Anomalous DnaA protein binding to the regulatory region of the *Escherichia coli* *aldA* gene

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A binding site for DnaA protein was identified in the regulatory region of the *aldA* gene of *Escherichia coli*. Specific binding was demonstrated by *in vitro* assays including filter binding as well as mobility shift in a polyacrylamide gel of the DnaA–DNA complex. In cells growing in minimal medium containing glucose, expression of β -galactosidase activity from an *aldA*–*lacZ* fusion gene was suppressed by oversupply of DnaA protein and was enhanced by reducing the free DnaA level. These results suggest that DnaA protein negatively regulates expression of the *aldA* gene under these conditions. Despite fairly strong binding, the bound DNA fragment had no consensus 9 bp DnaA binding sequence (DnaA box), and anomalous binding to an AT-rich sequence located close to the transcription start site was revealed by a footprinting experiment.

Keywords: DnaA, *aldA*, transcription, replication, *Escherichia coli*

INTRODUCTION

The DnaA protein of *Escherichia coli* is the initiator protein of chromosomal replication and binds to the five 9 bp repeats termed DnaA boxes, TTAT(C/A)CA-(C/A)A (Fuller *et al.*, 1984) or TT(A/T)TNCACA (Schaper & Messer, 1995), in the replication origin, *oriC*. Assisted by protein HU or IHF, cooperative binding of 20–40 monomers of ATP-bound DnaA protein per *oriC* stimulates duplex opening at the AT-rich 13-mer repeats in *oriC*, where several replication proteins are loaded to initiate DNA replication (Kornberg & Baker, 1992). Besides being present in *oriC*, DnaA boxes are found at many sites on the chromosome and some of them located close to promoters of genes are involved in regulation of expression of the genes (Messer & Weigel, 1997). Binding of DnaA to the promoter region mostly inhibits but in some cases stimulates transcription. In addition, binding of DnaA to some but not all binding sites located within genes terminates transcription (Messer & Weigel, 1997). DnaA binding to DnaA boxes plays an important role not only in replication initiation and

transcription control but also in the timing of initiation of replication. The chromosomal locus *datA*, which titrates a large amount of DnaA molecules, has been suggested to exert such a function by regulating the cellular concentration of free DnaA molecules during the cell cycle (Kitagawa *et al.*, 1996, 1998).

There is some uncertainty in predicting DnaA-binding activity from a nucleotide sequence, partly because it seems to be affected by some poorly characterized factor(s). It is known that a 9 bp sequence, which binds DnaA when located in certain surrounding sequences does not bind it in other places (Fuller *et al.*, 1984). In another case, weak binding has been observed of a 368 bp pBR322 fragment, which lacks strong homologies to the DnaA box (Fuller *et al.*, 1984). A relaxed consensus sequence, (T/C)(T/C)(A/T/C)T(A/C)C-(A/G)(A/C/T)(A/C), has been proposed (Schaefer & Messer, 1991), but it is not clear how many base changes from the original consensus sequence are allowed. It seems, therefore, to be necessary to characterize more actual DnaA-binding sites to establish the sequence features involved in the binding. Such studies would contribute to understanding the role of DnaA protein in the global control of gene expression and the cell cycle.

The *aldA* gene encodes an aldehyde dehydrogenase of broad substrate specificity (Hidalgo *et al.*, 1991; Limon *et al.*, 1997). The gene is induced by hydroxyaldehydes and 2-oxoglutarate, which are formed as metabolic

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intermediates of various carbohydrates and amino acids (Quintilla *et al.*, 1991). In addition, expression of the gene is activated by Crp-cAMP and is repressed under anaerobic growth conditions by ArcA-P, the phosphorylated response regulator of the ArcB/A two-component signal-transduction system (Pellicer *et al.*, 1999).

In this paper, we report that a DNA segment encompassing the *aldA* regulatory region has DnaA-binding activity. Despite a fairly strong binding, the region has no canonical DnaA box. A footprinting experiment suggested that DnaA initially interacts with an AT-rich site between the -10 promoter sequence and the transcription start point, leading to cooperative binding to a region of about 100 bp around the site at higher DnaA concentrations.

METHODS

Bacterial strains and plasmids. *E. coli* K-12 derivatives used were DH5 α [*supE44* Δ *lacU169* (ϕ 80*lacZ* Δ M15) *recA1* *endA1* *gyrA96* *thi-1* *relA1*] and CSH50 [*ara* Δ (*lac-pro*) *strA* *thi*].

Plasmid pRP270T was constructed by inserting a 261 bp *TaqI* fragment carrying the *aldA* regulatory region and a part of the coding sequence at the *SmaI* site of pUC119. pBRP270 was constructed by replacing the small *EcoRI*-*SalI* fragment of pBR322 with the *EcoRI*-*SalI* fragment of pRP270T, which carries the 261 bp *TaqI* fragment. pMAPCdSG10 (Fuller *et al.*, 1984) is a pBR322 derivative which has no DnaA boxes downstream of the replication origin. pFPMCLZ1 and pTOA50 have been described (Kitagawa *et al.*, 1996). pFCmLZ1 carries a promoter-less *lacZ* gene and was constructed by inserting a *ScaI*-*Asel* fragment of pMC1871 (Amersham Pharmacia Biotech) into the *Bsu36I* site of the mini-F plasmid pFCm (Kitagawa *et al.*, 1996). pFAL1 carries the promoter and the N-terminal three codons of the *aldA* gene fused in-frame to the *lacZ* gene on pFCmLZ1. This plasmid was made by inserting the *EcoRI*-*FokI* fragment of pRP270T into the *SmaI* site of pFCmLZ1 after treatment with the large fragment of DNA polymerase I. pROA200 (Kitagawa *et al.*, 1996) is a pUC119-based plasmid, which carries the *dnaA* gene of *E. coli* under control of the *tac* promoter in addition to the *lacI^q* gene. pROA199 was derived from pROA200 by removing an *EcoRI* fragment which contains the *dnaA* gene. pROAKK-EX (Kitagawa *et al.*, 1996) carries the chromosomal *data* region on the pACYC177-based vector pROAKK1.

Filter binding assay. Preparation of DnaA protein has been described (Kitagawa *et al.*, 1996). pRP270T was cleaved with *XbaI* and *SacI* to separate the insert fragment from the vector portion. The digest was labelled with [α -³²P]dCTP using the large fragment of DNA polymerase I. pMAPCdSG10 was linearized with *XbaI*, labelled similarly and used as a negative control. Mixtures of these three DNA fragments (0.26 nM each, 1.23–2.25 $\times 10^3$ c.p.m. fmol⁻¹) were subjected to the binding reactions with DnaA protein as described by Fuller *et al.* (1984), except that ATP (1 μ M) and sonicated salmon sperm DNA (0.5 μ g per 25 μ l) were present in the reaction mixtures. Bound DNA fragments were eluted and analysed as described by Fuller *et al.* (1984), except that radioimages were obtained and radioactive bands was quantified by a BAS 1000 Bio Imaging Analyzer (Fuji Photo Film).

Gel mobility shift assay. The DNA fragments analysed were

the 282 bp *XbaI*-*SacI* fragments of pRP270T carrying the *aldA* regulatory region and labelled at the 5' ends of the *XbaI* site using [γ -³²P]ATP and polynucleotide kinase. Labelled fragments (2500 c.p.m. per 10 fmol) were mixed with various amounts of DnaA in 6 μ l reaction mixtures containing 25 mM HEPES/KOH (pH 8.0), 6 mM magnesium acetate, 0.8 mM EDTA, 4 mM dithiothreitol, 0.01% Triton X-100, 80 μ g bovine serum albumin ml⁻¹, 80 μ M ATP, 80 μ g *E. coli* tRNA ml⁻¹ and 7% (v/v) glycerol. After incubation for 5 min at 20 °C, mixtures were subjected to 4% (w/v) polyacrylamide gel electrophoresis (acrylamide:bisacrylamide, 29:1) in 10 mM Tris/borate (pH 8.3), 0.2 mM EDTA. The gel was dried on a DE81 paper (Whatman) and autoradiographed. *E. coli* tRNA was used as a competitor to reduce nonspecific DNA binding; we found that it works similarly to the sonicated salmon sperm DNA used in the filter binding assay.

DNase I footprinting. The DNA fragments used were prepared from pRP270T. Radioactive label was introduced after *XbaI* treatment, either at the 3' end using [α -³²P]dCTP and the large fragment of DNA polymerase I, or at the 5' end using [γ -³²P]ATP and polynucleotide kinase. Labelled DNA was treated with *TaqI* and the 288 bp fragment containing the *aldA* regulatory region was purified by agarose gel electrophoresis. End-labelled fragments (1–3.5 $\times 10^5$ c.p.m. per 60–80 fmol) were combined with DnaA buffer or with 100–250 ng DnaA protein in five tubes containing 12 μ l solutions for the gel mobility shift assay described above. After electrophoresis and autoradiography, seven bands corresponding to the bands 0–6 in Fig. 2 were excised from the gel and treated in gel slices with DNase I under conditions which permit partial hydrolysis (Straney *et al.*, 1989). DNA fragments were eluted from gel slices, subjected to polyacrylamide gel electrophoresis under denaturing conditions, and radioimages were obtained using a BAS 1000 Bio Imaging Analyzer.

Assay of β -galactosidase. Plasmid-carrying cells of CSH50 were grown at 30 °C to exponential phase in M9 glucose medium supplemented with 40 μ g L-proline ml⁻¹ and 5 μ g thiamin ml⁻¹ and appropriate antibiotics (30 μ g ampicillin ml⁻¹, 10 μ g chloramphenicol ml⁻¹). IPTG was added where indicated at 0.5 mM and cells were harvested 6 h after addition of the inducer. The β -galactosidase activity was assayed as described by Miller (1972). Assays were performed six to nine times with at least three independent cultures.

RESULTS

Binding of DnaA to the *aldA* regulatory region

In surveying an aligned *E. coli* DNA library (Kohara *et al.*, 1987) by filter binding assays (Fuller *et al.*, 1984), we found that clone 270 has a fairly strong DnaA-binding activity. The binding activity of this clone (containing 16635 bp *E. coli* DNA sequence) was localized to a 261 bp *TaqI* fragment, which carries the regulatory region of the *aldA* gene. This fragment was subcloned into the *SmaI* site of pUC119 (plasmid pRP270T).

In the experiment shown in Fig. 1, various amounts of DnaA protein were added to reaction mixtures containing three labelled linear DNA fragments: vector pUC119, the insert fragment containing the *aldA* regulatory region, and pMAPCdSG10. The DnaA-DNA complexes formed were collected on nitrocellulose filters and the bound DNA fragments were recovered and

Fig. 1. Agarose gel electrophoresis of DNA bound to DnaA protein. (a) A filter binding assay was carried out with three labelled DNA fragments as described in Methods. DNA fragments retained on the filter were eluted and analysed. Concentrations of DnaA (nM) in reaction mixtures for lanes 1–7 were 0, 19, 38, 76, 114, 152 and 190, respectively. In lane M, a portion of the DNA fragment mixture used in the assay was directly loaded on the gel. Positions of the vector DNA (A), linear pMAPCdSG10 DNA (B) and the insert fragment carrying the *aldA* regulatory region (C) are indicated on the right. (b) Radioactive bands in (a) were quantified and plotted versus DnaA concentration. The ordinate represents the proportion of bound radioactivity in each DNA fragment.

analysed by agarose gel electrophoresis. pUC119 has two DnaA boxes downstream of the replication origin, which enhance plasmid replication by functioning as a loading site of primosomal proteins for synthesis of the lagging strand (Parada & Marians, 1991). pMAPCdSG10, on the other hand, has no DnaA box.

From the figure, it is evident that DnaA protein bound the insert fragment. The binding was more efficient than that to the vector DNA, especially at higher DnaA concentrations. As expected, pMAPCdSG10 did not bind DnaA.

From a similar assay in the absence of the competitor, the dissociation constant (K_d) of the 261 bp *TaqI* fragment was estimated to be $1.3 \pm 0.2 \times 10^{-8}$ M (data not shown). This value is only 1.5-fold higher than the K_d for an *oriC* fragment measured in a similar ex-

Fig. 2. Gel mobility shift assay of DnaA complexed to the *aldA* regulatory region. Amounts of DnaA protein added were 0, 10, 20, 30, 40, 50, 75 and 100 ng in lanes 1–8, respectively. Band 0 corresponds to free DNA fragment.

periment (Kitagawa *et al.*, 1996) and it indicates that the affinity of DnaA to the *TaqI* fragment is fairly strong.

Specific binding of DnaA to the *aldA* regulatory region was analysed by a gel mobility shift assay with purified DNA fragment and DnaA protein (Fig. 2). Discrete bands were resolved in the gel and slowly migrating species increased at higher DnaA protein concentrations. Shift to larger complexes occurred continuously until all DNA molecules stayed at the origin, although resolution of bands was incomplete in the upper part of the gel. These results suggest that a specific complex is formed first and then progressively converted to more complicated structures by cooperative binding of additional DnaA molecules.

Characterization of the binding site by a footprinting experiment

Despite the clear binding, the 261 bp *TaqI* fragment has no canonical 9 bp DnaA box sequence. In order to identify the interaction sites, DNase I footprinting was performed (Fig. 3). End-labelled fragments complexed with DnaA protein were first separated by non-denaturing polyacrylamide gel electrophoresis. Free DNA band and six shifted bands, 1–6, corresponding to those in Fig. 2 were partially hydrolysed in gel slices with

Fig. 3. Footprint of DnaA protein bound to the *aldA* gene regulatory region. Upper and lower strands denote the representation in Fig. 4. The fragment used was the *TaqI*-*XbaI* fragment of pRP270T labelled at the 3' end of the *XbaI* site (upper strand) or at the 5' end of the *XbaI* site (lower strand). In lanes 0–6, radioactive bands corresponding to bands 0–6 in Fig. 2 were analysed. Triangles at the top indicate increase of the bound DnaA protein. The leftmost lanes are ladders of the Maxam–Gilbert A+G reaction. Numbers on the left are the nucleotide coordinates, with the transcription start site being +1.

DNase I and subjected to polyacrylamide gel electrophoresis under denaturing conditions. The results are schematically summarized in Fig. 4.

Protection from the nuclease digestion first occurred at a site between the transcription start site and the –10 sequence (Pribnow Box) on the upper strand. Upon increase of the number of bound molecules, the protected region extended in both directions accom-

panied by periodic enhanced cleavage sites. Both protection and enhancement sites repeated at roughly 10 bp intervals and finally covered about 100 bp of sequence. Far upstream, at least beyond the *XmnI* site at position –58, seemed to have no effect on DnaA binding (data not shown).

It was difficult to find a distinct protection site that appeared in response to the band shift from band 2 and after. Rather, several bands seemed to diminish simultaneously and gradually upon the mobility shift to slower bands. This may suggest that ordered DnaA binding to fixed sites is not involved in the formation of complexes in bands 2–6.

Binding of DnaA to the *aldA* regulatory region *in vivo*

The region bound by DnaA overlapped with the regulatory region of the *aldA* gene. This gene encodes NAD-linked aldehyde dehydrogenase with a broad substrate spectrum and is under control of several transcriptional regulators that bind to sites within or near the region bound by DnaA (Limon *et al.*, 1997; Pellicer *et al.*, 1999) (Fig. 4). Therefore, we next examined whether DnaA binds to this region as well *in vivo*, using a system to measure the level of derepression of a *mioC*-*lacZ* fusion gene upon introduction of a plasmid carrying the *aldA* regulatory region. The mini-F-based plasmid pFPMCLZ1 has the promoter region and a portion of the coding sequence of the *mioC* gene fused in-frame to the *lacZ* gene. The fusion gene is negatively controlled by DnaA by its binding to the DnaA box at the promoter region, and therefore a change in cellular DnaA level can be detected by measuring the β -galactosidase activity (Hansen *et al.*, 1987; Kitagawa *et al.*, 1996). Presence of a DnaA-binding sequence in the same cell on a compatible plasmid should result in a derepression of the fusion gene.

The activity of β -galactosidase in a *lacZ* strain (CSH50) carrying pFPMCLZ1 and the vector plasmid pBR322 was 0.52 ± 0.05 U. On the other hand, the activity was increased to 1.56 ± 0.08 U in the strain carrying pFPMCLZ1 and pBRP270, which has the 261 bp *TaqI* fragment. This value was higher than the β -galactosidase activity in cells carrying pFPMCLZ1 and pTOA50, which is a chimeric plasmid of *oriC* and pBR322 (1.14 ± 0.18 U). It should be noted that the copy number of pTOA50 is 1.5–2-fold higher than that of pBRP270 (data not shown). These results suggest that the *aldA* regulatory region can titrate more DnaA molecules than *oriC* *in vivo*.

Negative control of *aldA* gene expression by DnaA

In order to examine the role of DnaA in *aldA* gene expression, the regulatory region and N-terminal three codons of the gene (–184 to 52) was fused in-frame to the *lacZ* gene on a mini-F plasmid. Activity of β -

Fig. 4. (a) Representation of the *aldA* control region. Transcription of the *aldA* gene proceeds toward the right from position +1. The promoter -35 and -10 boxes as well as the Shine-Dalgarno ribosome-binding sequence (RBS) are shown by unfilled boxes. Upstream regulatory sequence (URS) and binding sites of cAMP-Crp, IHF, ArcA-P and DnaA are also indicated. (b) Schematic representation of the DnaA protein footprint. Part of the *TaqI* fragment, which extends from bp -184 to 77, is shown. The -10 promoter sequence, ribosome-binding site and initiation codon are boxed and a 12 bp palindrome is marked with a line between the two strands. Thickness of the solid blocks and size of the arrows reflect relative strength of protection and cleavage, respectively. Changes of the footprinting pattern appear at lower DnaA concentrations at sites of thick blocks and large arrows.

Table 1. Negative control of *aldA-lacZ* fusion gene by DnaA protein

Strain*	IPTG	β -Galactosidase (U)
CSH50	—	0.1 $\pm$ 0.06
CSH50(pFCmLZ1)	—	0.1 $\pm$ 0.04
CSH50(pFAL1)	—	4.0 $\pm$ 0.2
CSH50(pFAL1, pROA199)	—	3.4 $\pm$ 0.3
	+	3.5 $\pm$ 0.4
CSH50(pFAL1, pROA200)	—	3.3 $\pm$ 0.3
	+	1.0 $\pm$ 0.2
CSH50(pFAL1, pROAKK1)	—	4.2 $\pm$ 0.3
CSH50(pFAL1, pROAKK-EX)	—	8.6 $\pm$ 0.1

* See Methods and text for descriptions of plasmids.

galactosidase in *lacZ* cells carrying this plasmid (pFAL1) was dependent on the insert fragment, inasmuch as the control strain with a plasmid that did not carry this fragment (pFCmLZ1) had only a background level of the β -galactosidase activity (Table 1). Induction of DnaA from pROA200 by the addition of IPTG resulted in repression of the β -galactosidase activity. The level of DnaA induction by this plasmid was about 10-fold as measured by an immunoblotting experiment (data not shown). The effect of the control plasmid pROA199 was negligible in either the presence or absence of IPTG. In contrast, pROAKK-EX, which titrates a large amount

of DnaA molecules to the *datA* locus on the plasmid (Kitagawa *et al.*, 1998), derepressed the β -galactosidase activity, as has been observed on other DnaA-regulated genes. The control strain carrying the vector pROAKK1 instead of pROAKK-EX did not affect the β -galactosidase activity. These results suggest that DnaA negatively regulates the *aldA* gene expression.

The minimal sequence required for efficient DnaA binding is not known but, supporting the footprinting data, similar results were obtained when the upstream *TaqI-XmnI* fragment (-184 to -59) was removed from pFAL1 (data not shown).

DISCUSSION

DnaA protein usually recognizes a 9 bp sequence (DnaA box), TTAT(C/A)CA(C/A)A (Fuller *et al.*, 1984) or TT(A/T)TNCACA (Schaper & Messer, 1995). Weak interaction to sites deviating from this sequence has been observed (Fuller *et al.*, 1984; Yung & Kornberg, 1989; Schaefer & Messer, 1991) and a relaxed DnaA box sequence proposed (Schaefer & Messer, 1991). The DnaA-DNA interaction seems to be strengthened when two or more weak binding sites are present on the same DNA fragment (Konopa *et al.*, 1999; authors' unpublished observation). In a recent study, a 6-mer sequence, AGATCT, has been shown to interact with ATP-bound DnaA when a canonical DnaA box is present in close proximity (Speck *et al.*, 1999). The *aldA*

regulatory region appears to be distinct from these examples in that the interaction is relatively strong and stable, despite the absence of any consensus sequence. A characteristic of the *aldA* promoter region is the presence of AT clusters between positions -10 and 30 . We assume that these AT clusters serve as the target sites of DnaA, inasmuch as major protection occurs at these sites. A palindromic sequence of 12 bp extends from the -10 promoter sequence to the first protection site, but its significance is unknown. The 368 bp pBR322 fragment which has a weak binding activity, despite the lack of strong homologies to the DnaA box, also has several AT clusters (Fuller *et al.*, 1984). DnaA-binding activity of this fragment, however, is very weak as compared to the origin fragment of pBR322 carrying two DnaA boxes.

Although several discrete bands appeared in a gel mobility shift assay, we could not attribute the mobility shift to the binding of DnaA to a new specific site. It seems that DnaA bound first at a site between the transcription start site and the -10 sequence on the upper strand triggered the cooperative binding upon increase of the number of bound molecules. Both protection and enhancement sites repeated at roughly 10 bp intervals and finally covered about 100 bp of sequence. A similar cooperative binding has been observed on a fragment containing a single canonical DnaA box (Fuller *et al.*, 1984). In this case, 20–30 DnaA monomers covering about 100 bp have been visualized by electron microscopy, but a gel mobility shift experiment has not been done and structures of intermediate complexes are unknown. The stoichiometry of binding is not certain in our study, because it appears that the binding is not saturated even at the highest amount of the input DnaA protein (Fig. 2).

DnaA-binding sites on the chromosome may function to control the frequency of initiation of DNA replication by titrating the initiator. The only example known at present to have such a function is the *datA* locus, which titrates an extremely large amount of DnaA molecules (Kitagawa *et al.*, 1998). Deletion of this locus causes overinitiation of DNA replication. The *aldA* regulatory region, however, may not have such physiological significance, inasmuch as growth of cells carrying pRP270T is not impaired. High-copy-number plasmids carrying *datA* severely retard cell growth rate by lowering the free DnaA concentration (Kitagawa *et al.*, 1998). In addition, gene dosage of the *aldA* region is lowest in growing cells due to its location on the genome (32 min) close to the *ter* site.

Negative control of *aldA* gene expression by DnaA was suggested from the derepression assay (Table 1). It should be noted, however, that such an effect was observed only in a minimal medium in the presence of glucose. Under these conditions, expression of *aldA* is limited (Limon *et al.*, 1997). In M9 medium containing rhamnose in place of glucose and supplemented with Casamino acids, or in L-broth, where expression of the gene is induced, neither increase nor decrease of cellular

DnaA concentration affected the level of β -galactosidase activity (data not shown). Formation of a specific transcription initiation complex or efficient transcription initiation may block the binding of DnaA to the regulatory region.

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REFERENCES

- Fuller, R. S., Funnell, B. E. & Kornberg, A. (1984). The DnaA protein complex with the *E. coli* chromosomal replication origin (*oriC*) and other DNA sites. *Cell* **38**, 889–900.
- Hansen, F. G., Koefoed, S., Sørensen, L. & Atlung, T. (1987). Titration of DnaA protein by *oriC* DnaA-boxes increases *dnaA* gene expression in *Escherichia coli*. *EMBO J* **6**, 255–258.
- Hidalgo, E., Chen, Y.-M., Lin, E. C. C. & Aguilar, J. (1991). Molecular cloning and DNA sequencing of the *Escherichia coli* K-12 *ald* gene encoding aldehyde dehydrogenase. *J Bacteriol* **173**, 6118–6123.
- Kitagawa, R., Mitsuki, H., Okazaki, T. & Ogawa, T. (1996). A novel DnaA protein-binding site at 947 min on the *Escherichia coli* chromosome. *Mol Microbiol* **19**, 1137–1147.
- Kitagawa, R., Ozaki, T., Moriya, S. & Ogawa, T. (1998). Negative control of replication initiation by a novel chromosomal locus exhibiting exceptional affinity for *Escherichia coli* DnaA protein. *Genes Dev* **12**, 3032–3043.
- Kohara, Y., Akiyama, K. & Isono, K. (1987). The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. *Cell* **50**, 495–508.
- Konopa, G., Szalewska-Palasz, A., Schmidt, A., Srutkowska, S., Messer, W. & Wegrzyn, G. (1999). The presence of two DnaA-binding sequences is required for an efficient interaction of the *Escherichia coli* DnaA protein with each particular weak DnaA box region. *FEMS Microbiol Lett* **174**, 25–31.
- Kornberg, A. & Baker, T. A. (1992). *DNA Replication*, 2nd edn. New York: Freeman.
- Limon, A., Hidalgo, E. & Aguilar, J. (1997). The *aldA* gene of *Escherichia coli* is under the control of at least three transcriptional regulators. *Microbiology* **143**, 2085–2095.
- Messer, W. & Weigel, C. (1997). DnaA initiator – also a transcription factor. *Mol Microbiol* **24**, 1–6.
- Miller, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Parada, C. A. & Mariani, K. J. (1991). Mechanism of DnaA protein-dependent pBR322 DNA replication. DnaA protein-mediated *trans*-strand loading of the DnaB protein at the origin of pBR322 DNA. *J Biol Chem* **266**, 18895–18906.
- Pellicer, M. T., Lynch, A. S., De Wulf, P., Boyd, D., Aguilar, J. & Lin, E. C. C. (1999). A mutational study of the ArcA-P binding sequences in the *aldA* promoter of *Escherichia coli*. *Mol Gen Genet* **261**, 170–176.
- Quintilla, F. X., Baldoma, L., Badia, J. & Aguilar, J. (1991). Aldehyde dehydrogenase induction by glutamate in *Escherichia coli*. Role of 2-oxoglutarate. *Eur J Biochem* **202**, 1321–1325.
- Schaefer, C. & Messer, W. (1991). DnaA protein/DNA inter-

action. Modulation of the recognition sequence. *Mol Gen Genet* **226**, 34–40.

Schaper, S. & Messer, W. (1995). Interaction of the initiator protein DnaA of *Escherichia coli* with its DnaA target. *J Biol Chem* **270**, 17622–17626.

Speck, C., Weigel, C. & Messer, W. (1999). ATP- and ADP-DnaA protein, a molecular switch in gene regulation. *EMBO J* **18**, 6169–6176.

Straney, D. C., Straney, S. B. & Crothers, D. M. (1989). Synergy

between *Escherichia coli* CAP protein and RNA polymerase in the *lac* promoter open complex. *J Mol Biol* **206**, 41–57.

Yung, B. Y. & Kornberg, A. (1989). The DnaA initiator protein binds separate domains in the replication origin of *Escherichia coli*. *J Biol Chem* **264**, 6146–6150.

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