NOTES

Ndd, the Bacteriophage T4 Protein That Disrupts the Escherichia coli Nucleoid, Has a DNA Binding Activity

JEAN-YVES BOUET,† HENRY M. KRISCH, AND JEAN-MICHEL LOUARN*  
Laboratoire de Microbiologie et de Génétique Moléculaire du CNRS, Toulouse, France

Received 8 January 1998/ Accepted 23 July 1998

Early in a bacteriophage T4 infection, the phage ndd gene causes the rapid destruction of the structure of the Escherichia coli nucleoid. Even at very low levels, the Ndd protein is extremely toxic to cells. In uninfected E. coli, overexpression of the cloned ndd gene induces disruption of the nucleoid that is indistinguishable from that observed after T4 infection. A preliminary characterization of this protein indicates that it has a double-stranded DNA binding activity with a preference for bacterial DNA rather than phage T4 DNA. The targets of Ndd action may be the chromosomal sequences that determine the structure of the nucleoid.

Within a few minutes after bacteriophage T4 infection, the spatial distribution of the Escherichia coli chromosome is dramatically altered (12, 13). The T4 ndd gene is responsible for this nuclear disruption phenomenon (17, 18), which converts the large, central nucleoid into numerous small DNA globules on the inner membrane (4). The highly basic 17-kDa Ndd protein has no significant homology with any other known protein (5). About 4,000 Ndd molecules are produced by T4-infected cells (11), but significantly lower levels of expression of the cloned ndd gene are nonetheless lethal to E. coli and induce a slow disorganization of the nucleoid (4). Ndd protein has little effect on bacterial gene expression but inhibits replication apparently by generating on the chromosome obstacles to progression of replication forks (4). Even after Ndd has caused extensive cell killing and disorganization of the nucleoids, no bacterial DNA cleavage or degradation is detected nor is the SOS system induced (4). These observations indicate that only the architecture of the nucleoid is affected and suggest that Ndd might interact directly with elements that determine the conformation and the location of the bacterial chromosome within the cell.

In this communication, we report that a high level of cloned ndd gene expression induces nuclear disruption as rapidly and completely as T4 infection, thus indicating that Ndd is the only T4 protein required for nucleoid disruption. Extracts from cells that overexpress Ndd protein were used to study Ndd activity in vitro. We present evidence that Ndd is a DNA binding protein with some specificity for sequences located on the bacterial chromosome.

Cloning a thermosensitive ndd allele under the control of a T7 promoter. Attempts to clone the wild-type ndd gene in pET11a vector (Stratagene) under the control of the tightly regulated T7 promoter (19) were unsuccessful, even in a host that did not carry the T7 RNA polymerase gene. A few Ndd protein molecules were probably made from this plasmid, and they sufficed to kill E. coli (4). However, we could clone the temperature-sensitive nddts2 allele (4, 15) under the control of this T7 promoter, provided the transformed cells were maintained at 42°C, a nonpermissive temperature for Nddts2 protein. The resulting plasmid, pJYB41, was then introduced into strain BL21(lDE3), which carries the T7 RNA polymerase gene under the control of the lac promoter so that the addition of IPTG (isopropyl-β-D-thiogalactopyranoside) induces T7 RNA polymerase synthesis. This strain (LN3243) now produces large amounts of soluble Nddts2 protein after a shift down to 30°C and addition of IPTG. The synthesis of the Nddts2 protein can be detected soon after induction by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and by 60 min, Nddts2 accounts for about 8% of the total protein stained with Coomassie blue (data not shown).

Overproduction of Nddts2 induces a rapid and complete disruption of the nucleoid. When Nddts2 protein is produced by LN3243 bacteria, a rapid disruption of the nucleoid occurs. This is illustrated in Fig. 1, where the DNA of such cells was stained with the fluorescent dye DAPI (4′,6-diamidino-2-phenylindole) (8) and photographed by fluorescence microscopy (as described previously [4]). Clearly, Nddts2 production for just 10 min is sufficient to cause the redistribution of the DNA from the center of the cells to globules at the periphery of the inner membrane (compare the blue color distributions in Fig. 1B and D). Thus, a high level of Ndd provokes the rapid and complete disruption of the nucleoid, and no other T4 gene products are necessary. This correlates well with the fact that nucleoid disruption during wild-type T4 infection is achieved within a few minutes (18), when about 4,000 molecules of Ndd per cell are produced (11), and further suggests that Ndd be required to act at many sites simultaneously to bring about the destruction of the nucleoid. The previous observation that induction of Ndd expression produced only slow nuclear disruption is explained by the lower level of the protein in these experiments compared to that present after T4 infection (4).

Ndd binds to double-stranded DNA (dsDNA) but not to single-stranded DNA (ssDNA). The highly basic composition

* Corresponding author. Mailing address: Laboratoire de Microbiologie et de Génétique Moléculaire du CNRS, 118, route de Narbonne, Toulouse 31062 Cédex, France. Phone: (33) 561-33-59-64. Fax: (33) 561-33-58-86. E-mail: louarn@ibcg.biotoul.fr.
† Present address: Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada.
were digested with *E. coli* DNA. Ampicillin-containing LB medium (100 μg/ml) was added, and growth was allowed to continue at 42°C for 30 min. Cells were then shifted to 30°C by dilution with cold medium and the concomitant addition of 1 mM IPTG. Ten minutes later, the cells were chilled on ice and observed by use of phase-contrast or fluorescence microscopy with a Leica DMRB microscope. (A) LN3245 cells, phase contrast; (B) LN3245 cells, fluorescence; (C) LN3243 cells, phase contrast; (D) LN3243 cells, fluorescence.

![Image](http://jb.asm.org/)

**FIG. 1.** Complete disruption of the chromosome by Nddts2. Strains LN3243 (Nddts2 producer) and LN3245 (control) were grown at 42°C to an OD₅₄₀ of 0.2. DAPI (2 μg/ml) was added, and growth was allowed to continue at 42°C for 30 min. Cells were then shifted to 30°C by dilution with cold medium and the concomitant addition of 1 mM IPTG. Ten minutes later, the cells were chilled on ice and observed by use of phase-contrast or fluorescence microscopy with a Leica DMRB microscope. (A) LN3245 cells, phase contrast; (B) LN3245 cells, fluorescence; (C) LN3243 cells, phase contrast; (D) LN3243 cells, fluorescence.

![Image](http://jb.asm.org/)

**FIG. 2.** Filter binding assay for Ndd DNA binding activity and specificity for *E. coli* DNA. *E. coli* DNA (circles) or T4 cytosine-containing DNA (squares) were digested with *Sau3AI* prior to end labeling with ³²P. Increasing amounts of these DNAs were incubated at room temperature for 20 min with Ndd-containing LN3243 (solid lines) or control LN3245 (dotted lines) protein extracts (15 μg of total protein) in 500-μl reaction mixtures containing 250 μl of 2× DNA binding buffer (40 mM Tris-HCl [pH 7.5], 20% glycerol, 100 mM KCl, 0.2 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 20 mM EDTA). Reaction mixtures were filtered through GF/C filters (Whatman). The filters were washed three times, dried, and immersed in liquid scintillation cocktail (Ready-Safe; Beckman). The DNA retained on each filter was monitored by scintillation counting (Beckman LS3801). The fraction of DNA retained is plotted as a function of the amount of DNA incubated in the reaction mixture.

![Image](http://jb.asm.org/)

**FIG. 3.** 3²P-end-labeled *Sau3AI* *E. coli* (from strain PB4144) genomic fragments were incubated with such extracts. Compared with control extracts, the Ndd-containing extracts had a 25- to 30-fold-increased capacity to retain this DNA (Fig. 2). If the reaction mixture was incubated at 44°C instead of room temperature, the DNA retained by Ndd2ts extract was reduced 3.2-fold. That the DNA binding activity of Nddts2 mutant protein is thermosensitive in vitro is also consistent with this DNA binding activity being involved in vivo nucleoid disruption by Ndd2ts, which is also thermosensitive in this mutant. When denatured DNA (*E. coli* genomic DNA heated at 100°C followed by a rapid cooling on ice to produce single-stranded DNA) was used in this binding assay, DNA retention was very low and no difference could be detected between the Ndd-containing and control extracts (data not shown). The absence of binding to single-stranded DNA argues strongly against Ndd having a nonrelevant DNA binding activity simply due to its basic character. The double-stranded DNA binding activity detected in cell extracts when the *ndd* gene is overexpressed is most likely a property of the Ndd protein itself, and this activity is probably involved in the Ndd effect on nucleoid structure.

Ndd has no endonuclease activity, and its binding to dsDNA is insensitive to topology. Upon incubation of purified covalently closed circular (CCC) or open circular (OC) plasmid DNA (pFGB68; kindly provided by F. Boccard) with Ndd2ts-containing extracts, neither interconversion between these species nor their transformation to linear DNA was detected (Fig. 3, lanes i and j). Thus, binding of Ndd to DNA is not accompanied by single-strand or double-strand cleavage. This result is consistent with the absence of DNA degradation observed in vivo after *ndd* gene expression induction (4).

OC and linear (L) forms were generated from supercoiled DNA of plasmid pFGB68. Mixtures of CCC, OC, and L forms were incubated with either a Ndd2ts-containing or a control extract and then filtered through GF/C filters. The DNA retained by the filters was subsequently eluted by soaking in 0.2% SDS at 30°C for 2 h, depolymerized by phenol-chloroform treatment, and analyzed by gel electrophoresis. CCC, OC, and L forms were present in the reextracted material in the same proportions as those in the original mixture (Fig. 3, compare lanes d to g with lane c). From this ensemble of data, we conclude that, in vitro, Ndd binding is not sensitive to the topological state of the dsDNA. The plasmid used, pFGB68, is a pUC19 derivative into which is cloned a 1.8-kb *E. coli* genomic segment that contains a large bacterial interspersed mosaic element (2, 9) with five natural repeats of repetitive extragenic palindromes. The repetitive extragenic palindromic...
DNA was electrophoresed in a 0.8% agarose gel (1 × TAE buffer [15]) to separate its supercoiled (CCC), OC, and L forms. These forms, either alone or mixed in known amounts, were incubated with extracts and subjected to the retention assay. Lanes a and m, DNA ladder in kilobases (Bethesda Research Laboratories); lane b, CCC plus OC DNA mixture; lane c, CCC, OC, and L DNA mixture; lanes d to g, the same DNA mixture as that in lane c, but incubated with Nddts2 extracts (in increasing amounts from lane d to g), filtered on GF/C membranes, eluted from the filter with 0.5% SDS, precipitated with ethanol, and dissolved in Tris-EDTA buffer prior to electrophoresis; lane h, same as that in lane f, but incubated with control extract; lanes i to l, CCC and OC DNA incubated with Nddts2 extract (lanes i and j) or control extract (lanes k and l) and then treated as described for lane d. A DNA size scale (in kilobases) is indicated on the right. Positions of CCC, OC, and L forms of pGFB68 are indicated on the left. N, Nddts2-containing extract; C, control extract.

FIG. 3. Ndd binds equally to different forms of dsDNA. Plasmid pGFB68 DNA was electrophoresed in a 0.8% agarose gel to separate its supercoiled (CCC), OC, and L forms. These forms, either alone or mixed in known amounts, were incubated with extracts and subjected to the retention assay. Lanes a and m, DNA ladder in kilobases (Bethesda Research Laboratories); lane b, CCC plus OC DNA mixture; lane c, CCC, OC, and L DNA mixture; lanes d to g, the same DNA mixture as that in lane c, but incubated with Nddts2 extracts (in increasing amounts from lane d to g), filtered on GF/C membranes, eluted from the filter with 0.5% SDS, precipitated with ethanol, and dissolved in Tris-EDTA buffer prior to electrophoresis; lane h, same as that in lane f, but incubated with control extract; lanes i to l, CCC and OC DNA incubated with Nddts2 extract (lanes i and j) or control extract (lanes k and l) and then treated as described for lane d. A DNA size scale (in kilobases) is indicated on the right. Positions of CCC, OC, and L forms of pGFB68 are indicated on the left. N, Nddts2-containing extract; C, control extract.

Ndd has a higher affinity for E. coli DNA than for T4 DNA. Since during the infectious cycle the replicating phage DNA occupies a central position within the cell, T4 DNA must be preferentially bound by Ndd to CCC and OC DNA mixture; lanes d to g, the same DNA mixture as that in lane c, but incubated with Nddts2 extracts (in increasing amounts from lane d to g), filtered on GF/C membranes, eluted from the filter with 0.5% SDS, precipitated with ethanol, and dissolved in Tris-EDTA buffer prior to electrophoresis; lane h, same as that in lane f, but incubated with control extract; lanes i to l, CCC and OC DNA incubated with Nddts2 extract (lanes i and j) or control extract (lanes k and l) and then treated as described for lane d. A DNA size scale (in kilobases) is indicated on the right. Positions of CCC, OC, and L forms of pGFB68 are indicated on the left. N, Nddts2-containing extract; C, control extract.

sequence contains inverted repeats that could be extruded as cruciforms from the negatively supercoiled plasmid DNA. If Ndd had a preferential affinity for such cruciforms, as has been found for the HU DNA binding protein (3, 14), preferential binding of Ndd to CCC pGFB68 DNA should have been detected.

What is the exact specificity of Ndd-DNA interactions? What are the components of the nucleoid with which Ndd interacts? How can these interactions destroy the architecture of the chromosome so rapidly? Answering these questions could provide significant insights into the organization of the bacterial chromosome.

We thank Koryn Péral and François Cornet for micrographs and for help in preparing the figures and D. Lane for careful reading of the manuscript and valuable comments.

J.-Y.B. acknowledges the Association pour la Recherche contre le Cancer (ARC) for a fellowship. This work was supported in part by an ATP “Virologie” from the Ministère de l’Education Nationale.

REFERENCES