mixture) was purified through chromatography on Bio-Gel A1.5m and gp*ntrC* (5 μl = 0.17 μg; mutant form, see legend to Table 1) was highly purified. Highly purified E from *E. coli* (1.5 μg) was kindly provided by R. Burgess and Eσ^{70} + E (1 μl = 3 μg) was purified through heparin agarose chromatography. The DNA template was plasmid pJES86. All transcription reactions contained the components present in coupled reactions except that amino acids were omitted and CTP was added separately. Reactions (24 μl) were initiated with [α-^{32}P]CTP (final CTP concentration, 0.04 mM; 10 μCi) and were incubated at 37°C for 6 min except in the case of reactions for lanes 9 and 10, which were incubated for 1.5 and 3.0 min, respectively. Then excess unlabeled CTP and heparin (final concentrations, 0.8 mM and 0.08 mg/ml, respectively) were added (final total volume, 25 μl) and incubation was continued for 6 min. Reactions were stopped by extraction with phenol/chloroform. An amount corresponding to 1/10th of the total reaction mixture was subjected to electrophoresis. Molecular size standards (lane 12, marked at left of gel) were produced by *Hin*dIII cleavage of phage 29 DNA (and were labeled by using the Klenow fragment of DNA polymerase I). The *glnA* transcript is marked by an arrow at the left of the gel. Lanes 13–17, S1 nuclease mapping of the startpoint for *glnA* transcription *in vitro*. The DNA probe of 490 bp was 5′ labeled at the *Eco*RI site (position +205 of Fig. 1) and extended to a *Dde* I site upstream of *glnA*. For lanes 14 and 15, transcript was synthesized as described above (except in a final vol of 75 μl) in the presence of gp*ntrA* + gp*ntrC* + E (lane 14) or gp*ntrA* + E (lane 15). Lane 13 shows the *in vivo* startpoint for strain SK214 as a position marker. It is marked by an arrow at the right of the gel. Lanes 16 and 17 are Maxam and Gilbert sequencing ladders for G (lane 17) or G + A (lane 16) of the bottom strand of Fig. 1. All lanes are from the same 8% sequencing gel (33), but lanes 12–17 were exposed to film for longer and were inverted relative to other lanes. The top of the gel is not shown.

Results). Consistent with the dependence of gp*ntrA* on E, expression of *glnA* in an *ntrA*$^+$ S30 extract, like that of *lacUV5*, is inhibited by rifampicin and by streptolydigin, both of which bind to the β subunit of E (reviewed in ref. 34). Furthermore, in an *ntrA*$^+$ S30 extract from a strain that carries a mutation to rifampicin resistance in *rpoB*, the gene encoding β, expression from both templates is resistant to rifampicin (data not shown). Thus, gp*ntrA* appears to be a new σ subunit for RNA polymerase rather than (a subunit of) a new form of RNA polymerase that is independent of E. We think that it is gp*ntrA* itself that stimulates *glnA* transcription rather than another protein whose synthesis depends on the *ntr* system: Addition of an *ntrA*$^+$ plasmid (*ntrA*$^+$ insert of 2.8 kbp) to an *ntrA*$^-$ S30 resulted in a 30-fold stimulation of *glnA* expression (data not shown), and because protein synthesis in S30 extracts is dependent on an exogenous DNA template, stimulation was presumably due to gp*ntrA* that was synthesized from the plasmid.

Sequences in the Promoter Region for *glnA*. We find no reasonable matches to the consensus Eσ^{70} promoter (35) upstream of the major *glnA* transcriptional startpoint (see Fig. 1; unpublished results). Rather, we find sequences (5′-TCGCT-3′ and 5′-TTGGCAC-3′ centered at −10 and −21, respectively; overlined in Fig. 1) that are similar to sequences identified as a nitrogen fixation consensus promoter in *Klebsiella pneumoniae* and *Rhizobium meliloti* (5′-TTGCA-3′ and 5′-CTGGCAC-3′ in the −10 and −20 regions, respectively; reviewed in refs. 7 and 28; see also ref. 29). Activation of transcription from nitrogen fixation promoters of *K. pneumoniae in vivo* is dependent on gp*ntrA* together with gp*nifA* (10, 28), a protein that is functionally and evolutionarily related to gp*ntrC* (28). We hypothesize that the *glnA* promoter sequence described above and closely related nitrogen fixation promoters constitute recognition sites for a holoenzyme form of RNA polymerase that contains gp*ntrA* as σ subunit. Consistent with this, in the presence of E our most purified gp*ntrA* fractions protect sequences in the −10 and −20 regions of the *glnA* promoter from cleavage by DNase I, whereas the Eσ^{70} form of RNA polymerase does not (unpublished results).

In the region between 34 and 144 base pairs upstream of the major startpoint for *glnA* transcription lie five binding sites for gp*ntrC*. By varying its concentration in DNase I protection experiments, we ascertained that gp*ntrC* had highest apparent affinity for the most upstream sites, 1 and 2, less for sites 3 and 4, and least for site 5, which is nearest the major startpoint of transcription (unpublished observations). Binding of gp*ntrC* to these sites occurs in the absence of gp*ntrA*. It is an attractive hypothesis that effects of gp*ntrC* on *glnA* transcription depend on positions of its five binding sites relative to other sites and on its different affinities for individual sites. For example, repression of transcription from the upstream promoter(s) by Eσ^{70} may occur when sites 1 and/or 2 are occupied, whereas activation of transcription from the downstream major promoter may occur only when sites 3, 4, and 5 are also occupied.

σ Factors in Enteric Bacteria and Their Phage. Grossman *et al.* (36) have demonstrated that the *htpR* (now *rpoH*) gene product of *E. coli*, which is a positive regulatory factor required for increased synthesis of heat shock proteins at high temperature (37), is a new σ subunit for RNA polymerase. Kassavetis and Geiduschek (38) have described a σ factor from *E. coli* phage T4, gp55, that allows the core form of RNA polymerase to selectively transcribe from T4 late promoters.

If gp*ntrA* is, in fact, a σ factor, its function has several interesting features. (*i*) If glutamine is provided in the growth medium, gp*ntrA* is dispensable—mutant strains lacking it are glutamine auxotrophs. (*ii*) The gp*ntrA* activity is dependent on an auxiliary protein, gp*ntrC* or gp*nifA*, to activate transcription at nitrogen-controlled promoters. The auxiliary protein gp*ntrC* is known to be a DNA-binding protein (21, 32) and probably stimulates initiation of transcription. (*iii*) Directly or indirectly, function of gp*ntrA* in activating transcription is subject to metabolic regulation by the state of nitrogen nutrition of the cell. We have evidence that metabolic control is mediated, at least in part, by the auxiliary protein gp*ntrC*.

We thank C. Meares, Sue Bernhard, and R. Burgess for the Eσ^{70} and E forms of *E. coli* RNA polymerase. We thank them and E. P. Geiduschek, C. Gross, and J. Erickson for valuable advice. We are particularly grateful to Stan Artz for materials and guidance in the use of the S30 system. This work was supported by National Institutes of Health Grant GM21307 to S.K., National Institutes of Health Traineeship GM 07377-06AI to K.S., and a National Science Foundation Graduate Fellowship to J.K.

1. Merrick, M. J. (1982) *Nature (London)* **297,** 362–363.
2. Magasanik, B. (1982) *Annu. Rev. Genet.* **16,** 135–168.
3. Garcia, E., Bancroft, S., Rhee, S. G. & Kustu, S. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1662–1666.
4. Kustu, S., Burton, D., Garcia, E., McCarter, L. & McFarland, N. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4576–4580.
5. McFarland, N., McCarter, L., Artz, S. & Kustu, S. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2135–2139.
6. Reitzer, L. J. & Magasanik, B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5554–5558.
7. Dixon, R. (1984) *Nucleic Acids Res.* **12,** 7811–7830.
8. Reitzer, L. J. & Magasanik, B. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1979–1983.
9. McCarter, L., Krajewska-Grynkiewicz, K., Trinh, D., Wei, G. & Kustu, S. (1984) *Mol. Gen. Genet.* **197,** 150–160.
10. de Bruijn, F. J. & Ausubel, F. M. (1983) *Mol. Gen. Genet.* **192,** 342–353.
11. Weaver, F. R. & Weissmann, C. (1979) *Nucleic Acids Res.* **7,** 1175–1193.
12. Gilman, M. Z. & Chamberlin, M. J. (1983) *Cell* **35,** 285–293.
13. Aiba, H., Adhya, S. & De Crombrugghe, B. (1981) *J. Biol. Chem.* **256,** 11905–11910.
14. Schmitz, A. & Galas, D. J. (1983) in *Methods of DNA and RNA Sequencing*, ed. Weissman, S. M. (Praeger, New York), pp. 305–347.
15. Artz, S. W. & Broach, J. R. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3453–3457.
16. Wei, G. R. & Kustu, S. (1981) *Mol. Gen. Genet.* **183,** 392–399.
17. McFarland, N., McCarter, L., Artz, S. & Kustu, S. (1982) *Mol. Gen. Genet.* **185,** 152–157.
18. Weinstock, G. M., Berman, M. L. & Silhavy, T. J. (1983) in *Gene Amplification and Analysis*, eds. Papas, T. S., Rosenberg, M. & Chirikjian, J. G. (Elsevier/North-Holland, New York), pp. 27–64.
19. Simons, R. W., Hoopes, B. C., McClure, W. R. & Kleckner, N. (1983) *Cell* **34,** 673–682.
20. New England Nuclear (1983) *New Product News* **2,** no. 2.
21. Ueno-Nishio, S., Mango, S., Reitzer, L. J. & Magasanik, B. (1984) *J. Bacteriol.* **160,** 379–384.
22. Burgess, R. R. & Jendrisak, J. J. (1975) *Biochemistry* **14,** 4634–4645.
23. Elliott, T. & Geiduschek, E. P. (1984) *Cell* **36,** 211–219.
24. Burgess, R. R. (1969) *J. Biol. Chem.* **244,** 6160–6167.
25. Chamberlin, M., Kingston, R., Gilman, M., Wiggs, J. & de Vera, A. (1983) *Methods Enzymol.* **101,** 540–568.
26. Remaut, E., Stanssens, P. & Fiers, W. (1981) *Gene* **15,** 81–93.
27. Hanau, R., Koduri, R. K., Ho, N. & Brenchley, J. E. (1983) *J. Bacteriol.* **155,** 82–89.
28. Ausubel, F.M. (1984) *Cell* **37,** 5–6.
29. Ow, D. W., Xiong, Y., Gu, Q. & Shen, S.-C. (1985) *J. Bacteriol.* **161,** 868–874.
30. Krajewska-Grynkiewicz, K. & Kustu, S. (1984) *Mol. Gen. Genet.* **193,** 135–142.
31. Kustu, S. G., McFarland, N. C., Hui, S. P., Esmon, B. & Ames, G. F.-L. (1979) *J. Bacteriol.* **138,** 218–234.
32. Ames, G. F.-L. & Nikaido, K. (1985) *EMBO J.* **4,** 539–547.
33. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
34. Zillig, W., Palm, P. & Heil, A. (1976) in *RNA Polymerase*, eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 101–125.
35. Hawley, D. K. & McClure, W. R. (1983) *Nucleic Acids Res.* **11,** 2237–2255.
36. Grossman, A. D., Erikson, J. W. & Gross, C. A. (1984) *Cell* **38,** 383–390.
37. Neidhardt, F. C. & van Bogelen, R. A. (1981) *Biochem. Biophys. Res. Commun.* **100,** 894–900.
38. Kassavetis, G. A. & Geiduschek, E. P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5101–5105.