Identification of a Surface of FNR Overlapping Activating Region 1 That Is Required for Repression of Gene Expression*

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A library of *Escherichia coli* fnr mutants has been screened to identify FNR (regulator of fumarate and nitrate reduction) variants that are defective repressors, but competent activators. All but one of seventeen variants had substitutions close to or within the face of FNR that contains activating region 1 (AR1). Activating region 1 is known to contact the α subunit of RNA polymerase to facilitate transcription activation. It is now evident that this face also has a role in FNR-mediated repression. Single amino acid substitutions at Lys54, Gly74, Ala95, Met147, Leu169, Arg197, or Leu229, and double substitutions at Ser133 and Ser145, Gly16 and Ile48, Tyr49 and Ser133, or Lys164 and Phe192, impaired FNR-mediated repression of ndh without greatly affecting activation from model Class I (FNR site at −71.5) and Class II (FNR site at −41.5) FNR-activated promoters. Although repression was impaired in a second group of FNR variants with substitutions at Leu44, Arg72 and Leu169, Phe92, or Ser176, transcription activation from the simple FNR-dependent promoters was severely reduced. However, expression from pYfYD (FNR sites at −40.5 and −93.5) and a derivative lacking the site at −93.5, pYfYD−/−, remained relatively high indicating that this second group have a context-dependent activation defect as well as a repression defect. The prediction that the substitutions affecting repression were likely to be in solvent exposed regions of FNR was supported by analysis of peptides produced by partial proteolysis of FNR. Thus, FNR-mediated repression at promoters with multiple FNR sites requires regions of FNR that are different from, but overlap, AR1.

The FNR protein of *Escherichia coli* is a global transcription regulator, controlling the expression of genes in response to oxygen starvation. FNR is predicted to be structurally related to the cAMP receptor protein and acts mainly as an activator of genes involved in anaerobic energy generation (1). Generally, FNR activates transcription by binding to a site centered at about −41 in target (Class II) promoters where it makes multiple direct activating contacts with RNA polymerase involving two discrete activating regions of FNR (Fig. 1A, 2 and 3). Activating region 1 (AR1) appears to contact the C-terminal domain of the RNA polymerase α subunit, thereby preventing inhibition of transcription activation caused by an un tethered α subunit. Activating region 3 (AR3) contacts the σ70 subunit of RNA polymerase to activate transcription (2). At promoters in which the FNR site is centered at −61 or beyond (Class I) activation depends on a different AR-α subunit contact (3).

As well as acting as an activator of anaerobic gene expression, FNR also acts as a repressor of some aerobic genes (1). Unlike the FNR-activated promoters, there is no discernible pattern of FNR site positioning among FNR-repressed promoters, although a common feature is the presence of multiple FNR sites upstream of the transcription start (1). The best characterized of the FNR-repressed genes is *ndh*, which encodes a non-proton-translocating NADH dehydrogenase that is the major primary dehydrogenase of the aerobic respiratory chain (4). The *ndh* promoter has two FNR sites centered at −50.5 and −94.5, and both contribute to FNR-mediated repression (5–7). The mechanism of FNR-mediated repression appears not to be due to simple promoter occlusion, but rather displacement of the RNA polymerase α subunit leads to inhibition of transcription (7). FNR sites located beyond the region of DNA occupied by RNA polymerase have also been shown to be necessary for efficient repression of the fnr and *narX* promoters (8). Furthermore, FNR occupation of the far upstream FNR site of the *yfiD* promoter (FNR sites at −40.5 and −93.5) down-regulates *yfiD* expression (9). These observations indicated that FNR-mediated repression of these promoters requires multiple FNR binding sites and thus repression may arise as a consequence of interactions between two or more FNR dimers, or between FNR dimers (or tetramers) and RNA polymerase. Therefore a library of fnr mutants was screened for FNR variants defective in repression. Two types of variant were identified: type A was a poor repressor but good activator; type B was a poor repressor but failed to activate transcription from simple FNR-activated promoters. However, the type B variants did activate transcription from the complex *yfiD* promoter. All the variants isolated, except one, contained amino acid substitutions overlapping the face of FNR that contains AR1, indicating that this surface may have a role in repression as well as in activation and anti-inhibition.

EXPERIMENTAL PROCEDURES

Error-prone Polymerase Chain Reaction Mutagenesis—Random mutations in the *fnr* gene carried by pGS24 (a derivative of pBR322 containing the *fnr* gene in a HindIII-BamHI fragment) were introduced using *Taq* DNA polymerase and the following synthetic primers, as described previously (2): 5′-GGTTATATCGATAGATGTTGAACTTTGCCGG (fnr coordinates 1–23) and 5′-CGTTAGGAGATCCAGCC-GTTACGG (1625–1641), where the unique HindIII and BamHI targets are underlined. Following digestion with HindIII and BamHI, the polymerase chain reaction products were ligated into the corresponding sites of pBR322. Plasmids were isolated by standard methods, and mutations in the *fnr* gene were defined by Applied Biosystems cycle sequencing with the aid of two primers: 5′-AATATGGAATCCCAAGGAAACCTCGATGGTAGCTGAAATCCC-TTTGCCGG (fnr coordinates 1–23) and 5′-GGAAACCTCGATGGTAGCTGAAATCCC-TTTGCCGG (520–536) and 5′-GGAAACCTCGATGGTAGCTGAAATCCC-TTTGCCGG (520–536) and 5′-GGAAACCTCGATGGTAGCTGAAATCCC-TTTGCCGG (520–536) and 5′-GGAAACCTCGATGGTAGCTGAAATCCC-TTTGCCGG (520–536).
derivative of JRG1728 (ΔlacIPOZYA1X74 galU galK rpsL Δara-leu Δ(ynr-R-fnr-rac-try)17 add-230::Tn9) containing a compatible yfiD-lac reporter plasmid, pGS1000 (10). Transformants were tested for enhanced yfiD-lac expression on L-agar containing: 5-bromo-4-chloro-3-indolyl β-galactosidase (40 µg/ml), ampicillin (200 µg/ml), and tetracycline (35 µg/ml). The pBR322 derivatives containing the mutant fnr genes were isolated and were then used to transform JRG3917, a JRG1728 derivative containing a pRW2-based ndh-lacZ reporter, pdh (2), and the degree of repression conferred by the FNR variants was estimated by measuring β-galactosidase activity as above. As anaerobic ndh-lacZ expression of fnr strains is enhanced during growth on rich medium (6), the effect of the FNR variants on ndh-lacZ expression was also determined following growth on a glucose minimal medium supplemented with leucine (12) and appropriate antibiotics. Similarly, two simple FNR-activated promoters, the Class I FNR-activated reporter (pBR222) and Class II FPNR (FNR site at −71.5) and Class II FPNR (FNR site at −41.5), as well as pSyl promoter mutants with impaired FNR sites at either −93.5 (pSylD−/−; pGS1062) or −40.5 (pSylD+/−; pGS1063) fused to lac in plasmid pBR322 were used to determine the effects of the selected amino acid substitutions on FNR-mediated activation (9, 13). Western blotting of the soluble fraction of sonic cell-free extracts with polyclonal anti-FNR serum has been described previously (14). The relative amount of each FNR variant was estimated by qualitative densitometry using a Vilber-Lourmat imaging system.

Partial Proteolysis—The FNR protein was purified from a glutathione S-transferase-FNR fusion protein as described (15). Aliquots (20 µl, 3.4 mg/ml) of the isolated FNR were incubated at 30 °C for up to 2 h in the presence of either 0.68 unit of trypsin (Sigma), 1 unit of chymotrypsin (Sigma), or 0.1 unit of V8 protease (Sigma). The peptides generated were then fractionated by SDS-polyacrylamide gel electrophoresis and measured using a Vilber-Lourmat imaging system.

RESULTS

Identification of Repression Defective FNR Variants—Previous attempts to isolate repression defective FNR variants using the FNR-repressible ndh promoter as a screen were unsuccessful, yielding only FNR proteins with reduced affinity for their DNA target and therefore compromised in both activation and repression (16). However, recent analysis of the regulation of yfiD expression in E. coli (9) has provided an opportunity to develop a better screen. The yfiD gene has an unusual promoter architecture for an FNR-activated gene, with two FNR sites centered at −40.5 and −93.5 relative to the transcript start. Multiple FNR sites are usually found in FNR-repressed promoters; for example, the ndh gene has FNR sites centered at −50.5 and −94.5 (1). Expression of yfiD is dependent upon an activating FNR dimer centered at −40.5, but occupation of the upstream FNR site (−93.5) down-regulates expression (9). Therefore, screening a library of randomly mutagenized FNR genes for those that allow increased yfiD-lac expression (i.e. FNR variants that still activate from position −40.5, but fail to act as repressors −93.5) should ensure that the FNR protein tested is not compromised in DNA binding activity.

Error-prone polymerase chain reaction mutagenesis was used to generate a library of randomly mutated fnr genes in pBR322. Transformants of JRG3701 (Δfnr Δlac, containing a compatible yfiD-lacZ reporter plasmid, pGS1000) were screened for elevated yfiD-lac expression on 5-bromo-4-chloro-3-indolyl β-galactoside plates. Approximately 4000 colonies were screened, and 19 were found to contain plasmids that enhanced yfiD expression (Table 1). Estimation of the β-galactosidase activities of anaerobic cultures indicated that all of the FNR variants encoded by the plasmids were defective in the down-regulation of yfiD expression. Most of the FNR variants were produced in normal amounts, as judged by Western blotting (Table 1). Expression of fnr is autoregulated, partially by binding at a site overlapping the transcript start such that FNR acts as a molecular brick. Hence, the amount of each FNR variant may reflect their relative affinities for this site as well as any effects that the particular substitutions may have on protein stability. Even those produced at lower levels were expressed well enough for the screening protocol, because, although chromosomal expression yielded least FNR (<5% of that obtained with pGS24), it was still sufficient to allow regulation of yfiD expression comparable with that observed with multicopy fnr (Table 1). This reflects the relative abundance of the reporter plasmid (2–5 copies per cell) compared with the fnr-encoding plasmid (15–20 copies per cell). Therefore, the failure to down-regulate yfiD is probably not due to a lack of FNR in the cell.

Nucleotide sequence analysis revealed that most of the FNR variants encoded by the plasmids contained substitutions in the face of FNR that contains AR1 (Table 1, Fig. 1A). The only exception was the variant M147T (which was isolated twice) representing a replacement buried in the dimer interface (helix oα). There is no obvious reason why such a substitution should result in the properties observed; however, it should be noted that the M147T variant still contained significant repressing ability (Table 1). Many of the substitutions (K54E, Y69C, R72L, G74C, F92S, A95P) were clustered in a series of loops that form the AR1 side of the FNR β-roll. A second cluster was evident in the region encompassing oα to α5 (S178F, F191L, L193P, R197H). Four variants (G74C, F92S, A95P, and L193P) had been identified in a previous screen, and it was suggested that the defect in yfiD down-regulation was due to an altered AR1-containing surface in these variants (9). Five of the 17 variants identified contained two substitutions, but in each case at least one of the replacements was of a residue predicted to be close to or part of the AR1-containing surface.

The pBR322 derivatives encoding FNR variants defective in yfiD down-regulation were transferred into JRG3917 (Δfnr Δlac, containing a compatible FNR-repressed ndh-lacZ reporter, pdh; Ref. 2). All the variants failed to repress ndh expression normally indicating that the yfiD and ndh promoters may share a common mechanism of FNR-mediated repression (Table 1). As anaerobic expression of ndh is known to respond to nutrient quality in fnr strains (6), ndh-lacZ expression was also determined for cultures grown in a defined minimal medium (Table 1). The data obtained confirmed the FNR repression defects observed in rich medium. Tests with equivalent strains carrying the FNR-activated Class II FPNR- lacZ reporter plasmid, which should be substantially unaffected by substitutions in AR1, indicated that all but four of the seventeen variants were capable of activating transcription. It was expected that all the variants would be competent activators of Class II promoters, because the basis of the original screen depended on FNR activation from a site at −40.5 in the yfiD promoter. Therefore, the response of the simple Class II promoter FPNR defines two types of FNR variant: Type A, which fails to repress but activates normally, and Type B, which has both repression and activation defects. This assignment was confirmed using a yfiD−/− reporter (pGS1062) with an impaired FNR site at −93.5 (Table 1). The Type B variants exhibited much reduced activity at this promoter compared with the Type A proteins. Studies with the Class I promoter FF-71.5pmelR, which requires an AR1-o contact for activation, indicated that the Type A substitutions could be neutral for (R197H), or improve (K54E, A95P) or impair (I45T, Y69C, S133P, G74C, M147T, L193P, L239P, L239E), the AR1 contact. However, any improvement in the AR1 contact was insufficient...
Expression of β-galactosidase driven by the indicated FNR variants was measured in JRG1728 (ΔlacΔfur) transformed with ppyfID, pndh, FPpemelR, or FF-71.5pemelR fused to lac. Values (which varied by no more than 10%) are the mean of duplicate assays of at least two independent anaerobic cultures of each strain, grown on rich medium in sealed bottles at 37 °C for 16 h. Expression of ndh-lacZ was also determined in minimal medium to eliminate the enhancement of ndh expression associated with anaerobic growth on rich medium in the absence of FNR. Aerobic expression from the test promoters for all the variants was similar to that observed for FNR: 100 Miller units for ppyfID; 2100 for pndh; 110 for ppyfIDΔ/+; 150 for FPpemelR; and 150 for FF-71.5pemelR. Expression of each variant was assessed by Western blotting using anti-FNR serum and is given as a percentage (+20%) of that observed with cultures expressing fnr from an equivalent plasmid (pGS24, row labeled FNR). The figures in parentheses indicate the number of independent isolates.

| FNR variant | Codon change | Expression Anaerobic β-galactosidase activity |
|-------------|---------------|------------------------------------------|
| Δfur        | S13P          | 70%                                      |
|             | S145N         | 70%                                      |
|             | I45T          | 70%                                      |
|             | K54E          | 70%                                      |
|             | G74C          | 70%                                      |
|             | A95P          | 70%                                      |
|             | M147T (2)     | 70%                                      |
|             | Y69C          | 70%                                      |
|             | S133P         | 70%                                      |
|             | F191L (2)     | 70%                                      |
|             | L193P         | 70%                                      |
|             | R197H         | 70%                                      |
|             | L239P         | 70%                                      |
|             | K164E         | 70%                                      |
|             | L239E         | 70%                                      |
|             | Stop-14       | 70%                                      |
|             | L34P          | 70%                                      |
|             | R72L          | 70%                                      |
|             | L193P         | 70%                                      |
|             | F92S          | 70%                                      |
|             | S178F         | 70%                                      |

Substitutions Effecting FNR-mediated Repression Are Located in Surface-exposed Regions—There is no structure available for FNR, but there is evidence to indicate that the predicted similarity with the cAMP receptor protein is well founded (1). An essential feature of any regions of FNR involved in contacting other components of the transcription machinery is that they must be solvent exposed. Partial proteolysis of isolated FNR with trypsin (28 possible cleavage sites, to allow activation from a yfID reporter with an impaired FNR site at −40.5, yfIDΔ/+ (not shown). Furthermore, the variants G74C and L193P, which activated the Class II promoter well, but were poor at Class I, indicated that the anti-inhibition contact made between the RNA polymerase α subunit and the AR1 face of FNR (required at Class II promoters) is different to the activating AR1α subunit contact made at Class I promoters.

**Fig. 1.** Positions of amino acid replacements that compromise FNR-mediated repression and locations of solvent exposed regions of FNR. A, predicted structure of an FNR monomer based on that of the cAMP receptor protein showing the positions of amino acid replacements that impair FNR-mediated repression of ndh-lacZ and yfID-lacZ expression. The helix-turn-helix motif (αD-αF) in the DNA-binding domain, the essential cysteine residues that act as ligands for the [4Fe,4S] cluster (ringed), and the previously identified activating regions AR1 and AR3 (or 85-loop) are also indicated. B, surface-exposed regions of FNR. Predicted structure of an FNR monomer showing the sites of cleavage by trypsin and V8 protease.
**TABLE II**

Partial proteolysis of FNR

Polypeptide products identified following digestion of FNR (68 μg) with trypsin (a) or V8 protease (b). The FNR protein used was FNR-572 released from a glutathione S-transferase-FNR fusion protein (15) as a result, the isolated FNR protein contains an additional 15 N-terminal amino acids (GSPGISGGGGGILDS). Lowercase letters indicate that the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence; – indicates that the sequence corresponds to the N-terminal of the amino acid residue was not detected but is from the known sequence.

| Band and apparent M<sub>r</sub> | N-terminal sequences | Cleavage position |
|-----------------------------|---------------------|------------------|
| (a)                         |                     |                  |
| 1 37,000                    | GSPGIS              | –                |
| 2 36,000                    | gspGISG             | –                |
| 3 32,000                    | GSPGISGG            | –                |
| 4 28,000                    | GSPGISGGG           | –                |
| 5 25,000                    | SYTITEQG            | Tyr<sup>79</sup> |
| 6 22,500                    | SYTITEQGDEQITGF     | Lys<sup>77</sup> |
| 7 21,000                    | HLAGdLVG            | Phe<sup>92</sup> |
| 8 17,000                    | SYTITEQGDEQITGF     | Lys<sup>77</sup> |
| 9 14,500                    | GSPGISGGGGGGLDS     | –                |
| 10 13,000                   | GSPGISGGGGGGLDS     | –                |
| (b)                         | SYTITEQGDEQITGF     | Lys<sup>77</sup> |
| 1 33,000                    | KRIIRRI             | Glu<sup>4</sup> |
| 2 28,000                    | LDQLDLN1ERKKP1Q     | Glu<sup>48</sup> |
| 3 17,000                    | KRIIRICoG           | Glu<sup>17</sup> |
| 4 15,500                    | KRIIRIQSGG          | Glu<sup>17</sup> |

**DISCUSSION**

Transcription can be repressed either passively by promoter occlusion, i.e. when a regulator blocks access of RNA polymerase to the promoter, or actively, in which the regulator makes direct contact with RNA polymerase to inhibit transcription initiation (17). The observation that FNR and RNA polymerase can simultaneously interact with the FNR-repressible ndh promoter suggested that, in this case, repression is unlikely to be mediated simply by promoter occlusion (6, 7). The ndh promoter has two FNR sites centered at 50.5 and 94.5, and it has been proposed that FNR occupation of these sites prevents RNA polymerase α subunit from interacting with DNA (7). Such a repression mechanism may require direct protein contacts between the two FNR dimers and/or FNR and RNA polymerase. Previous attempts to identify FNR variants compromised for repression of ndh-lacZ were unsuccessful, because the transformants recovered contained plasmids encoding FNR proteins with defects in DNA binding (16). However, the yfdD-lacZ reporter provided an opportunity to screen out FNR variants defective in DNA-binding, because this promoter, although down-regulated by FNR occupation of a site centered at 95.5, requires an activating FNR dimer (at 40.5) for expression.

Using yfdD-lacZ as an initial screen 17 FNR variants defective in repressing both yfdD and ndh promoters were identified. All but one of the variants (M147T) had substitutions near or within the face of FNR that contains AR1 (Fig. 1), a region of the protein known to contact the α subunit of RNA polymerase and thereby facilitating transcription activation (3). Two types of contact can be made depending on the architecture of the
activated promoter. At Class I promoters (FNR site at or beyond −61) AR1 of the downstream FNR monomer makes an activating contact with the α subunit, whereas at Class II promoters (FNR site at or about −41) AR1 makes an anti-inhibition contact (3). It is now apparent that the same face of FNR can be involved in repressing transcription at promoters that contain multiple FNR sites and that the regions involved are solvent exposed. This is supported by the observations that (i) the positions of two substitutions (Arg72 and Phe191, indicated in yellow on Fig. 2) are common to FNR variants with altered AR1 or repression properties; (ii) all the repression defective variants (with the exception of the M147T) contain a altered AR1 or repression properties; (iii) most of the repression defective variants display altered activation from a model Class I (AR1-dependent) promoter (Table I); and (iv) that several amino acids close to those substituted in repression defective variants (Leu34, Gly74, Phe92, Ser178) are sufficiently exposed to allow proteolytic attack (Glu18, Lys77, Phe92, and Arg184). Thus, the simplest explanation for the repression defective phenotype displayed by the FNR variants is that they possess an altered surface that is different from but overlaps AR1 which has a role in repressor of promoters with multiple FNR sites.

It has been suggested recently that transcription regulators can be viewed as catalysts (20). It is envisaged that activators lower the activation energy associated with one or more steps in the reaction pathway leading to transcription initiation, whereas repressors could increase the energy barriers to be overcome in forming an open complex, thereby inhibiting transcription initiation (20). Thus, it is possible that the specific configurations of two FNR dimers at the ndh and yfiD promoters effectively jam RNA polymerase in one of the intermediate states between the closed and open complex. Indeed, FNR-mediated inhibition of open complex formation at the ndh promoter has been observed previously (6). The data presented here suggest that this could be achieved by direct protein–protein contacts involving the face of FNR containing AR1.

The architecture of a promoter is clearly crucial in determining the effect of a particular regulator (or combination of regulators) on transcription (21). It is not yet established if the repression specific components of the AR1 face participate in FNR-FNR or FNR-RNA polymerase interactions, but it is likely that the various contacts made by the AR1 containing face are subtly different. Indeed, there is good evidence to suggest that, for the cAMP receptor protein the anti-inhibition and activating contacts made by AR1 are different (22). The GalR protein is perhaps the best example of an “active” repressor (20). Like FNR, GalR can act either as an activator (of gal promoter 2, galP2) or as a repressor (of gal promoter 1, galP1) and a characteristic GalR-RNA polymerase-gal ternary complex is formed at each promoter because of putative GalR-RNA polymerase interactions. Mutations in the region of the C-terminal domain of the α subunit of RNA polymerase thought to be involved in GalR-mediated activation also relieve GalR-mediated repression (20). However, the activator complex at galP2 is an open complex, whereas the repressing complex at galP1 is a closed complex and thus the context of the GalR-RNA polymerase contacts are different. These context effects are proposed to be sufficient to allow a single regulatory protein to act as both a repressor and an activator while maintaining similar regulator-polymerase contacts (20). Therefore, it is suggested that the context in which FNR finds itself at the yfiD and ndh promoters favors the formation of a ternary complex incorporating FNR-polymerase contacts that render the complex incompetent for transcription activation.

In conclusion, the FNR variants identified here provide the first indication that specific regions (amino acids) of FNR that overlap AR1 may be required for transcription repression at promoters with multiple FNR sites. Mutational analysis of the C-terminal domain of the RNA polymerase α subunit should determine whether FNR can repress transcription via direct contact between the AR1 containing face and RNA polymerase or whether FNR-FNR contacts are the key to FNR-mediated repression at promoters sharing the ndh architecture.

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