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Identity of the 17-Kilodalton Protein, a DNA-Binding Protein from *Escherichia coli*, and the *firA* Gene Product

REIN AASLAND,[1]* JACK COLEMAN,[2] ASKILD L. HOLCK,[1]† CASSANDRA L. SMITH,[3] CHRISTIAN R. H. RAETZ,[2] AND KJELL KLEPPE[1]‡

Laboratory of Biotechnology, University of Bergen, N-5029 Bergen, Norway[1]; Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706[2]; and Departments of Microbiology and Psychiatry, College of Physicians and Surgeons of Columbia University, New York, New York 10032[3]

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The 17-kilodalton protein, a DNA-binding protein encoded by the *skp* gene of *Escherichia coli*, was found to be identical to histonelike protein I, the product of the *firA* gene. This conclusion was reached after chromosomal localization, using the recently constructed high- and low-resolution *E. coli* restriction maps, and by direct comparison of the N-terminal amino acid sequence of histonelike protein I and the 17-kilodalton protein.

A number of low-molecular-weight, DNA-binding proteins with properties similar to those of the eucaryotic histones have been found in bacteria (for review, see reference 3). Convincing evidence that these proteins actually fold the bacterial DNA into nucleosomelike structures in vivo is, however, lacking. Several of the histonelike proteins have molecular sizes of 16 to 17 kilodaltons (kDa). Among these are the basic proteins histonelike protein I (HLP-I) (FirA) (12, 14) and the 17-kDa protein (7), as well as the neutral proteins known as H-NS (10), H1a (2, 9, 19), and B1 (20). It has been suggested that the latter three proteins are identical (9). In this report, we present evidence that the 17-kDa protein is identical to HLP-I (FirA).

The 17-kDa protein, which is a constituent of bacterial chromatin (6, 17; A. L. Holck, unpublished data) has been purified and characterized, and its N-terminal amino acid sequence has been determined (7). Subsequently, the *skp* gene encoding the 17-kDa protein was isolated and sequenced (5). The protein has an isoelectric point of about 9.5 and behaves as a tetramer upon gel filtration. The protein binds to DNA with moderate affinity and is present in exponentially growing cells at about 4,000 monomeric molecules per cell. The amino acid sequence of the *skp* gene revealed the presence of a putative signal sequence. However, only trace amounts of the protein were found in membrane preparations. The protein did not affect in vitro transcription when either lambda or φX174 DNA was used as a template. In contrast, mutations in *firA* affect transcription and render rifampin-resistant *rpoB* mutants sensitive. It is therefore thought that the FirA protein (HLP-I) interacts with RNA polymerase (12). Furthermore, unlike HLP-I, the 17-kDa protein is heat labile and unable to bind to DEAE-Sepharose (7). These observations led us to conclude that the 17-kDa protein was different from HLP-I. It is possible, however, that these discrepancies were due to the different protocols used for purification of the 17-kDa protein (7) and HLP-I (12).

Location of the *skp* gene on the physical map of the chromosome. The approximate location of the *skp* gene on the *Escherichia coli* K-12 EMG2 chromosome was identified by taking advantage of the low-resolution *Not*I restriction map (18). A plasmid containing the *skp* gene was used as a probe in a hybridization experiment with chromosomal DNA digested by *Not*I and *Sfi*I and separated by pulsed-field gel electrophoresis (Fig. 1). The results showed that the gene resides on the *Not*I D fragment and the *Sfi*I D fragment (275 and 345 kilobases, respectively), both mapped in the 0- to 6-min region of the chromosome (18; C. L. Smith, unpublished data).

The *skp* gene was more precisely mapped by using the high-resolution restriction map of *E. coli* K-12 W3110 (8). Restriction site data were generated by using the *skp* probe in hybridization experiments with DNA from *E. coli* B, using the eight enzymes that were used by Kohara et al. (8). These data did not conform exactly to any region between 0 and 6 min. However, an extremely strong similarity was seen in the 210- to 220-kilobase region on this map, corresponding to about 4 min on the genetic map. The restriction map of this region closely resembled the recently refined map of the 4-min region (1) (Fig. 2). The most prominent discrepancy between these sets of data was the absence of a *Pst*I site in the *lpxA* gene in the map of Kohara et al. (8). This region is also known to contain the *firA* gene (11). These results help validate the similarities in the low- and high-resolution restriction maps even though the maps were made with use of two different *E. coli* K-12 strains and the *skp* gene was isolated from the even more distantly related *E. coli* B strain.

Identity of the *skp* and *firA* genes. The N-terminal amino acid sequence of the purified FirA protein (HLP-I) was recently determined (1). The *firA* sequence is identical to the N-terminal amino acid sequence of the mature 17-kDa protein (7). Furthermore, the nucleotide sequence of a 155-base-pair *Pst*I fragment of λ *fir*-24 (J. Coleman, unpublished data) is identical to nucleotides 44 through 198 in the *skp* sequence (5). Thus, by the same criteria by which HLP-I was identified as the *firA* gene product (12), the 17-kDa protein was found to be the product of the *firA* gene. Work in one of our laboratories on the genes involved in lipid A biosynthesis led to the identification of an open reading frame immediately upstream of *lpxA*. This open reading frame is capable of

* Corresponding author.
† Present address: Norwegian Food Research Institute, Osloveien 1, N-1430 Ås, Norway.
‡ This paper is dedicated to the memory of Kjell Kleppe, who died on 19 June 1988.

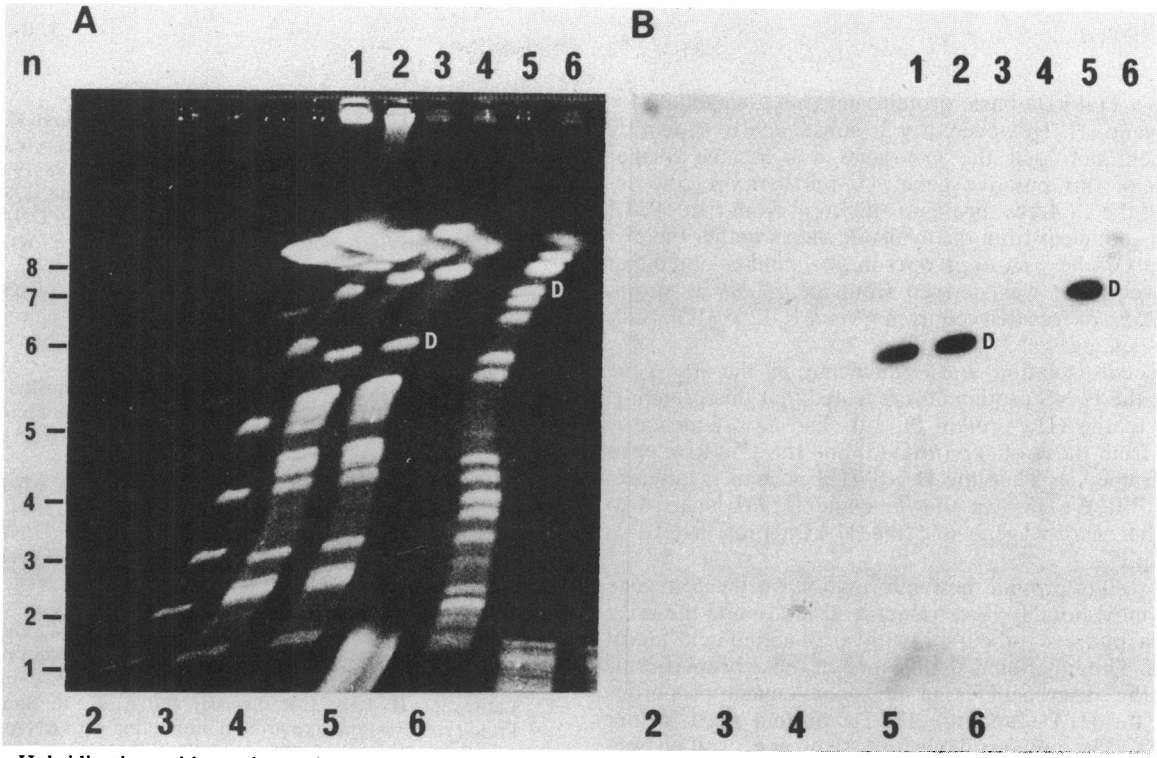

FIG. 1. Hybridization with a *skp* probe to a blot of *Not*I- and *Sfi*I-digested *E. coli* EMG2 DNA separated by pulsed-field gel electrophoresis. Lanes: 1, some of the smaller yeast chromosomes; 2, linear concatemers of lambda cI857 DNA starting from the 48.5-kilobase monomer (n = 1); 3 and 4, 5 and 6, total digests of chromosomal *E. coli* DNA with *Not*I and *Sfi*I, respectively. The DNA in lane 5 was degraded during preparation. (A) Image of DNA stained with ethidium bromide after separation by pulsed-field gel electrophoresis as previously described (18). (B) Autoradiogram of the blotted DNA after hybridization to ^{32}P-labeled pGAH317 (5) containing the cloned *skp* gene (corresponding to the 2.3-kilobase left half of the segment shown in Fig. 2B). Hybridization was carried out as described by Maniatis et al. (15) in the presence of 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and 8.25% dextran sulfate at 42°C with the plasmid radiolabeled by the random-primer technique (4). The blot was stringently washed in 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C for 30 min. The hybridizing *Not*I D and *Sfi*I D fragments are indicated (D).

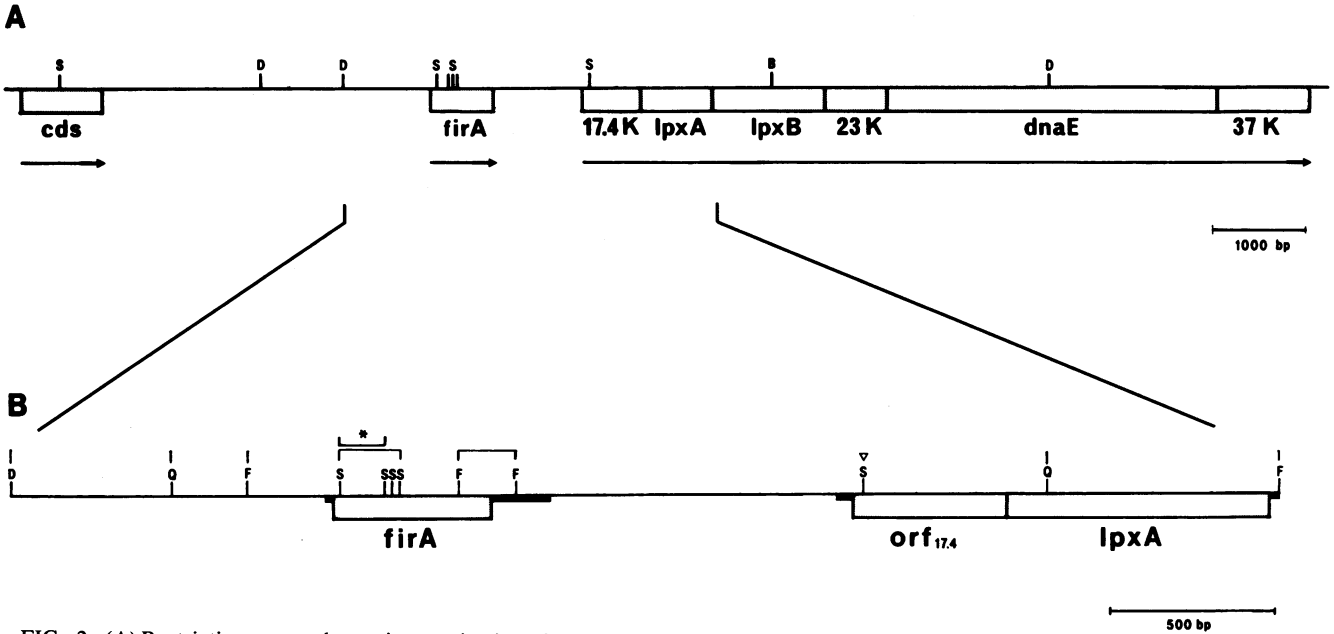

FIG. 2. (A) Restriction map and genetic organization of the 4-min region of the *E. coli* genome as previously described (1). Arrows indicate the direction of transcription. The three open reading frames are indicated by the molecular sizes (in kilodaltons [K]) of the putative proteins. (B) A more detailed restriction map of the *firA-lpxA* region. The data represented by bars below letters are consistent with data obtained by hybridization with pGAH317 to *E. coli* B DNA digested with the eight enzymes used by Kohara et al. (8). The enzymes *Bam*HI, *Eco*RI, *Bgl*II, and *Pvu*II do not cut in the region shown. These data are also consistent with the published sequences including *skp* (*firA*) (5), $orf_{17.4}$ and *lpxA* (1), and flanking sequences (indicated by the thick line). The asterisk indicates the 155-base-pair (bp) *Pst*I fragment from λ *fir*-24 that was sequenced. The bars above letters indicate restriction sites corresponding to the map generated by Kohara et al. (8). Their map does not resolve the four closely spaced *Pst*I sites and the two *Eco*RV sites indicated by horizontal lines. Furthermore, the *Pst*I site indicated by the triangle is not present on their map. Transcription of the three genes is from left to right. The restriction enzymes are *Bam*HI (B), *Hin*dIII (D), *Kpn*I (Q), *Eco*RV (F), and *Pst*I (S).

encoding a 17.4-kDa basic protein and was a candidate for the *firA* gene (1). However, it was subsequently shown by complementation that the *firA* gene was located further upstream of this putative gene (1). Furthermore, the sequence of the 17.4-kDa protein is different from that of *firA* (*skp*). It is not clear from the available data whether the *firA* gene is part of the *lpxA-dnaE* operon. It should be noted that the *skp* sequence was derived from an *E. coli* B strain, whereas λ *fir*-24 was derived from *E. coli* K-12 W3110. Still, the two sequences are identical.

The recent isolation and sequencing of the *hns* gene encoding the H-NS protein (16) revealed that this protein is identical to the H1a protein (9, 10). The *hns* sequence is different from those of *skp* (*firA*) (5) and the 17.4-kDa open reading frame (1). Thus the H-NS–H1a protein is different from HLP-I. We suggest that *firA* and HLP-I be used to denote the *skp-firA* gene and the 17-kDa protein–HLP-I, respectively.

These results provide new information on the *firA* gene product, most notably its tetrameric structure and the presence of a putative precursor with a 20-amino-acid signal sequence. The functional significance of these properties as well as the discrepancies in physicochemical properties reported for HLP-I and the 17-kDa protein need further evaluation. The now-available *firA* sequence, well-characterized temperature-sensitive *firA* mutants (13, 14), and purified HLP-I and specific antisera should make it possible to pursue investigation of the anticipated role of HLP-I in transcription and its role as a histonelike protein.

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