Nucleic Acids Research, Vol. 20, No. 19 5035–5039

Specificities of three tight-binding Lac repressors

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Received July 21, 1992; Revised and Accepted September 5, 1992

ABSTRACT

Tight binding mutants of Lac repressor exhibit complex repression phenomena. In this work, *in vivo* Lac operator binding of three such mutants of *E.coli* Lac repressor (X86: ser 61-leu, I12: pro 3-tyr and the double mutant I12X86: pro 3-tyr, ser 61-leu) was analyzed. Repression of β-galactosidase synthesis controled by ideal *lac* operator and its 27 symmetric operator variants containing each possible base-pair at each single half-operator position in the presence of the tight-binding Lac repressor mutants was determined. The average increase of repression with all operator variants was about 3 fold with the X86 mutant. It was about 4 fold with the I12 mutant and about 2 fold with the double mutant I12X86 as compared to wildtype Lac repressor. The X86 mutant showed the same increase of affinity to all operator variants, whereas the I12 and I12X86 mutants exhibited lower repression with some variants than with most others. These results suggest that the X86 mutant has gained no additional specificity. In contrast the I12 mutant and the I12X86 mutant exhibit a relaxed specificity for certain base pairs in positions 1 and 3 of *lac* operator. This suggests that the extreme N-terminus of Lac repressor may interact with the inner base-pairs in the minor groove.

INTRODUCTION

Specific protein-DNA interaction is crucial for gene control in eukaryotes as well as prokaryotes. In *E.coli* the Lac repressor-operator system is one of the best analyzed protein-DNA recognition systems. Extensive biochemical and genetic studies with Lac repressor indicate that the protein is composed of three major domains : A small N-terminal domain, the headpiece (about 60 amino acids), recognizes *lac* operator, the core-protein (about 270 amino acids) binds inducer and leads to aggregation of dimers (1) and the C-terminal 30 residues, a leucine mini zipper, mediate tetramer assembly (2). Adler *et al.* (3) proposed that the sequence from amino acid 17 to 25 constitutes a DNA-recognizing α-helix. NMR-analysis of the *lac* operator-repressor complex confirmed this prediction (4, 5). Genetic analyses of amino acid-exchanges in the recognition helix and symmetric base-pair substitutions of ideal *lac* operator revealed specific contacts between amino acids and base-pairs and determined the orientation of the recognition helix within the major groove of the DNA which is opposite to that of λ repressor and λ *cro* protein (6–10).

Apart from the above described mutants coding for changes in the recognition helix, there exist some Lac repressor mutations outside the helix-turn-helix motif which cause a tighter binding to DNA in general (11–15). A serine to leucine exchange at position 61 creates the well known tight-binding mutant X86 (13–16). It was originally isolated by F.Jacob after X-irradiation (16). Jobe and Bourgeois (13) determined an *in vitro* binding constant of this mutant to *lac* operator-DNA of 10^{-15} M. This is 40 times higher than that of wildtype Lac repressor. Pfahl (14) showed that the binding affinity of the X86 mutant is also increased for nonspecific DNA. In the presence of excess *E.coli* DNA, the *in vitro* association rate of the X86 repressor mutant and *lac* operator is fivefold slower than with wildtype Lac repressor. Schmitz *et al.* (15) described another mutant with a proline to tyrosine exchange at position 3 of Lac repressor (I12 mutant), whith similar properties as the X86 mutant. They also report a 10.000 fold increased *in vitro* affinity of the double mutant I12X86 for both operator- and non-operator DNA.

In order to understand better the molecular mechanism by which these mutations enhance DNA binding and to identify possible contacts between these residues and operator DNA, I analyzed their interactions with 28 symmetric *lac* operator variants *in vivo*.

MATERIALS AND METHODS

Bacterial strain

Strain DC 41-2 Δ (*lac pro*), *thi galE smR recA* (6) was used for β-galactosidase assays.

Plasmids

All exchanges in the *lacI* gene were cloned into plasmid pWB 1000 (7), which codes for tetrameric Lac repressor with the help of synthetic oligonucleotides. The control repressor mutant Δ1 was obtained after digestion of plasmid pWB 1000 DNA with *NaeI* and *HindIII*, a fill in reaction and ligation such that the DNA coding for the helix-turn-helix motif was deleted from codon 14 to 60 (6). Ideal *lac* operator and its symmetric variants were cloned into the unique *XbaI* site of pWB 300 as synthetic oligonucleotides (6). The base pairs are numbered from the centre of symmetry of the operator as proposed by Lehming *et al.*(6). The nomenclature of the operator containing plasmids is as follows : The first number (3) indicates that these plasmids are derivaives of pWB 300 (6). The second number refers the operator position which was altered while the third number indicates the particular base with A=1, C=2, G=3 and T=4. The plasmids containing operator and repressor are mutually compatible and stable together. They carry different origins of replication and antibiotic resistances (6, 7).

DNA sequencing

All repressor and operator-variants were verified by DNA-sequencing according to Sanger *et al.* (17).

β-galactosidase assays

DC 41-2 cells were transformed successively with about 10 ng DNA each of pWB 1000 or one of its derivatives and pWB 300 carrying one of the operator variants according to the method of Hanahan (18). β-galactosidase assays were performed as described by Miller (19). Values for specific β-galactosidase activity reported in table 1 are averages of two measurements of at least 2 independent transformants. The specific β-galactosidase activities in the presence of tetrameric wildtype Lac repressor have been reported. The values from Lehming *et al.* (8) were confirmed with the exception of plasmid pWB 314. Here I measure a basal level 7 times lower and the repressed level 14 times lower then reported previously. The value of repression is defined as the quotient of the specific activity of β-galactosidase in the presence of the Lac repressor mutant Δ1 carrying a deletion of the helix-turn-helix motif (specific activity 'unrepressed', table 1) and the specific activity of β-galactosidase in the presence of wildtype Lac repressor or one of the mutants.

RESULTS

Lehming *et al.* (6) established a two plasmid system which allows the analysis of contacts between amino acids in the recognition helix of Lac repressor mutants and symmetric *lac* operator variants *in vivo*. One of the plasmids (pWB 300 or its derivates) carries a modified *lac* operon consisting of the natural *lac* promotor, a unique *XbaI* site, which replaces *lac* operator O_1 and the *lacZ* gene. Ideal *lac* operator and its symmetric variants were cloned into the unique *XbaI* site. The other plasmid carries the *lacI* gene, which codes for tetrameric Lac repressor or its mutants. Although the three wildtype *lac* operators cooperate in repression in the wildtype situation (20), the remaining two wildtype *lac* operators (O_2 is located 401 bp downstream from O_1 within the *lacZ* gene and O_3 92 bp upstream from O_1) in this case do not significantly contribute to repression, because Lac Repressor and its mutants are highly overexpressed to concentrations of about 4000 molecules per cell (6). In fact, the difference in repression between tetrameric and dimeric Lac repressor, which cannot participate in loop formation between two operators is less than a factor of 2 (7).

I used this system to examine the specificities of the three tight-binding mutants X86 (16), I12 (15) and I12X86 (15). The specific activities of β-galactosidase obtained under the control of the cloned operator variants and in the presence of wildtype Lac repressor or its tight-binding mutants are given in table 1 (specific activities unrepressed, of wildtype, X86, I12 and I12X86 Lac repressor). It has been observed before (6), that single base-pair exchanges within the *lac* operator-sequence, which is embedded in the promoter and is partly transcribed, affect β-galactosidase expression even under unrepressed conditions. Here I observed maximally a 30-fold difference between the unrepressed β-galactosidase activities of plasmids 333 and 371 respectively.

Table 1 also summarizes the repression values obtained with wildtype or one of the three tight binding Lac repressor mutants and ideal *lac* operator or one of its 27 symmetric variants (for the definition of the term repression, see materials and methods). Since the specific activity of β-galactosidase is proportional to the fraction of *lac* operator which is not occupied by Lac repressor (6), the repression value of Lac repressor or one of its mutants with each *lac* operator variant reflects the binding affinity for this operator variant: The larger the repression value the higher the binding affinity for an operator variant.

To analyze the increased or decreased affinities of the tight-binding Lac repressor mutants for ideal *lac* operator and its 27 symmetric variants, I calculated the ratios of the specific β-galactosidase activities in the presence of wildtype Lac repressor and each of the three tight-binding mutants with each of the *lac* operator variants. These ratios are given in the last three lanes of table 1. A ratio below 1.0 indicates reduced binding affinity of the tight-binding mutant for the respective operator variant as compared to wildtype Lac repressor and a ratio greater than 1.0 indicates an increased binding affinity of the tight-binding mutant for this operator variant. These ratios signal reduced or increased affinities of the tight-binding mutants for a particular *lac* operator variant.

Repression with the X86 mutant was in all cases higher than with wildtype Lac repressor. The increase of repression ranges from 1.5 fold to 5.2 fold with the symmetric *lac* operator variants. The average increase of affinity of X86 Lac repressor to all operator variants is about 3 fold. The increases of affinity of the X86 mutant for the three operator variants with all possible exchanges at a single position differ by no more than a factor 2 (operator variants 21 and 22). Each value of specific activity of β-galactosidase in table 1 is a arithmetic average of 2−3 independent transformants. These values contain an estimated limit of error of about 25%. If two values of specific activities of β-galactosidase (repressed/unrepressed) are considered the errors may sum up maximally to 50%, which corresponds to a factor of 2. Thus, the 2 fold difference in the increases of affinity of the X86 mutant for the operator variant 21 and 22 is within the limits of error of this *in vivo* system.

Repression mediated by the I12 and I12X86 mutants was in most cases also increased in comparison to wildtype Lac repressor (table 1) but there are some exceptions common to both mutants (see asterisks in table 1): The I12 mutant binds ideal *lac* operator with similar affinity as wildtype Lac repressor but recognizes a guanine at position 1 of the operator 9 fold better than wildtype Lac repressor. The I12X86 mutant binds ideal *lac* operator half as well as wildtype Lac repressor but shows a 15 fold enhanced affinity for the operator variant 13 with the guanine at position 1. A similar effect was observed with *lac* operator variants with base substitutions at operator position 3: Compared to wildtype Lac repressor both mutants exhibit a reduced affinity for guanine (operator variant 33), the I12X86 mutant also for adenine (ideal *lac* operator) at this position and they recognize a thymine (operator variant 34) 8 fold (I12) and 27 fold(I12X86) better than wildtype Lac repressor. The plasmid pWB 333 has the highest unrepressed specific activity of β-galactosidase which implies an increase of promoter strength or messenger stability caused by the 8 central G-C pairs of this operator variant. The ratio of β-galactosidase under unrepressed and repressed conditions though should not be influenced by the absolute level of expression. With the *lac* promoter mutant L8 induced and repressed expression are similarly reduced such that the ratio of induction is the same as with wildtype *lac* promoter (21). Since the X86 mutant still binds this operator variant with 3 fold higher affinity than wildtype Lac repressor, I conclude that the reduced affinities of the I12 and I12X86 mutants are not artificially lowered by an altered equilibrium in the competition between RNA polymerase and

Table 1. Repression of wildtype and three tight-binding *Lac* repressor mutants with various *lac* operator variants

Plasmid	Operator	spec.act. unrepressed	spec.act. wt lacI	spec.act. X86 lacI	spec.act. I12 lacI	spec.act. I12X86 lacI	wildtype repression	X86 repression	I12 repression	I12X86 repression	spec.act. wt spec.act. X86	spec.act. wt spec.act. I12	spec.act. wt spec.act. I12X86
310	AATTGTGAGC+GCTCACAATT	6700	21	9	23	42	319	744	291	160	2,33	0,91*	0,50*
311	AATTGTGAGA+TCTCACAATT	7300	1900	1260	1140	1350	4	6	6	5	1,51	1,67	1,41
313	AATTGTGAGG+CCTCACAATT	6100	750	330	94	100	8	18	65	61	2,27	7,98*	7,50*
314	AATTGTGAGT+ACTCACAATT	3200	480	180	140	90	7	18	23	36	2,67	3,43	5,33
321	AATTGTGAAC+GTTCACAATT	24100	270	104	63	240	89	232	383	100	2,60	4,29	1,13
322	AATTGTGACC+GGTCACAATT	17500	520	100	52	250	34	175	337	70	5,20	10,00	2,08
324	AATTGTGATC+GATCACAATT	5500	640	160	120	240	9	34	46	23	4,00	5,33	2,67
332	AATTGTGCGC+GCGCACAATT	4900	1200	580	720	450	4	8	7	11	2,07	1,67	2,67
333	AATTGTGGGC+GCCCACAATT	30000	3000	1000	4600	12100	10	30	7	2	3,00	0,65*	0,25*
334	AATTGTGTGC+GCACACAATT	4700	1300	424	240	190	4	11	20	25	3,07	5,42*	6,84*
341	AATTGTAAGC+GCTTACAATT	4800	350	100	80	93	14	48	60	52	3,50	4,38	3,76
342	AATTGTCAGC+GCTGACAATT	11100	650	170	150	225	17	65	74	49	3,82	4,33	2,89
344	AATTGTTAGC+GCTAACAATT	5200	750	280	150	123	7	19	35	42	2,68	5,00	6,10
351	AATTGAGAGC+GCTCTCAATT	9200	210	107	43	73	44	86	214	126	1,96	4,88	2,88
352	AATTGCGAGC+GCTCGCAATT	8200	470	200	134	200	17	41	61	41	2,35	3,51	2,35
353	AATTGGGAGC+GCTCCCAATT	3600	360	160	53	100	10	23	68	36	2,25	6,79	3,60
361	AATTATGAGC+GCTCATAATT	9200	1100	520	390	840	8	18	24	11	2,12	2,82	1,31
362	AATTCTGAGC+GCTCAGAATT	6300	1100	730	465	1400	6	9	14	5	1,51	2,37	0,79
364	AATTTTGAGC+GCTCAAAATT	8000	1300	440	660	970	6	18	12	8	2,95	1,97	1,34
371	AATAGTGAGC+GCTCACTATT	1000	175	68	52	106	6	15	19	9	2,57	3,37	1,65
372	AATCGTGAGC+GCTCACGATT	8000	200	83	85	300	40	96	94	27	2,41	2,35	0,67
373	AATGGTGAGC+GCTCACCATT	7100	170	95	240	140	42	75	30	51	1,79	0,71	1,21
381	AAATGTGAGC+GCTCACATTT	2900	70	43	16	45	41	67	181	64	1,63	4,38	1,56
382	AACTGTGAGC+GCTCACAGTT	8300	62	39	17	100	134	213	488	83	1,59	3,65	0,62
383	AAGTGTGAGC+GCTCACACTT	5200	57	28	21	80	91	186	248	65	2,04	2,71	0,71
392	ACTTGTGAGC+GCTCACAAGT	4300	51	20	15	122	84	215	287	35	2,55	3,40	0,42
393	AGTTGTGAGC+GCTCACAACT	4600	110	33	21	116	42	139	219	40	3,33	5,24	0,95
394	ATTTGTGAGC+GCTCACAAAT	2000	25	9	10	23	80	222	200	87	2,78	2,50	1,09

* an asterisk indicates possible specifity changes.

repressor for this promoter-operator construct. It seems noteworthy that in the case of ideal *lac* operator and in the case of variant 33 the affinity of the I12 mutant is decreased by the additional introduction of the X86 mutation by the same factor by which the affinity of wildtype Lac repressor is enhanced by the X86 mutation. With operators 313 and 334 on the other hand the presence or absence of the X86 mutation has not much influence on the affinity of the I12 mutant for the respective operator variants. Affinities of both mutants for all other triplets of operator variants with all possible base substitutions at a single position do not differ by more than a factor 2, with one exception: The affinity of the I12 mutant for adenine at position 7 is about 5-times higher than for guanine. Thus, the effects of single symmetric base substitutions at operator positions 1 and 3 on affinities of the I12 and I12X86 mutants are substantial and reflect a relaxation of specificity contributed by the proline to tyrosine exchange at amino acid position 3 of Lac repressor.

DISCUSSION

How can amino acid residues outside the helix-turn-helix motif participate in specific and non-specific DNA binding and how can these residues confer an increased affinity for DNA to the whole protein?

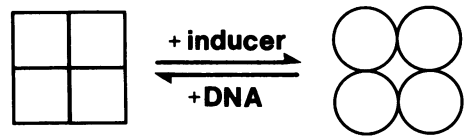

Figure 1. Schematic representation of the two states of Lac repressor, which depend on the binding of different ligands. The circles and squares symbolize the four identical subunits of tetrameric Lac repressor. The binding of inducer or DNA can presumably change the quarternary structure of the whole protein.

The two possible answers to these questions are closely related to two aspects of specific recognition. In a more general way specific recognition describes a close fit between the complementary surfaces of DNA and protein. This description is not equivalent to the indirect readout hypothesis proposed for *Trp* repressor/operator interactions (22), because this hypothesis requires water bridges between amino acids and base-pairs. At the general level of specificity a minor change of the quarternary structure may result in a slight adjustment of the binding surface. If a protein is able to bind either DNA or alternatively an inducer molecule like *Tet* and Lac repressors, it must be able to adopt two different conformations depending on the presence or absence

of DNA or inducer molecules. The equilibrium can be illustrated by the simple scheme in figure 1. If these repressors are really allosteric proteins as originally proposed by Monod *et al.* (23), one can assume that intermediate forms exist, which resemble one structure more than the other. Thus it is possible that a mutation in the proposed transmitter region between the DNA binding headpiece and the inducer binding core could result in the stabilisation of such an intermediate form, which could have a large effect on the position of the equilibrium in the upper scheme.

My results with the X86 Lac repressor are best explained by such a mechanism. This Lac repressor mutant has an increased affinity to DNA in general. The mutation is caused by an amino acid exchange at position 61 of Lac repressor. The proposed transmitter region of Lac repressor comprises amino acids 51 to 75 (1).

Previous *in vitro* binding studies with the X86 tight-binding Lac repressor (10) have also shown that the specific interactions between amino acid side chains of the recognition helix and base pairs are not affected by the presence of the X86 mutation. We exchanged tyrosine 17 and glutamine 18 the first and the second amino acid in the recognition helix for proline and lysine. A mutant with these substitutions can no longer bind to ideal *lac* operator but recognizes the oparator variant 4251, which carries cytosine in position 4 (instead of a guanine) and adenine in position 5 (instead of thymine). In electrophoretic shift assays with 4251 operator sequences we observed no differences in the behavior of the P_1K_2 Lac repressor and the P_1K_2 X86 Lac repressor respectively. We conclude that the X86 mutation does not result in additional specific contacts to *lac* operator (10).

At the level of atomic groups specific recognition refers to the interactions of amino acid side chains and functional groups of base-pairs projecting into the solvent. Crystallographic and genetic studies on DNA-binding proteins and their operators revealed numerous hydrogen bonds or hydrophobic contacts between individual bases and mostly amino acids of the helix-turn-helix motifs (for a review : 24). In particular amino acids 1,2,5,6 and 9 of the recognition helix are involved in such contacts because they lie on the solvent exposed surface of this protruding α-helix (the second of the helix-turn-helix motif) and may penetrate into the major groove of the DNA. There are also reports of specific hydrogen bonds between amino acids outside the helix-turn-helix motif and operator DNA. In λ repressor (25) for example, glutamine 33, which is the first residue of the helix preceeding the recognition helix forms a hydrogen bond with base-pair 2 of λ operator. Lysine 4 and asparagine 55, both also outside the helix-turn-helix motif form two hydrogen bonds with base-pair 6 of λ operator (25). The participation of the amino acids in positions 4 and 55 in operator recognition was first recognized through tight-binding λ repressor mutants with amino acid exchanges at these positions (26) and N-terminal deletion mutants of λ repressor (27). The latter study proposed an N-terminal 'arm', which partially determines the specificity for λ operators. This hypothesis was recently confirmed by a high-resolution analysis of a cocrystal of the N-terminal domain of λ repressor and λ operator (28), which revealed multiple hydrogen bonds between lysines 3 and 4 and guanines in the major groove of the operator DNA. An N-terminal 'arm' like structure, which presumably is involved in operator recognition is also known from *Trp* repressor (29, 30). NMR studies on Lac repressor and *lac* operator (4, 5) indicated a close vicinity between amino acids 5, 6, and 7 and the inner base-pairs of *lac* operator. Amino acids 3 and 4 were not considered in these studies.

Kleina and Miller (31) described several mutations at the very N-terminal end of Lac repressor which result in a tight-binding phenotype : proline 3 to phenylalanine , valine 4 to alanine, proline, lysine, tyrosine or cysteine. My results with the I12 mutant and the I12X86 double mutant indicate that both bind much better to a guanine at operator position 1 and a thymine at operator position 3 than wildtype Lac repressor. I connect this broadening of specificity to the proline 3 to tyrosine exchange, because the X86 repressor has no additional or altered operator specificity. The X86 mutation decreased apparently the affinity of the I12 mutant to most *lac* operatror variants except for the operator variants 13 and 34 which are better recognized than by wildtype Lac repressor. Neither the NMR studies nor genetic anlysis could exclude a N-terminal arm in Lac repressor similar to λ repressor, where also the third and fourth residue are involved in operator recognition. Work is in progress to identify more Lac repressor mutants at positions 3 and 4 with altered binding specificities.

ACKNOWLEDGEMENTS

I thank Daniela Tils and Gudrun Zimmer for excellent technical help, Brigitte von Wilcken-Bergmann, Reimund Fickert, Stefan Oehler, Bob Jack and Benno Müller-Hill for discussions and critical reading this manuscript. This work was supported by Deutsche Forschungsgemeinschaft through Schwerpunkt Protein Design (Mu 575/5-1).

REFERENCES

1. Müller-Hill, B. (1975) *Prog. Molec. Biol.*, **30**, 227–252.
2. Alberti, S., Oehler, S., Wilcken-Bergmann, B. v., Krämer, H. and B. Müller-Hill. (1991) *The New Biol.*, **3**, 57–62.
3. Adler, K., Beyreuther, K., Fanning, E., Geisler, N., Gronenborn, B., Klemm, A., Müller-Hill, B. Pfahl, M. and A. Schmitz (1972) *Nature*, **237**, 322–327.
4. Boelens, R., Scheek, R.M., van Boom, J.H. and R. Kaptein (1987) *J. Mol. Biol.*, **193**, 213–216.
5. Lamerichs, R.M.J.N., Boelens, R., van der Marel, G.A., van Boom, J.H. and R. Kaptein (1990) *Eur. J. Biochem.*, **194**, 629–637.
6. Lehming, N., Sartorius, J., Niemöller, M., Genenger, G., Wilcken-Bergmann, B. v. and B. Müller-Hill (1987) *EMBO J.*, **6**, 3145–3153.
7. Lehming, N., Sartorius, J., Oehler, S., Wilcken-Bergmann, B. v. and B. Müller-Hill (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 7947–7951.
8. Lehming, N., Sartorius, J., Kisters-Woike, B., Wilcken-Bergmann, B. v. and B. Müller-Hill (1991) In F. Eckstein and D.M.J. Lilley (eds.), Nucleic Acids and Molecular Biology, Vol. 5, p. 114–125. Springer Verlag Berlin Heidelberg.
9. Ebright, R. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 303–307.
10. Kolkhof, P., Teichmann, D., Kisters-Woike, B., Wilcken-Bergmann, B. v. and B. Müller-Hill (1992) *EMBO J.*, **11**, 3031–3038.
11. Betz, J.L. (1987) *J. Mol. Biol.* , **195**, 495–504.
12. Betz, J.L. and J.S. Sadler (1976) *J. Mol. Biol.*, **105**, 293–319.
13. Jobe, A. and S. Bourgeois (1972) *J. Mol. Biol.*, **72**, 139–152.
14. Pfahl, M. (1976) *J. Mol. Biol.*, **106**, 857–869.
15. Schmitz, A., Coulondre, C. and J.H. Miller (1978) *J. Mol. Biol.*, **123**, 431–456.
16. Chamness, G.C. and C.D. Willson (1970) *J. Mol. Biol.*, **53**, 561–565.
17. Sanger, F., Nicklen, S. and A.R. Coulsen (1977) *Proc. Natl. Acad. Sci. USA* , **74**, 5463–5467.
18. Hanahan, D (1985) In Glover, D.M. (ed.), DNA cloning. A practical approach. IRL Press, Oxford, pp. 109–135.
19. Miller, J.H. (1972) In Experiments in molecular genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 352–355.
20. Oehler, S., Eismann, E.R., Krämer, H. and B. Müller-Hill (1990) *EMBO J.*, **9**, 973–979.

21. Miller, J.H. (1978) In Miller, J.H. and Reznikoff, W.S. (eds.), The Operon. Cold Spring Harbor Laboratory Press. New York, pp. 31–88.
22. Otwinowski, Z., Schevitz, R.W., Zhang, R.-G., Lawson, C.L., Joachimiak, A., Marmorstein, R.Q., Luisi, B.F. and P.B. Sigler (1988) *Nature*, **335**, 321–329.
23. Monod, J., Changeux, J.-P. and F. Jacob (1963) *J. Mol. Biol.*, **6**, 306–329.
24. Harrison, S.C. and A.K. Aggarwal (1990) *Annu. Rev. Biochem.*, **59**, 933–969.
25. Jordan, S.R. and C.O. Pabo (1988) *Sience*, **242**, 893–899.
26. Nelson, H.C. and R.T Sauer (1986) *J. Mol. Biol.*, **192**, 27–38.
27. Eliason, J.L., Weiss, M.A. and M. Ptashne (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 2339–2343.
28. Clarke, N.D., Beamer, L.J., Goldberg, H.R., Berkower, C. and C.O. Pabo (1991) *Science*, **254**, 267–270.
29. Carey, J. (1989) *J. Biol. Chem.*, **264**, 1941–1945.
30. Hurlburt, B.K. and C. Yanofsky (1992) *Nucl. Ac. Res.*, **20**, 337–341.
31. Kleina, L.G. and J.H. Miller (1990) *J. Mol. Biol.*, **212**, 295–318.