DNA Specificity of the Bicoid Activator Protein Is Determined by Homeodomain Recognition Helix Residue 9

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Summary

Formation of anterior structures in the Drosophila embryo requires the product of the gene bicoid. The bicoid protein contains a homeodomain and may exert its effects in early development by regulating transcription of the gap gene, hunchback (hb). Consistent with this view, we have demonstrated that DNA-bound Bicoid fusion proteins stimulate gene expression. We used the gene activation phenotype in yeast to study DNA recognition by the Bicoid homeodomain. We found that a single amino acid replacement at position 9 of the recognition helix was sufficient to switch the DNA specificity of the Bicoid protein. The altered specificity Bicoid mutants recognized DNA sites bound by Ultrabithorax, fushi tarazu, and other related homeodomain proteins. Our results suggest that DNA specificity in Bicoid and Antennapedia class proteins is determined by recognition helix residue 9.

Introduction

Pattern formation in Drosophila embryos appears to be initiated by a small number of maternal-effect genes. One of these genes, bicoid, is necessary for establishing anterior-posterior polarity (Nüsslein-Volhard et al., 1987). Mutants that lack functional bicoid product fail to develop head and thoracic structures. bicoid mRNA is synthesized in nurse cells during oogenesis and transported into the developing oocyte where it is localized in the anterior (Berleth et al., 1988). After egg deposition, bicoid mRNA is translated and the protein (here called Bicoid) accumulates in the anterior of the egg (Driever and Nüsslein-Volhard, 1988a). Bicoid is present in embryonic nuclei where it may stimulate zygotic expression of hunchback (Driever and Nüsslein-Volhard, 1988b, 1989), and other gap genes whose expression is necessary to establish pre-segmental domains in the immature egg (reviewed in Ingham, 1988).

Like many other proteins important for development, Bicoid contains a conserved 60 amino acid sequence known as the homeodomain (reviewed in Gehring, 1987). Sequence similarity of the homeodomain to the helix-turn-helix DNA binding motif of many bacterial proteins (Pabo and Sauer, 1984), suggests that the homeodomain may also bind DNA (Laughon and Scott, 1984). Indeed, many Drosophila homeodomain proteins bind to specific DNA sequences in vitro (Desplan et al., 1985; Hoey and Levine, 1988; Beachy et al., 1988; Driever and Nüsslein-Volhard, 1989); in some cases, DNA binding has been shown to require the homeodomain (Desplan et al., 1988; Müller et al., 1988b; Hoey et al., 1988). Similarly, some mammalian transcription factors also contain homeodomain sequences (Scheidereit et al., 1988; Müller et al., 1988a) that are involved in DNA binding (Ko et al., 1988; Sturm and Herr, 1988).

Many bacteriophage repressor proteins use the second α-helix of the helix-turn-helix motif (the recognition helix) to contact DNA. Sequence specificity is determined primarily by hydrogen bonding of side chains of residues 1 and 2 of the recognition helix to functional groups of base pairs in the major groove (Lewis et al., 1983; Wharton and Ptashne, 1985; Hochschild and Ptashne, 1986; Wharton and Ptashne, 1987; Jordan and Pabo, 1988). Additional contributions to specificity are made by polar interactions or hydrogen bonds by residues 5 and 6 (as in λ repressor, Hochschild et al., 1988; or as in 434 repressor, Wharton and Ptashne, 1987; Aggarwal et al., 1988). Studies of λ and 434 repressor-DNA cocrystals reveal hydrogen bonds between the amino-terminal residues of the two helices in the motif that may help position the recognition helix correctly in the major groove (Aggarwal et al., 1988; Jordan and Pabo, 1988). Several nonspecific contacts with the sugar-phosphate backbone, including one made by the amino acid at recognition helix position 9, may contribute to the stability of the repressor-operator complex.

The amino acid sequence of the Bicoid homeodomain differs from that in proteins of the Antennapedia (Antp) sequence family (Scott et al., 1989). Several of these differences lie in positions which, by analogy with the bacterial helix-turn-helix proteins, might be important for DNA recognition. Amino acids 1 and 2 of the putative Bicoid recognition helix (homeodomain helix 3) differ from those found at equivalent positions in Antp class proteins, and might be predicted to make site-specific contact with DNA. Other residues that are important in bacteriophage repressors and that differ between Bicoid and Antp class homeodomain proteins include recognition helix residues 9, which could make nonspecific DNA contacts, and a residue near the amino terminus of the first helix of the motif (Bicoid residue 127). The position of residue 127 suggests that it might interact with the first residue in the recognition helix, and thus help position the recognition helix for favorable contact with DNA.

Bicoid binds to variants of a 9 bp consensus sequence, TCTAATCCC, that are found upstream of the transcription start site of the zygotically expressed form of hunchback (Driever and Nüsslein-Volhard, 1989). This hunchback element (or Bicoid binding site) is distinct from sites bound by other Drosophila homeodomain proteins. These sites, TCAATAAAT (Desplan et al., 1986) and (TAA), (Beachy et al., 1988) are bound by proteins encoded by engrailed, even-skipped, and the Antp class genes Ultrabithorax, Abdominal-A, and fushi tarazu (Desplan et al., 1986; Hoey and Levine, 1988; Beachy et al., 1988; Karsch and Bender, 1988).
personal communication; Samson et al., 1989). These sites are not recognized by the Bicoid protein (Samson et al., 1989; this paper). It seemed possible that Bicoid and other homeodomain proteins distinguish between these sites using residues in the recognition helix which differ, and are in positions corresponding to those important for repressor-DNA recognition.

In this study, we employed fusion proteins that contain the DNA binding domains of the bacterial LexA repressor or the yeast GAL4 activator (Brent and Ptashne, 1985; Ma and Ptashne, 1987a). Whenever examined, fusion proteins that carry a DNA binding domain from the native protein in addition to the LexA or GAL4 domain stimulate expression of target genes that carry binding sites for the native protein (Brent and Ptashne, 1985; Hope and Struhl, 1986; Fitzpatrick and Ingles, 1988; Sameen et al., 1988). We took advantage of this fact to examine gene regulation and DNA binding by Bicoid.

Here we show that DNA-bound LexA-Bicoid and GAL4-Bicoid fusion proteins stimulate gene expression in yeast. Bicoid fusion proteins activated target genes that carried upstream LexA operators, GAL4 sites, or Bicoid binding sites. We used the gene activation phenotype to establish which residues in the Bicoid homeodomain are required for DNA recognition. Unlike bacterial helix-turn-helix proteins, amino acids in positions 1, 2, or 5 of the putative recognition helix do not appear to be important. Instead, "helix swap" experiments showed that Bicoid's DNA specificity is determined by recognition helix amino acid 9. Our results also suggest that for Antp class and other closely related homeoproteins, DNA specificity inheres in residue 9.

Results

DNA-Bound Bicoid Activates Gene Expression

We tested whether Bicoid regulates gene expression by producing, in yeast, Bicoid fusion proteins that contained the DNA binding domains of either LexA or GAL4. Hybrid genes were constructed by fusing a bicoid cDNA (Berleth et al., 1988) to sequences encoding the amino-terminal 87 residues of LexA or the amino-terminal 147 residues of GAL4 (Figure 1). The fusion proteins were expressed from an ADH1 promoter on 2 μM-based plasmids carrying the yeast HIS3 gene. All but the first two amino acids of Bicoid were included in the fusions. To test whether DNA-bound LexA-Bicoid activates gene expression, yeast cells were co-transformed with target genes that carried an appropriate binding site positioned upstream of the transcription start site (Figure 2). Changes in β-galactosidase activity of cells grown in liquid culture.

Bicoid fusion proteins stimulated gene expression in yeast (Tables 1a and 1b). Target genes lacking upstream binding sites were not stimulated by Bicoid fusion proteins, while those carrying a LexA operator were stimulated about 50-fold by LexA-Bicoid, and those carrying a GAL4 binding site were stimulated about 20-fold by GAL4-
Helix 3 in the Bicoid Homeodomain Is Required for DNA Recognition

Amino acid substitutions were made within the putative recognition helix of the Bicoid homeodomain at positions we thought were likely to make base-specific contacts with DNA. To destroy DNA recognition, these residues were changed to alanines, small residues that do not hydrogen bond to base pairs in DNA, and have been shown not to disrupt the recognition helices of bacteriophage repressors (Hochschild et al., 1986; Wharton and Ptashne, 1987; Hochschild and Ptashne, 1986). By analogy with repressor proteins, the Bicoid recognition helix has been predicted to encompass, minimally, residues 138–147 in the native protein. Here, these ten amino acids will be referred to as positions 1 through 10.

To confirm that the mutant LexA-Bicoid proteins were produced in yeast, we tested their ability to stimulate gene expression of LexA operator-containing targets (Table 1c). Only one mutant protein did not satisfy this criterion (mutant A5A6A7); we assume this protein was unstable or defective in its activation function. As expected, none of the mutant proteins activated expression of target genes that did not contain upstream binding sites.

The mutant proteins were then assayed for activation of target genes that carried Bicoid binding sites (Table 1c). LexA-Bicoid mutant A5 contained a Thr→Ala substitution at position 1 and still activated a Bicoid site target. LexA-Bicoid mutant A6 contained a Lys→Ala substitution at position 5 and also activated a Bicoid site target but at reduced levels. However, activation by this mutant was also reduced using a LexA site target, perhaps because the protein is less stable or impaired in its activation function. Finally, a LexA-Bicoid mutant that contained a Lys→Ala substitution at position 9 did not activate a Bicoid site target, perhaps because the protein is less stable or impaired in its activation function.

Altered Specificity Bicoid Mutants

To identify those residues in Bicoid required for DNA specificity, we carried out “helix swap” experiments similar to those described by Wharton and Ptashne (1985). In these experiments, we changed the predicted solvent exposed residues in the Bicoid recognition helix to those found in the recognition helix of Antennapedia class proteins (Figure 3). The mutant proteins were tested for their ability to distinguish between two sites known to bind different classes of homeodomain proteins in vitro. As before, our assay measured expression of target genes bearing these upstream sites. Bicoid target genes contained 10 copies of the hunchback element (TCTAACCC), and Antp class...
target genes contained 12 copies of an Antp class binding site (TCAATTAATGA).

Results are given in Table 2. In the yeast strain used here, wild-type LexA-Bicoid and the various mutant proteins stimulated expression of LexA target genes to comparable levels, although these levels were uniformly lower than those obtained previously (see legend to Table 2). As expected, the mutant proteins did not stimulate target genes lacking upstream binding sites. Wild-type LexA-Bicoid activated Bicoid site targets but did not activate Antp class targets above background levels. LexA-Bicoid mutant E1R2, which contains a Thr→Glu substitution at position 1 and an Ala→Arg substitution at position 2, retained its ability to stimulate expression of target genes containing Bicoid sites and did not stimulate target genes containing Antp class sites. However, LexA-Bicoid mutant R2-E1R2Q9 which contained, in addition to the changes at positions 1 and 2, a Leu→Arg substitution at position 3 in helix 2 and a Lys→Gln substitution at position 9, lost the ability to stimulate target genes carrying Bicoid sites but gained the ability to stimulate target genes carrying Antp class sites. A LexA-Bicoid mutant bearing only three

Table 2. Gene Activation by Mutant Bicoid Derivatives

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Results are given in units of β-galactosidase activity. Experimental conditions are described in the legend to Table 1. Target plasmids (see Figure 2) were all pLR1Al derivatives and produced no detectable background activity, except for the Antp class binding site target plasmid which regularly produced about 30 U of background β-galactosidase activity. In these experiments yeast strain YM709 was used throughout because of very high background levels of β-galactosidase activity produced in strain MGLD4-4a when transformed with the Antp site target plasmid.
of these changes, E_F-Q_P exhibited a similar phenotype. This mutant also stimulated expression of target genes that carried the Antp type I(TAA) site (data not shown) recognized by Ultrathorax protein and other Antp class proteins (Beachi et al., 1988; Samson et al., 1989). The results above implicate the importance of residue 9 in DNA specificity. This was tested directly by constructing LexA-Bicoid mutant Q_P, which bears a single amino acid substitution of Lys → Glu at position 9 in the recognition helix. This mutant did not stimulate expression of Bicoid site targets but strongly stimulated expression of targets carrying Antp class sites. For each mutant bearing this Lys → Glu substitution at position 9, levels of activation of Bicoid targets were decreased more than 500-fold, while levels of activation of Antp class targets were increased more than 20-fold (Table 2). If one subtracts background levels obtained with the Antp class target (~30 units), then position 9 mutants activated Antp class targets more than 600-fold relative to wild-type LexA-Bicoid.

Discussion

Bicoid is a DNA Binding-Dependent Gene Activator

Bicoid is a gene activator. LexA-Bicoid and GAL4-Bicoid activated expression of target genes that contained LexA binding sites or GAL4 binding sites, and did not stimulate gene expression from target genes that lacked binding sites. The fusion proteins also activated expression of target genes carrying Bicoid binding sites. Since, in yeast, LexA sites and GAL4 sites are only bound by proteins that contain the cognate binding domains, we conclude that stimulation of gene expression by these Bicoid fusion proteins requires their binding to DNA (Brent and Ptashne, 1985; Giniger et al., 1985; Keegan et al., 1986). These results support the notion that activation of targets with Bicoid sites in our yeast system and in Drosophila cells (Driever and Nüsslein-Volhard, 1989) is a consequence of binding by Bicoid to these sites.

We do not know how Bicoid turns on transcription. Bicoid contains an acidic region (residues 345–390, net charge -9), a glutamine-rich region (or M-repeat, McGinnie et al., 1984), and a region that contains alternating histidines and prolines (or PRD repeat, Frigerio et al., 1986). Acidic regions, glutamine-rich regions, and proline-rich regions have all been shown to function as transcription activation domains (Hope and Struhl, 1986; Ma and Ptashne, 1987a, 1987b; Courey and Tjian, 1988; Williams et al., 1988). We observed activation by a GAL4-Bicoid derivative that contained only Bicoid residues 3–251. This protein lacked the acidic region and the polyglutamine tract but retained the PRD repeat. It is possible, therefore, that transcription activation by native Bicoid is due to the PRD repeat. Alternatively, it is possible that gene activation by this Bicoid derivative is due to phosphate groups. Bicoid is likely to be differentially phosphorylated during development (Driever and Nüsslein-Volhard, 1989); it is possible that the phosphate groups may substitute for acidic amino acids by providing equivalent negative surface charge (Lech et al., 1988; Sorger and Pelham, 1988).

Oligomerization of Bicoid

The DNA-bound form of LexA-containing proteins is a dimer (Brent and Ptashne, 1981, Brent, 1982). As previously noted for other fusion proteins, the amino terminus of LexA does not dimerize efficiently, and the fact that LexA-Bicoid recognizes LexA operators suggests that the Bicoid moiety contributes to dimerization (Brent and Ptashne, 1985; Hope and Struhl, 1986; Lech et al., 1988; Samson et al., 1989). Whether dimerization occurs on or off the DNA, the results with LexA-Bicoid thus suggest that in Drosophila embryos, native Bicoid binds DNA as an oligomer. Our data suggest Bicoid oligomers may exhibit another form of cooperativity. In yeast, Bicoid strongly stimulated targets that carried ten hunchback elements, but did not detectably stimulate targets that carried two hunchback elements. This result, which is similar to results observed with a number of homeodomain proteins in transient expression experiments (Desplan et al., 1988; Jaynes and O'Farrell, 1988; Driever and Nüsslein-Volhard, 1989), suggests that either binding to the hunchback elements or gene activation by the DNA-bound proteins is highly cooperative. LexA-Bicoid dimers activate a single LexA site target more strongly than they activate a target with two Bicoid sites, which suggests that the cooperativity facilitates binding to the Bicoid sites rather than gene activation once the protein is bound to DNA. Clusters of Bicoid binding sites occur upstream of the hunchback element (Berleth et al., 1988); cooperative binding of Bicoid oligomers to these sites provides a mechanism by which the continuous Bicoid gradient might be converted into the three-cell-wide boundary observed for hunchback expression (Tautz, 1988).

Unexpected DNA Contact by the Bicoid Recognition Helix

While nothing so simple as a code has emerged, a large body of work in recent years has suggested that interaction of helix-turn-helix proteins with DNA is governed by some common rules (for review, see Pabo and Sauer, 1984; Ptashne, 1986; Schleif, 1988). Protein monomers typically associate into dimers, in which each symmetrically disposed monomeric subunit interacts with symmetric or near symmetric operator half-sites that lie in adjacent major grooves. In the case of lambdoid phage repressors and cro proteins, and the E. coli CAP protein, the second a-helix of the helix-turn-helix motif (the recognition helix) protrudes into the major groove of DNA, and the amino-terminal end of the helix makes base-specific contacts. Sequence alignments (Laugton and Scott, 1984; Scott et al., 1989) and NMR studies (Ottig et al., 1988) have suggested that Drosophila homeodomain proteins contain a helix-turn-helix motif. If the Bicoid homeodomain interacts with DNA, as do bacteriophage repressors, we reasoned that base-specific contacts would be made by amino acids 1 and 2 of the recognition helix, with possible contributions from residues at positions 5, 6, and 9.
To test this idea, we made a series of mutant proteins in which residues in the putative Bicoid recognition helix were replaced by alanines. The LexA-Bicoid mutants were examined for their ability to stimulate expression of target genes that carried upstream Bicoid binding sites. These experiments benefited from the fact that the Bicoid derivatives were bifunctional for DNA recognition. By comparing activation of target genes that carried LexA sites or Bicoid sites with those of "wild-type" LexA-Bicoid, we could ascertain whether a given mutation affected Bicoid DNA specificity, or affected some other property of the protein (such as nuclear transport or protein stability). Results indicated that positions 1, 2, and 5 were not important for recognition of Bicoid sites. However, a mutation that bore alanine at position 9 no longer activated Bicoid targets. These experiments suggested that residue 9 in the recognition helix is important for either specific or nonspecific interaction with DNA.

To probe the function of individual amino acids in site-specific DNA recognition, we performed "helix swap" experiments (Wharton et al., 1984; Wharton and Ptashne, 1985). First, we established that Bicoid does not recognize Antp class sites and that proteins of the Antp class genes, Ultrabithorax and Abdominal-A, do not recognize Bicoid sites (this paper; Samson et al., 1989). Based on these facts, we attempted to change the DNA specificity of Bicoid by replacing amino acids in its recognition helix with those found at equivalent positions in the Antp class proteins. A series of LexA-Bicoid derivatives that contained changes at recognition helix residues 1, 2, and 9, and Bicoid residue 127 (in helix 2) were tested for recognition of Bicoid and Antp class sites. Proteins that contained changes at positions 1 and 2 still recognized Bicoid sites whereas all proteins with changes that included a Lys-+Gln substitution at position 9 no longer activated Bicoid targets. Based on the identity of position 9, there are at least three other classes of homeodomain proteins: proteins such as paired product, which has a Ser at this position, proteins such as cut product, which has His at this position, and POU class proteins, which have Gys at this position (Frigero et al., 1986; Blochinger et al., 1988; Finney et al., 1988; Herr et al., 1988). If these proteins contact DNA like the proteins studied here, then all members of a class should bind similar sequences, members of different classes should recognize different sequences, and in each case some of the DNA specificity should derive from contacts between residue 9 and the binding site.

Experimental Procedures

Strains, Media, and β-Galactosidase Assays
Escherichia coli strains were JM101 (supE thi lac-proAB [Ptra3D6 proAB lacP ZAM15]), BW313 (HfrKL16 P046 [lysA[6-1] drit ung1 thi relA]). CS570 (Ala-leu-thi ara strAfrA [proAB Flacig ZAM15 tra3D6]), BMH 71-18 ([ lac-proAB] thi supF P046 [ZAM15 proAB]), BMH 71-18 mutS ([ lac-proAB] thi supE Flacig ZAM15 proAB), BMH 71-18 mutS ([ lac-proAB] thi supE Flacig ZAM15 proAB mutS216:3nt), NKG 3 ([ lac-proAB] tro gae sth Placop ZAM15 proAB). Saccharomyces cerevisiae strains used were MGLD4-4a (a ura3-52 leu2 his3 trp1 lys2 cyr), RBY50 (a Dgah ura3-52 leu2 his3), and YM109 (a ura3-52 his3-200 ade2-101 His2-807 trp1-111 tyr1-111 leu2-807 can4-542 galo6-538). Bacteriological work was done using standard techniques (Miller, 1972; Ausubel et al., 1987). Yeast media preparation and cell growth were carried out as described in Sherman et al. (1978). Yeast transformations were done using the LlOAC procedure (Ito et al., 1983). β-Galactosidase assays of liquid cultures were carried out as described by Harashman (1985) using at least three individual yeast transformants. Cells were grown in complete minimal medium (carbon source and histidinone) to mid-logarithmic stage and one ml was collected by centrifugation. The cell pellet was resuspended in 200 μl of 0.1 M Tris-Cl (pH 7.5), 0.01M Triton X-100 and frozen at ~80°C. After thawing slowly on ice, β-galactosidase assays were carried out on permeabilized cells, and cell debris was then removed by centrifugation. Units are expressed as (1000)A420/(minutes of reaction)(cell volume)/OD660 and varied up to about 20% between individual isolates.

Plasmid Construction
DNA manipulations were carried out by standard methods (Maniatis et al., 1980; Ausubel et al., 1987). Oligonucleotides were synthesized by the phosphite triester method using a Bioscribe Synthesis 8000 DNA Synthesizer and purified using Applied Biosystems oligonucleotide purification cartridges. A GAL4-fusion plasmid, pMA424 (Ma and Ptashne, 1987b) was ob-
tained from Jun Ma. A LexA-fusion plasmid, pSH2-1, was constructed by insertion of an EcoRI–SacI (~3 kb) fragment of pMA424 containing a polylinker, ADH1 terminator, and yeast 2 µm replicator into the EcoRI–SacI backbone of pMA47 (Ma and Plasmide, 1987). The ADH1 promoter–LexA fragment in pMA47 was originally derived from pRBlO27 (Brent and Ptashne, 1985). The second BamHI site upstream of the ADH1 promoter in this plasmid (pSH1-1) was then destroyed creating pSH2-1. Plasmid pSH2-1 is essentially identical to pMA424 except that the DNA binding region of GAL4 (residues 1-147) has been replaced by the DNA binding region of LexA (residues 1-87). Both plasmids contain the HIS3 gene for selection in yeast. Unique sites in these plasmids for fusions to LexA or GAL4 are EcoRI, BamHI, and SalI. The sequence of the polylinker from EcoRI to SalI is GAATTCGGGCAGTATGCGAC.

The reading frame in the polylinker in each of the plasmids (pSH2-1, pMA424) is the same, and is indicated by the underline. Fusion of a nearly full length bicoid cDNA (Berleth et al., 1988) to LexA in pMA457 was accomplished in several steps. First, the cDNA (in EcoRI site of a Bluescript vector) was digested with Hpal, made flush with T4 DNA polymerase, and then digested with SalI. The 743 bp fragment was then ligated to pMA424 that had been previously digested with BamHI, Klenow treated, and SalI digested. The resulting plasmid was then digested with SalI, and the remainder of the bicoid cDNA sequence (including more than 1 kb of 3′ flanking sequences) was inserted as a SalI–SalI (~1.8 kb) fragment. The final plasmid, pSH1-1, encodes an in-frame fusion of GAL4 (residues 1-147) to residue 3 (Gln) of the bicoid coding sequence separated by an 18 bp polylinker sequence. A BamHI–SacI fragment containing bicoid and about 2.5 kb of adjacent vector sequences was removed and inserted into the BamHI–SacI backbone of pSH2-1. The resulting plasmid, pSH1-1, contains an in-frame fusion of LexA (residues 1-87) to residue 3 of Bicoid via a 21 bp linker. The top strand sequence across the junction, and the corresponding amino acid sequence of the LexABicoid fusion is as follows: CCT(LexA)-CGACCCAGGTGGCGGCCTGGTTBGAACCGTCGACGTCGTCA-CAAG(Bicoid3)-P-RPEFPGI-Q. The top strand sequence across the junction, and corresponding amino acid sequence of the GAL4–Bicoid fusion is as follows: TCG(GAL4)-CGCGAGACCCTGCGCGGATC-CAA(Bicoid3), P-RPEFPGI-Q. The two strands and the corresponding amino acid sequence of the LexA–Bicoid fusion as well as the Bicoid–LexA fusion are identical.

All target plasmids (except pSV15) were derivatives of LR1.11 (West et al., 1985) and contain the yeast URA3 gene, 2 µm replicator, and a GAL4–LexA fusion gene downstream of a GAL4 promoter from which the GAL7 promoter-LexA fragment in pSH1-1 was then digested and cloned into the EcoRI site of pMA47. The other plasmids were all derivatives of LR1 Al (West et al., 1985) and contain the HIS3 gene for selection in yeast. The LexA insert in pMA47 was then destroyed by digestion with EcoRI and BamHI. The DNA binding region of LexA was then replaced by the DNA binding region of GAL4 (residues 1-147) to create the GAL4–LexA expression plasmid pMA424 (Brent and Ptashne, 1985). The GAL4–LexA plasmid was then digested with SalI, and the resulting plasmid was then digested with BamHI, Klenow treated, and SalI digested. The resulting plasmid was then digested with SalI, and the remainder of the bicoid cDNA sequence (including more than 1 kb of 3′ flanking sequences) was inserted as a SalI–SalI (~1.8 kb) fragment. The final plasmid, pSH1-1, encodes an in-frame fusion of GAL4 (residues 1-147) to residue 3 (Gln) of the bicoid coding sequence separated by an 18 bp polylinker sequence. A BamHI–SacI fragment containing bicoid and about 2.5 kb of adjacent vector sequences was removed and inserted into the BamHI–SacI backbone of pSH2-1. The resulting plasmid, pSH1-1, contains an in-frame fusion of LexA (residues 1-87) to residue 3 of Bicoid via a 21 bp linker. The top strand sequence across the junction, and the corresponding amino acid sequence of the LexABicoid fusion is as follows: CCT(LexA)-CGACCCAGGTGGCGGCCTGGTTBGAACCGTCGACGTCGTCA-CAAG(Bicoid3)-P-RPEFPGI-Q. The top strand sequence across the junction, and corresponding amino acid sequence of the GAL4–Bicoid fusion is as follows: TCG(GAL4)-CGCGAGACCCTGCGCGGATC-CAA(Bicoid3), P-RPEFPGI-Q. The two strands and the corresponding amino acid sequence of the LexA–Bicoid fusion as well as the Bicoid–LexA fusion are identical.


