Unusual Oligomerization Required for Activity of NtrC, a Bacterial Enhancer-Binding Protein

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Nitrogen regulatory protein C (NtrC) contacts a bacterial RNA polymerase from distant enhancers by means of DNA loops and activates transcription by allowing polymerase to gain access to the template DNA strand. It was shown that NtrC from *Salmonella typhimurium* must build large oligomers to activate transcription. In contrast to eukaryotic enhancer-binding proteins, most of which must bind directly to DNA, some NtrC dimers were bound solely by protein-protein interactions. NtrC oligomers were visualized with scanning force microscopy. Evidence of their functional importance was provided by showing that some inactive non–DNA-binding and DNA-binding mutant forms of NtrC can cooperate to activate transcription.

When phosphorylated at aspartate 54 (D54), the bacterial enhancer-binding protein NtrC activates transcription by the σ^{54}-holoenzyme form of RNA polymerase (Fig. 1) (*1*, *2*). To do so, it catalyzes the isomerization of closed complexes between this polymerase and a promoter to open complexes in a reaction that depends on hydrolysis of the β-γ bond of adenosine triphosphate (ATP) or guanosine triphosphate. Unphosphorylated NtrC is a dimer in solution (*1*–*3*), but it is known that single phosphorylated dimers are not sufficient to activate transcription or hydrolyze ATP (*1*, *4*–*7*). Both reactions are greatly stimulated by enhancers, which are composed of two binding sites for dimers of NtrC, and it has been assumed that a tetramer was sufficient for transcriptional activation (*2*, *6*). However, it was not clear from previous studies whether the active entity at an enhancer contained only the two DNA-bound dimers or also contained additional dimers that were not bound directly to DNA. Because certain mutant forms of NtrC fail to activate transcription at concentrations that are apparently sufficient to occupy the *glnA* (glutamine synthase) enhancer but can do so at higher concentrations (*8*), we hypothesized that transcriptional activation might depend on formation of an oligomer larger than a tetramer, in which the additional dimer or dimers were held by protein-protein interactions to those directly bound to the enhancer. We report the visualization of large oligomers with scanning force microscopy (SFM) (*9*) and present evidence that they are required functionally for activation of transcription (Fig. 1).

To test the utility of SFM for determining the relative sizes of DNA-bound oligomers of NtrC (*10*), we collected images of NtrC-DNA complexes with well-defined size and stoichiometry (*1*) and of RNA polymerase-promoter complexes and assessed the relationship between the volumes of the proteins and their molecular masses. The standard complexes (*11*, *12*) were: (i) single dimers of NtrC bound to a single strong NtrC-binding site, (ii) two NtrC dimers (tetramers) bound to a "strong" enhancer derived from the *glnA* enhancer (two identical strong binding sites for NtrC; Fig. 1), and (iii) the σ^{70}-holoenzyme form of RNA polymerase bound to the rightward promoter (P_R) of bacteriophage λ (Fig. 2A). Each DNA-bound protein complex could be identified unambiguously by the length of the fragment to which it was bound and its position along this fragment (Fig. 2B). The volumes of these protein complexes (*13*) were a linear function of their molecular masses (Fig. 2E) (*14*). Because we anticipated that larger complexes of phosphorylated NtrC (P-NtrC) bound to the strong enhancer would carry three or four NtrC dimers, we expected their molecular masses (315 or 420 kD, respectively) would fall within the linear range (105 to ≈459 kD) (*15*, *16*).

An average of the data from three experiments indicated that most (72%) of the DNA molecules carrying unphosphorylated (inactive) NtrC at the enhancer carried tetramers, whereas the remainder (22%) carried mainly single dimers (Fig. 2D and Table 1). Tetramers sometimes had a bilobed appearance (Fig. 2, B and C), presumably when they were optimally oriented relative to the scanning tip. Most important, few (6%) of the DNA molecules carrying unphosphorylated NtrC at the enhancer carried oligomers containing more than two dimers (Table 1).

Commensurate with the ability of P-NtrC to activate transcription, the distribution of complexes bound to the enhancer spread noticeably toward larger sizes when NtrC was phosphorylated (Fig. 2H), and larger complexes were visible in individual images (Fig. 2, F and G). However, because of the variation in the measured volumes of each of the standard protein species bound to DNA (Fig. 2E) and the lability of large oligomers of P-NtrC at the enhancer (*10*), the peaks representing various multimer states were not clearly separated in histograms. Hence, we determined the percentage of complexes that carried more than two dimers of NtrC (Table 1) (*17*). This

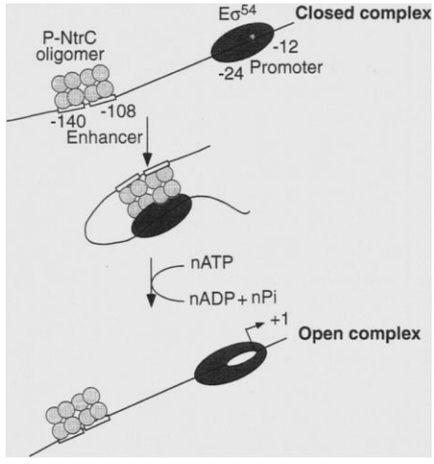

Fig. 1. Transcriptional activation by NtrC at the *glnA* promoter of *S. typhimurium*. Conserved promoter sequences recognized by σ^{54}-holoenzyme ($E\sigma^{54}$) lie at sites −12 and −24 with respect to the startsite of transcription at +1. Boxes represent the two 17-bp NtrC-binding sites that constitute the *glnA* enhancer; they are centered at −108 and −140. (Top) $E\sigma^{54}$ by itself can bind to the *glnA* promoter in a closed recognition complex, in which the DNA remains double-stranded. NtrC binds to the enhancer, but only the phosphorylated form (P-NtrC) can activate transcription. We demonstrate that active oligomers of P-NtrC must contain not only the two dimers bound to the enhancer but an additional dimer or dimers bound to these by protein-protein interactions. (Middle) P-NtrC contacts $E\sigma^{54}$ by means of a DNA loop. (Bottom) In a reaction that requires hydrolysis of ATP, P-NtrC catalyzes the isomerization of closed complexes between polymerase and the promoter to open complexes, in which the DNA around the transcriptional startsite is locally denatured and the correct strand can be used as template.

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was 26% (80/309) for protein phosphorylated with a low molecular mass phosphate donor, carbamoyl phosphate, and 43% (32/74) for protein phosphorylated with the physiological donor nitrogen regulatory protein B (NtrB). The large oligomers of P-NtrC at the enhancer appeared to be built up off the DNA rather than being spread out along it, and in agreement with this, some of the oligomers tethered together two enhancer-bearing DNA fragments (*18*). Such tethering was observed only when NtrC was phosphorylated.

To investigate whether large oligomers of NtrC were functionally required for transcriptional activation, we performed in vitro complementation studies to see whether DNA-binding and nonbinding mutant forms of NtrC could cooperate in forming active oligomers. The members of each pair were chosen because they activated transcription poorly or undetectably by themselves. The first pair was NtrC with an $Asp^{54} \rightarrow Glu^{54}$ mutation ($NtrC^{D54E}$) and P-$NtrC^{3Ala}$. $NtrC^{D54E}$, which has some negative charge where the phosphoryl group is normally located, activates transcription poorly and only at concentrations much higher than those required to occupy an enhancer (*8*). $NtrC^{D54E}$ cannot be phosphorylated and formed few large oligomers at the strong enhancer (Table 1). P-$NtrC^{3Ala}$, which essentially fails to bind to DNA, also activates transcription poorly (*19*) and failed to bind to the strong enhancer (Table 1). At very high concentrations, P-$NtrC^{3Ala}$ can form oligomers in solution and can apparently contact σ^{54}-holoenzyme without being tethered to DNA (*19*, *20*).

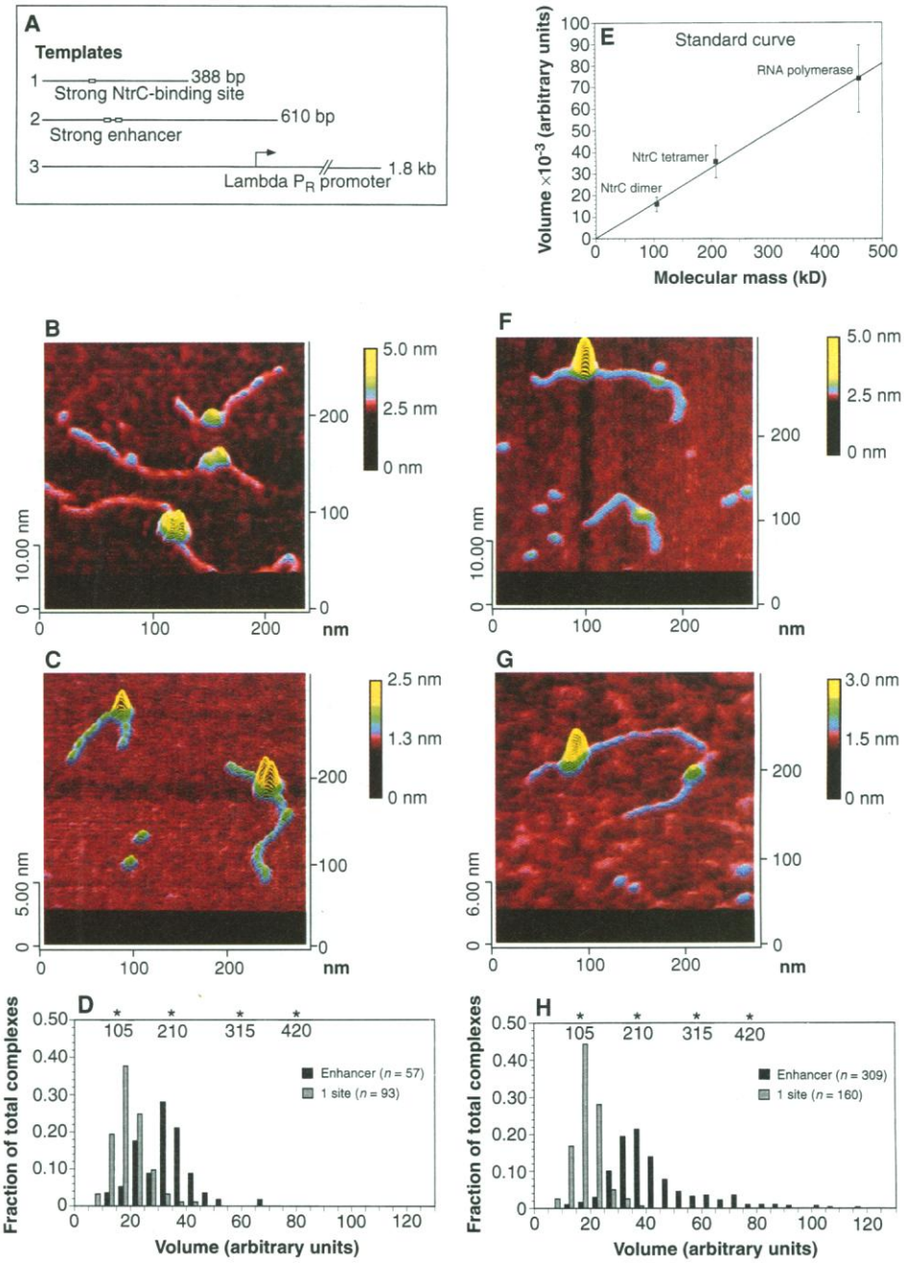


Fig. 2. SFM of NtrC and P-NtrC complexes at the strong enhancer. (**A**) DNA fragments are shown. NtrC was bound to either a single strong binding site [indicated by a box (*11*)] on a 388-bp fragment (template 1) or the strong enhancer (two strong NtrC-binding sites separated by 32 bp; see legend to Fig. 1) on a 610-bp fragment (template 2). RNA polymerase (σ^{70}-holoenzyme) was bound to the lambda P_R promoter on a 1.8-kb fragment (template 3). (B, C, F, and G) SFM images show nucleoprotein complexes. Images have been processed only by flattening to remove background slope. The *z* dimension (height), which is different for different panels, is indicated by the color code on the bar at the right; the mica surface is at half-maximal height. Images are displayed as line plots at a 60° tilt angle to emphasize topography. (**B**) Standard complexes are shown. At the top, middle, and bottom, respectively, are a single NtrC dimer bound to template 1, two NtrC dimers (a tetramer) bound to template 2, and RNA polymerase bound to template 3 (partially shown). (**C**) Two NtrC dimers are bound to the strong enhancer (right) and a single dimer is bound to a single strong site (upper left). (**F** and **G**) Large oligomers of P-NtrC (carbamoyl phosphate as donor) are bound to the strong enhancer. Each panel includes a single (unphosphorylated) dimer bound to a single site (lower right) that can be used for size comparison. (D and H) Histograms indicating the fraction of the total number of NtrC-DNA complexes as a function of volume. Black bars represent complexes of NtrC (**D**) or P-NtrC (**H**) bound to the strong enhancer. Gray bars represent standard complexes of unphosphorylated NtrC on a single strong site and are a marker for the volume and distribution of single dimers in each experiment. The total number of complexes on each template is given at the top right of the panel. The bin for an average dimer of unphosphorylated NtrC at a single site (105 kD) and the expected bins for 210-, 315-, and 420-kD proteins (legend to Table 1) are indicated by asterisks above the panels. Note the bimodal distribution of complexes at the enhancer in (D) (*7*) and the shift toward larger oligomers at the enhancer (H). (**E**) Volumes of the three standard proteins (*11*, *12*) as a function of their molecular masses. Volumes (arbitrary units) (*13*) were determined by averaging 171 NtrC dimers (105 kD) (*15*) bound to template 1, 81 RNA polymerase molecules (459 kD) (*16*) bound to template 3, and 123 NtrC tetramers (210 kD) bound to template 2. Before averaging the volumes of tetramers at the enhancer, we subtracted the volumes of dimers (*14*). The error bars are the standard deviations for each average volume.

At a concentration of 10 nM, $NtrC^{D54E}$ had little ability to activate transcription from a template (0.5 nM) carrying the strong enhancer (empty square on the y axis in Fig. 3A) and the same was true of P-$NtrC^{3Ala}$ at concentrations up to 600 nM (diamonds) (*18*). If the concentration of $NtrC^{D54E}$ was maintained at 10 nM and P-$NtrC^{3Ala}$ was added to it, transcriptional activation was greatly stimulated over that given by either protein alone (solid squares), commensurate with the ability of the two proteins to cooperate in forming large oligomers (Table 1). If the two proteins were allowed to undergo subunit exchange before transcriptional activation was assayed, synergistic effects were lost and residual activation was similar to that given by P-$NtrC^{3Ala}$ alone (empty squares). This concurs with the previous finding that heterodimers between DNA-binding and nonbinding forms of NtrC have essentially lost the ability to bind to DNA and hence cannot be tethered to the enhancer (*3*), and with the fact that P-$NtrC^{3Ala}$ was at concentrations in excess of $NtrC^{D54E}$. Complementation by P-$NtrC^{3Ala}$ protein persisted at 20 to 50 nM $NtrC^{D54E}$, concentrations at which the enhancer was fully occupied, on the basis of deoxyribonuclease I protection studies (*8*, *18*). As expected, background activation by $NtrC^{D54E}$ alone was higher at these higher concentrations.

To overcome two potential caveats in taking the previous experiment as evidence that large oligomers are required for transcriptional activation (*21*), we showed that active oligomers could be formed by two inactive partners. In this case we used NtrC protein with mutations of $Asp^{54} \rightarrow Asn^{54}$ or $Asp^{54} \rightarrow Ala^{54}$ ($NtrC^{D54N}$ or $NtrC^{D54A}$, respectively), which have no negative charge at the position of the native phosphoryl group, as the DNA-bound form (*8*). Like $NtrC^{D54E}$, these two proteins cannot be phosphorylated, but bind normally to the enhancer. Unlike $NtrC^{D54E}$, however, they have no detectable ability to activate transcription (triangle and diamond on the y axis in Fig. 3B). We used P-$NtrC^{A216V,\ 3Ala}$ as the form incapable of DNA binding. Like the NtrC mutant $Ala^{216} \rightarrow Val^{216}$ ($NtrC^{A216V}$) protein from which it was derived (*15*), P-$NtrC^{A216V,\ 3Ala}$ had no detectable ability to activate transcription (crosses, Fig. 3B), despite the fact that it retained essentially normal adenosine triphosphatase (ATPase) activity in solution (*18*). However, P-$NtrC^{A216V,\ 3Ala}$ could cooperate with $NtrC^{D54N}$ or $NtrC^{D54A}$ [triangles and diamonds, respectively, Fig. 3B (note the change in scale)] to form active oligomers. The maximum activity of the oligomers formed was 10 to 15% that of oligomers formed by the transcriptionally active proteins $NtrC^{D54E}$ and phosphorylated $NtrC^{3Ala}$.

Large oligomers of P-NtrC, which are probably octamers (*22*), appear to be required for ATPase activity (*1*, *4*, *5*, *15*). Correlations between their formation and the ATPase activity and transcriptional activation capacity of the protein are striking not only for wild-type NtrC but for $NtrC^{D54E}$. Wild-type NtrC forms oligomers larger than tetramers at the strong enhancer only when it is phosphorylated (Fig. 3 and Table 1); it has ATPase activity and activates transcription only under the same circumstances (*4*). Moreover, the ATPase activity of P-NtrC in solution is known to be markedly stimulated by the enhancer, but much less so by a single binding site for a dimer, presumably because the enhancer facilitates the formation of large oligomers (*1*, *5*, *15*). The $NtrC^{D54E}$ protein, which cannot be phosphorylated, forms few oligomers larger than tetramers at the strong enhancer (Table 1); the protein has very low ATPase activity in solution and poor ability to activate transcription (*8*). The ATPase activity of $NtrC^{D54E}$ (200 nM) is stimulated less than twofold by the enhancer (*20*), commensurate with the fact that the enhancer has little effect on the formation of large oligomers at this protein concentration.

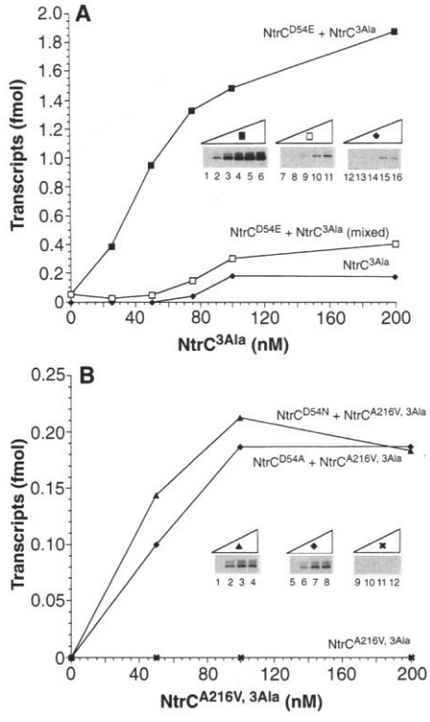
Unlike the case for NtrC and other activators of σ^{54}-holoenzyme (*23*), no eukaryotic enhancer-binding protein or upstream activator thus far characterized facilitates the isomerization of closed complexes between an RNA polymerase and a promoter to open complexes, and none has an ATPase activity. Rather, the eukaryotic proteins appear to affect steps earlier or later in the transcription process (*24*). Although efficient transcriptional activation by eukaryotic enhancer- or upstream activator-binding proteins often requires multiple molecules, these are arrayed on separate DNA-binding sites. More analogous to the

Table 1. Characterization of NtrC complexes at the strong enhancer [610-bp DNA fragment (template 2 of Fig. 2A)] by SFM.

Protein	Estimated % of complexes (*26*) as			Total
	One dimer	Two dimers	>Two dimers	
NtrC*	22	72	6	455
P-NtrC†	8	63	29	383
$NtrC^{D54E}$‡	23	73	5	164
$NtrC^{D54E}$ + P-$NtrC^{3Ala}$	24	62	14§	82

*Data were taken from the histogram (black bars) of Fig. 2D and two additional experiments. †NtrC was phosphorylated with the low molecular mass donor carbamoyl phosphate or the physiological donor NtrB, which depends on ATP (*12*, *17*). ATP was present in both cases. ‡NtrB was present for one experiment (91 complexes; 19% dimers, 78% tetramers, and 3% larger oligomers) and not for the other (73 complexes; 27% dimers, 66% tetramers, and 7% larger oligomers). §The increase over large oligomers for unphosphorylated NtrC and $NtrC^{D54E}$ was significant at the 95% confidence level using a properly corrected chi-squared test.

Fig. 3. Complementation between mutant forms of NtrC at the strong enhancer. Formation of open complexes was assessed in a single-cycle transcription assay with plasmid pJES534 (0.5 nM) as template (*7*, *15*, *27*). (**A**) The DNA-bound form of NtrC was $NtrC^{D54E}$ (*14*) (10 nM) and the nonbound form was P-$NtrC^{3Ala}$ (*27*), which was used at the concentrations indicated. Activity was as much as 18-fold higher than the sum of the activities of the individual proteins, and maximum template utilization was 15% (1.9 fmol/12.5 fmol total). Control reactions contained P-$NtrC^{3Ala}$ alone or a combination of the two proteins after subunit exchange. (Inset) Lanes 1 through 6, $NtrC^{D54E}$ (10 nM) plus P-$NtrC^{3Ala}$ (0, 25, 50, 75, 100, and 200 nM, respectively); lanes 7 through 11, $NtrC^{D54E}$ (10 nM) plus P-$NtrC^{3Ala}$ (25, 50, 75, 100, and 200 nM, respectively) after subunit exchange; lanes 12 through 16, P-$NtrC^{3Ala}$ alone (25, 50, 75, 100, and 200 nM, respectively). (**B**) The DNA-bound forms of NtrC were the inactive $NtrC^{D54N}$ and $NtrC^{D54A}$ proteins (10 nM). Both were complemented by the inactive P-$NtrC^{A216V,\ 3Ala}$. P-$NtrC^{A216V,\ 3Ala}$ alone lacked detectable activity. (Inset) Lanes 1 through 4, $NtrC^{D54N}$ (10 nM) plus P-$NtrC^{A216V,\ 3Ala}$ (0, 50, 100 and 200 nM, respectively); lanes 5 through 8, $NtrC^{D54A}$ (10 nM) + P-$NtrC^{A216V,\ 3Ala}$ (0, 50, 100, and 200 nM, respectively); lanes 9 through 12, P-$NtrC^{A216V,\ 3Ala}$ alone (0, 50, 100, and 200 nM, respectively).



case for NtrC, formation of activating hetero-oligomers at eukaryotic enhancers sometimes entails participation of both DNA-bound and nonbound partners (*25*). Remarkably, the estrogen receptor can activate transcription either by binding to specific sites in DNA or by associating solely by protein-protein interactions with one or more unidentified adaptor proteins that are specifically DNA-bound.

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10. Large oligomers were not detected in gel mobility shift assays, presumably because dimers not directly DNA bound (see text) dissociated during the course of electrophoresis (*7*). P-NtrC apparently formed large oligomers at the *glnA* enhancer in electron micrographs [W. Su, S. Porter, S. Kustu, H. Echols, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5504 (1990)], but their size could not be reliably determined, principally because images were distorted by staining and shadowing materials. Large oligomers of P-NtrC could not be studied by scanning transmission electron microscopy, because they were obscured by the background of free protein necessary to form them (M. Simon and I. Rombel, unpublished data). Deposition of samples for SFM takes <1 min. This technique can be used to obtain topographic images of biomolecules directly without the use of external means of contrast, and three-dimensional information can be obtained without complicated image reconstruction. Although SFM images are distorted in ways that alter the dimensions of biological molecules, these distortions are constant within a set of scans made with the same sample deposition and the same tip; hence, it should be possible to determine the relative sizes of biomolecules from images collected at the same time (*9*).
11. Wild-type and mutant forms of NtrC from *Salmonella typhimurium* were overproduced and purified essentially as described (*4*, *7*, *15*, *18*, *19*). Templates 1, 2, and 3 (Fig. 2A) were prepared from plasmids pJES483 (*18*), pJES534 (*7*), and PRLM4, respectively, and were isolated from agarose gels [F. Ausubel *et al.*, *Current Protocols in Molecular Biology* (Wiley, New York, 1996)]. The boxes represent 17–base pair (bp) NtrC-binding sites (5′-TGCACTA-AAATGGTGCA-3′).
12. After incubation of proteins with DNA for 25 min at room temperature, complexes were diluted 15- to 30-fold and deposited onto mica essentially as described (*9*). Complexes from different binding reactions were mixed at the dilution step and deposited within 20 s of mixing. SFM images (nonoverlapping 2-μm fields) were collected with a NanoScope III (Digital Instruments, Santa Barbara, CA) operated in the tapping mode with commercial silicon nitride tips. NtrC (final dimer concentration of 200 to 400 nM) was bound to DNA fragments (30 nM) in a volume of 10 μl of a buffer suitable for open complex formation (*7*, *18*). For cases in which NtrC was phosphorylated, binding mixtures also contained 4 mM ATP and either 20 mM carbamoyl phosphate or 20 to 40 nM NtrB dimer because phosphorylation is transient and must be maintained in situ (*1*, *2*). For experiments in which $NtrC^{D54E}$ and $NtrC^{3Ala}$ were combined, $NtrC^{D54E}$ was first bound to DNA (in the presence of ATP), and then $NtrC^{3Ala}$ and NtrB were added. The σ^{70}-holoenzyme form of RNA polymerase (260 nM final concentration; Epicenter Technologies, Madison, WI) and the 1.8-kb DNA fragment (140 nM final concentration) were combined in buffer in a volume of 10 μl and incubated for 15 min at 37°C.
13. The sizes of protein oligomers on DNA were determined using National Institutes of Health Image software. The average height and area of a manually defined object encircling the nucleoprotein complex were used to calculate a volume in arbitrary pixel units. The volume of an adjacent background region containing DNA was then subtracted to determine the volume of the protein oligomer.
14. Under conditions comparable to those used in these experiments (*7*), the strong enhancer is not fully occupied by tetramers of NtrC, resulting in a bimodal distribution of NtrC complexes at the enhancer (black bars in Fig. 2D). Volumes of dimers were subtracted before averaging those of tetramers by assuming that the peak corresponding to dimers had the same position and shape as that for dimers bound to the single strong site (gray bars in Fig. 2D).
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17. Because we observed the larger NtrC complexes with both phosphorylating agents (*12*), we infer that they do not contain NtrB. The decrease in the proportion of single dimers upon phosphorylation agrees with the observation that phosphorylation increases the cooperativity of binding of two NtrC dimers to the enhancer (*6*, *7*).
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21. Caveat 1: Because a single DNA-bound dimer of NtrC can be complemented by additional dimers of $P\text{-}NtrC^{3Ala}$ (*7*), template molecules that carried only a single dimer of $NtrC^{D54E}$ at the enhancer (for example, the 20% observed by SFM) would have contributed to increased activity in the presence of $P\text{-}NtrC^{3Ala}$. Caveat 2: Even if the enhancer was fully occupied by two dimers of $NtrC^{D54E}$, the only function of this protein may have been to tether two more active dimers of $P\text{-}NtrC^{3Ala}$ to the DNA. A tetramer of the latter may have accounted for increased activity.
22. In addition to symmetry considerations, there are two lines of evidence that active oligomers are octamers: (i) The phosphorylated $NtrC^{S160F,\ 3Ala}$ protein, which activates transcription very well but fails to bind to DNA, is an octamer, as assessed by gel filtration chromatography (*20*), whereas the unphosphorylated protein, which activates very poorly (*7*), is a dimer; and (ii) results of complementation tests between active and inactive forms of NtrC and $NtrC^{3Ala}$ proteins at a single strong binding site are most easily reconciled with those at the strong enhancer if one or two inactive dimers can be assimilated into active octamers but three or four cannot (I. Rombel and S. Kustu, unpublished data).
23. Like NtrC, at least two other activators of σ^{54}-holoenzyme (DctD and XylR) must apparently form oligomers to activate transcription and hydrolyze ATP [J. H. Lee, D. Scholl, B. T. Nixon, T. R. Hoover, *J. Biol. Chem.* **269**, 20401 (1994); J. Perez-Martin and V. de Lorenzo, *Cell* **86**, 331 (1996); *J. Mol. Biol.* **258**, 575 (1996)], but it is not known whether the active oligomers of these other proteins are composed only of "entities" (not necessarily dimers) that are DNA bound or must also contain additional entities that associate with these by protein-protein interactions. There are arguments for and against a requirement for oligomer formation by FhlA, a third σ^{54}-dependent activator [reviewed in S. Hopper and A. Bock, *J. Bacteriol.* **177**, 2798 (1995)].
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26. For experiments with NtrC, the volume of a complex carrying two dimers was interpolated from a line connecting the average volume of standard complexes carrying single NtrC dimers on template 1 of Fig. 2A and the average volume of standard complexes carrying σ^{70}-holoenzyme on template 3; it ranged from 1.95 to 2.17 times the average volume of a single dimer. The standard deviation (27%) was taken as the average of the standard deviations for the single NtrC dimer and σ^{70}-holoenzyme in four experiments. For experiments with $NtrC^{D54E}$, in which there were too few RNA polymerase complexes to use the method above, the volume of two NtrC dimers was assumed to be twice the average volume of a single dimer on template 1. The standard deviation was that for single NtrC dimers in each experiment. The volume limit for NtrC tetramers was operationally defined as the average volume of a tetramer plus or minus one standard deviation. Larger oligomers or those carrying only a single NtrC dimer were defined as those that fell above or below this limit, respectively. This method of estimating the proportion of single dimers gave essentially the same results as that described in (*14*).
27. The template was plasmid pJES534 (*7*), which carries the strong enhancer ≈450 bp upstream of the *glnA* promoter. Unless otherwise noted, conditions were chosen to minimize subunit exchange between NtrC dimers. The non-DNA-binding form was phosphorylated before being added to the other components (*18*). Carbamoyl phosphate was at 10 mM and the final concentrations of σ^{54} [purified from *S. typhimurium* as described (*19*)] and core RNA polymerase (from *Escherichia coli*; kindly supplied by D. Hager and R. Burgess, University of Wisconsin, Madison) were 50 and 30 nM, respectively. Complete subunit mixing between homodimeric species of NtrC for control experiments was achieved as described (*3*, *7*).

28. Supported by NIH grants GM17078 (C.W. and the late Harrison Echols), GM32543 (C.B.), and GM38361 (S.K). We thank K. Yamamoto for advice on eukaryotic transcriptional activation and R. Chambers and N. Pace for criticism of the manuscript.

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