Gene Activation and DNA Binding by Drosophila Ubx and abd-A Proteins

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Summary

The Ubx and abd-A gene products are required for proper development of thoracic and abdominal structures in Drosophila. We expressed LexA-Ubx and LexA-abdA fusion proteins in yeast. These proteins activated expression of target genes that carried either upstream LexA operators or upstream Ubx binding sites. Both proteins contain homeodomains. Experiments with mutant fusion proteins show that the homeodomain is not required for the proteins to form dimers or enter the nucleus, and that, when DNA binding is provided by the LexA moiety, the homeodomain is not required for gene activation. Our results suggest that the homeodomain is necessary for these proteins to bind Ubx sites, but that the homeodomain does not contact DNA exactly like bacterial helix-turn-helix proteins. Finally, our data suggest that gene activation by these proteins is a simple consequence of their binding to DNA, while negative gene regulation requires that these proteins act together with other Drosophila gene products.

Introduction

Early Drosophila development depends on the action of a gene hierarchy that directs the progressive subdivision of the embryo and its differentiation into parasegments. It is now thought that establishment of the dorsal–ventral and anterior–posterior body axes result from the action of maternal effect genes, while the latitudinal subdivision of the embryo into parasegments is mediated by the sequential action of gap genes, pair-rule genes, and segment polarity genes (Nusslein-Voichard and Wieschaus, 1980; Akam, 1987). Subsequent proper development of each parasegment requires expression of the homeotic genes. Ultrabithorax (Ubx) and abdominal-A (abd-A) are homeotic genes located in the bithorax complex. Mutations in Ubx and abd-A transform parasegments into more or less exact replicas of others (Lewis, 1978). The products of these genes have specific realms of activity within the embryo: proper formation of structures specific to parasegments 5–13 requires Ubx, while formation of structures specific to parasegments 7–14 requires abd-A (Lewis, 1978; Morata et al., 1983; Sánchez-Herrero et al., 1985; Akam, 1997).

Current evidence is consistent with the idea that Ubx and abd-A proteins may control development by stimulating or repressing gene expression. One fact that suggests this idea is that both proteins contain homeodomains. These domains are conserved stretches of amino acids, initially found in developmentally important Drosophila proteins (Birglin, 1988; Scott et al., 1989). Sequence similarity and physical data suggest that the C-terminal part of homeodomains folds into a helix-turn-helix motif like the DNA binding motif found in many prokaryotic proteins (Laughon and Scott, 1984; Scott et al., 1989; Otting et al., 1988), and led to the hypothesis that homeodomains might specify the binding of proteins that contain them to specific sites on DNA. It has also been proposed that homeodomains are important for proteins containing them to be transported to the nucleus, and to form oligomers (Hall and Johnson, 1987; McGinnis et al., 1984).

Experiments in vitro suggest that Ubx and abd-A proteins indeed bind to specific sites on DNA. Purified Ubx protein binds to sites upstream of the Ubx and Antennapedia coding sequences (Beachy et al., 1986). Recent experiments by Simon and Bender show that abd-A protein also binds DNA fragments containing these sites (personal communication). If the two proteins in fact bind the same sites, it is likely that binding depends on their homeodomains, since 56/61 of the amino acids within the two homeodomains are identical, but the proteins have no significant sequence similarity outside of this region (Karch and Bender, personal communication).

There is more direct evidence that Ubx and abd-A proteins regulate gene expression in living cells. Endogenous expression of Ubx protein in the visceral mesoderm of Drosophila embryos increases expression of a Ubx-lacZ fusion transgene (Bienz and Tremml, 1988). Expression of Ubx protein in HeLa cells stimulates expression of cotransfected target genes that carry upstream Ubx protein binding sites (Thali et al., 1988). In Ubx mutant embryos, Antp RNA is expressed in parasegments 5 and 6, but Ubx RNA is not, which suggests that Ubx protein normally represses Antp expression in these parasegments (Hafen et al., 1984; Carroll et al., 1986). Similarly, in abd-A mutant embryos, the amount of Ubx mRNA is increased in cells that normally produce abd-A protein (Struhl and White, 1985); in wild-type embryos, expression of a Ubx-lacZ fusion gene is diminished in cells that produce abd-A protein (Bienz and Tremml, 1988).

In our experiments, we wished to address three important issues. First, does gene regulation by these proteins depend on their binding to DNA in the vicinity of the regulated genes? Second, if the proteins regulate expression of nearby genes when bound to DNA, is that regulation positive or negative? Third, is the homeodomain required...
for the proteins to bind to specific sequences, or does it serve other functions? In order to answer these questions, we studied gene regulation by Ubx and abd-A proteins in yeast, in the absence of other Drosophila products. Our experiments relied on the fact that transcription of many yeast genes requires the binding of activator proteins to sites upstream of the transcription start site. If an appropriate binding site is positioned upstream of a gene, transcription can also be stimulated by chimeric proteins that contain a DNA binding domain fused to a transcriptional activator (Brent and Ptashne, 1985; Ma and Ptashne, 1987; Struhl, 1987; Lech et al., 1988).

We expressed LexA-Ubx and LexA-abdA fusion proteins in yeast and monitored their effect on expression of target genes. When the target genes carried upstream LexA operators or Ubx binding sites, the fusion proteins activated their expression. These results suggested that DNA binding by the fusion proteins was required for gene activation. We then generated a set of mutant LexA-abdA and LexA-Ubx fusion proteins. We used the yeast gene activation phenotype of these mutants to study the function of their homeodomains. Studies with the mutant proteins show that the homeodomain is not required for gene activation, nor is it required for the proteins to enter the nucleus or to form dimers and bind LexA operators. Our experiments suggest that the homeodomain is required for the fusion proteins to bind to their native sites, but that specific DNA recognition by the homeodomain differs from DNA recognition by analogous bacterial regulatory proteins. Finally, our results suggest that, in Drosophila, activation of gene expression occurs when Ubx and abd-A proteins bind DNA upstream of a gene, but that repression of gene expression may require additional Drosophila proteins.

Results

Plasmids directing the synthesis of LexA-Ubx and LexA-abdA proteins ("maker plasmids") were constructed and introduced into yeast (Figure 1). Western gel analysis (Figure 2) shows that, in yeast, LexA-Ubx and LexA-abdA have apparent molecular weights of 42 kd and 44 kd, close to their predicted molecular weights of 45 kd and 43 kd. To test the effects of these proteins on gene expression, we introduced LexA-Ubx or LexA-abdA maker plasmids into yeast, together with "target" plasmids, which contained a CYCl-lacZ or GAL1-lacZ "target gene" and an upstream LexA operator. Yeast colonies that contained maker and target plasmids were selected on solid medium and grown in liquid medium under continuous selection for both maker and target. The amount of β-galactosidase produced by log-phase cultures of these cells was used to measure expression of the lacZ fusion gene.

Figure 3 (lanes a, b, c, and d) shows that LexA-Ubx and LexA-abdA both activated gene expression from targets that contain LexA operators (LexA targets), but not from targets that do not contain LexA operators. The fusion proteins activated gene expression from targets that contain a LexA binding site 128, 178 (Figure 3), and 350 (not shown) nucleotides upstream of the transcription start site. Both proteins stimulated expression of LexA targets that were integrated into the chromosome (not shown). Both proteins stimulated gene expression of both LexAop-CYC1-lacZ and LexAop-GAL1-lacZ target genes, demonstrating that activation was not specific to a given promoter (Figure 4).
Gene Regulation and DNA Binding by LexA-Ubx and LexA-abdA

Figure 3. β-Galactosidase Assays

Targets pLG670Z (a), 1107 (b), pSH1521 (c), JK101 (d), p206 (e), and p209 (f), are described in Experimental Procedures, as are the maker plasmids for LexA-Ubx, LexA-abdA, LexA, LexA-GAL4, and the parental pAAH expression plasmid. pLG670Z bears a CX74acZ fusion gene, but does not contain the CYCl UAS. 1107 is a derivative of pLG670Z that bears a LexA operator upstream of the fusion gene. The targets derived from LRlA206 were pSH15-21 (which contains a Bicoid site), JK101 (which contains a LexA binding site), ~206 (which contains a 45-mer U-A Ubx binding site) and p209 (which contains a 77-mer U-B Ubx binding site). Since we used medium containing glucose, the GAL1 UAS upstream of these binding sites remained inactive (Yocum et al., 1984). The numbers correspond to units of β-galactosidase activity. Values greater than 50 were rounded to the closest multiple of 5. NT not tested.

We repeated the experiments described above with Ubx target plasmids. These targets were similar to those previously described, except that they contained sites called U-A or U-B that lie upstream of the Ubx coding sequence and bind Ubx protein in vitro (Beachy et al., 1988). Both LexA-Ubx and LexA-abdA activate gene expression from these targets (Figure 3). Both fusion proteins stimulate expression of CYC1-lacZ fusion genes if and only if those genes carry upstream Ubx sites (data not shown). Both fusion proteins stimulate gene expression from targets that contain the U-A Ubx site in the opposite orientation (data not shown). LexA-Ubx and LexA-abdA do not stimulate gene expression of GAL1-/lacZ targets that carry upstream sites that are bound by bicoid protein in vitro (Bicoid targets) (Driever and Niisslein-Volhard, 1989), while a LexA-Bicoid fusion protein does (Hanes and Brent, submitted).

In the Ubx protein used in our experiments, the homeodomain spans amino acids 294–354; in the abd-A protein used, it spans residues 137–197 (see Experimental Procedures). To explore the role of this domain in gene regulation and DNA binding, we utilized naturally occurring restriction sites to generate five C-terminal deletions of LexA-Ubx, and three C-terminal deletions and an internal deletion of LexA-abdA (Figure 4). We called the resulting proteins LexA-Ubx383, LexA-Ubx308, LexA-Ubx260, LexA-Ubx183, LexA-Ubx130, LexA-abdA285, LexA-abdA184, LexA-abdA72, LexA-abdAΔ67-179 (the naming scheme is explained in Experimental Procedures). We also used site-directed mutagenesis to construct a mutation called LexA-abdA*, in which Glu179 and Arg180, the presumptive first two amino acids of the homeodomain recognition helix, were replaced by alanines (Figure 4, Experimental Procedures). As judged by immunoblot analysis, all proteins except LexA-abdAΔ72 were produced in yeast, and their migrations on polyacrylamide gels were consistent with their predicted molecular weights (not shown).

We measured gene activation by these mutant fusion proteins on LexA and Ubx targets (Figure 4A). The amount of gene activation by LexA fusion proteins depends both on their intrinsic strength as activators and on the degree to which they occupy their binding sites upstream of target genes. Because we lacked a precise measure of site occupancy by the mutant proteins, we interpreted the mutant phenotypes using strictly qualitative criteria, dividing them into three classes based on their ability to stimulate gene expression from different targets (see Discussion).

Class 1 Mutants

LexA-Ubx383 stimulated gene expression both from LexA targets and from Ubx targets (Figure 4A). The fact that this protein behaved indistinguishably from wild-type LexA-Ubx indicates that the seven C-terminal amino acids of LexA-Ubx are required neither for gene activation nor for binding DNA. Similarly, LexA-abdA285 stimulated expression of LexA and Ubx targets, which shows that the 46 C-terminal amino acids of LexA-Ubx are required neither for gene activation nor for binding Ubx sites (Figure 4B). Finally, LexA-abdA* directed the synthesis of approximately half the amount of β-galactosidase as LexA-abdA, both from Ubx targets and from LexA targets (Figure 4A). Even though gene activation by this protein was lower than that observed for wild-type LexA-abdA, the fact that the ratio of LexA target activation to Ubx target activation by the two proteins was the same suggests that LexA-abdA* binds normally to Ubx sites. All class 1 mutants activated gene expression from JK101nt, a LexA target integrated into the chromosome; LexA-abdA and LexA-abdA* activated this target equally (not shown).

Class 2 Mutants

LexA-Ubx308 and LexA-Ubx260 activated gene expression from LexA targets but not from Ubx targets (Figure 4A). The simplest interpretation of this phenotype is that
Figure 4. The Fusion Proteins and Their Properties

(A) The LexA domain is depicted by the open box on the left, the homeodomain by the solid box. Presumptive α helices in the homeodomain are indicated by solid lines drawn above the solid box. The mutation introduced into LexA-abdA*, which changes Glu and Arg at position 1 and 2 of helix 3 to alanines, is shown by the symbol L. Except for LexA-abdA72, which was not detected, immunoblot analysis using anti-LexA antibodies showed that the predicted size of the proteins in MGLD4-4a is consistent with their migration on polyacrylamide gels (not shown). The amount of β-galactosidase produced from a target containing a LexA operator (JK101), and from a target containing Ubx sites (p209), was determined in the presence of the different proteins. Suppression of amber mutations was tested as described in the text, and was monitored on X-gal plates. Symbols: +, blue on X-gal plates; -, white on X-gal plates; NA, suppression test not applicable. In all cases, stimulation of target gene expression depended on the presence of LexA or Ubx sites in the target. (B) shows the sequence of the Ubx homeodomain. The Ubx protein in our experiments is 369 amino acids long; amino acids 294-354 comprise the homeodomain (Weinzierl et al., 1987; O'Connor and Bender, personal communication; D'Connor et al., 1988). Predicted α helices in the Ubx protein homeodomain, positioned by analogy with the α helices of the Anfp protein homeodomain (McGinnis et al., 1984; Otting et al., 1988) are underlined and labeled H1, H2, and H3. The abd-A protein homeodomain is identical to that in Ubx protein at 56/61 positions; all of the residues in helix 3 are identical (Karch and Bender, personal communication). The LexA-abdA* mutation changes the first two residues of the abd-A protein recognition helix to alanines.

these proteins contain a region sufficient for gene activation, but do not contain the portion of the protein necessary to bind Ubx sites. LexA-abdAA67-179 showed the same behavior on LexA and Ubx targets (Figure 4A). All class 2 mutants activated gene expression from the integrated LexA target JK101int; on this target, LexA-Ubx and LexA-Ubx260 showed identical activity (not shown). None of the class 2 proteins contained an intact homeodomain (Figure 4A).

Class 3 Mutants

Although the proteins were synthesized in yeast, LexA-Ubx183, LexA-Ubx130, and LexA-abdA184 did not stimulate gene expression from targets that contained either LexA operators or Ubx sites (Figure 4). We did not ascertain whether these proteins bound DNA in vivo. Immuno-

Suppression of the class 1 amber mutants, LexA-Ubx383 and LexA-abdA285, had no effect on their gene
activation phenotypes; both proteins still stimulated expression both from LexA and from Ubx targets (Figure 4A), which suggests that small insertions at these positions in Ubx and abd-A proteins (see Experimental Procedures) do not affect their DNA binding or gene activation. There were no class 2 amber mutants. Two class 3 amber mutants, LexA-Ubx103 and LexA-abdA72, became class 1 mutants when suppressed; that is, when suppressed, they activated gene expression from LexA and Ubx targets (Figure 4), again suggesting that small insertions at these positions in the proteins do not affect gene activation or DNA binding. Suppression did not correct the inactivity of the two other class 3 amber mutants, LexA-Ubx130 and LexA-abdA184.

Discussion

LexA-abdA and LexA-Ubx Are DNA Binding–Dependent Gene Activators

LexA-Ubx and LexA-abdA proteins activate expression of genes that carry either upstream LexA or Ubx binding sites. Both proteins stimulate expression of CYC7-lacZ and GAL7-lacZ genes that carry upstream binding sites at various positions. Target gene activation is not dependent on the orientation of the binding site. The properties of LexA-Ubx and LexA-abdA thus resemble the properties of other DNA binding–dependent transcription activators (Brent and Ptashne, 1985; Lech et al., 1988; Ptashne, 1988). By analogy with these other proteins, we presume that stimulation of gene expression by LexA-Ubx and LexA-abdA occurs because these proteins bind DNA upstream of target genes and stimulate their transcription. Similar activation of yeast gene expression by fusion proteins that carry the DNA binding domain of GAL4 fused to the fushi tarazu protein has recently been reported (Fitzpatrick and Ingles, 1985).

Activation of LexA Targets Does Not Require the Homeodomain

Many mutant proteins that did not contain intact homeodomains activated expression of LexA targets. In fact, in two cases (LexA-Ubx260 and LexA-abdA*), the mutant proteins activated expression of an integrated LexA target as well as the wild-type proteins. These facts show that, when DNA binding is provided by the LexA moiety, the homeodomain is not required for gene activation. Although many of the mutant proteins differed in the strength with which they activated plasmid-borne target genes, we did not measure the extent to which the mutant proteins occupied their binding sites, and we were therefore reluctant to ascribe much importance to quantitative differences in gene activation.

The fact that LexA-Ubx260 activates expression of LexA targets shows that amino acids 57–260 of Ubx protein are sufficient for gene activation. Similarly, the fact that LexA-abdAA67-179 activates LexA targets indicates that amino acids 17–66 and 180–285 are sufficient for gene activation. Inspection of the Ubx and abd-A protein sequences (Kornfeld et al., 1989; Akam, 1987; Karch and Bender, personal communication) shows that they do not contain the obvious acidic stretches sometimes sufficient for function of eukaryotic activators (Ptashne, 1988). Interestingly, all active abd-A protein derivatives contained a stretch between 227 and 258 in which 26 out of 31 amino acids are glutamines; such glutamine-rich stretches have been proposed to be important in gene activation by the eukaryotic activator Sp1 (Courrey and Tjian, 1980). However, we favor the idea that gene activation by these proteins depends on phosphate groups, which might mimic acidic amino acids by contributing equivalent negative surface charge (Lech et al., 1988; Sorger et al., 1988; Lech, Besmond, and Brent, unpublished data; Fanning and Brent, unpublished data).

Gene activation by LexA fusion proteins is a complex phenotype that requires that the proteins enter the nucleus, form dimers, and bind to sites on DNA. The homeodomain has been proposed to be involved in both nuclear entry and dimerization (Hall and Johnson, 1987; McGinnis, 1985). The fact that the class 2 mutants activate gene expression of LexA target genes shows that, whatever it may contribute to either process, it is not necessary for either.

The Homeodomain Is Required for Activation of Ubx Targets

LexA-Ubx activates gene expression from targets that carry upstream sites to which Ubx protein binds in vitro, presumably because it binds to those sites in yeast. LexA-abdA has the same properties, which argues that abd-A protein binds to the same sites as Ubx protein. Class 2 mutant proteins do not contain intact homeodomains and do not activate Ubx targets, presumably because they do not contact Ubx protein binding sites in yeast. Ubx and abd-A proteins do not share significant homology, outside of their homeodomains (Karch and Bender, personal communication). Taken together with this fact, our results strongly suggest that the DNA binding region of Ubx and abd-A proteins is the homeodomain.

In other proteins, the homeodomain also seems to confer DNA specificity (Desplan et al., 1985, 1988; Müller et al., 1988; Mihara and Kaiser, 1988). Sequence comparison (Laughon and Scott, 1984; Bürglin, 1988; Scott et al., 1989) and physical data (Müller et al., 1988) suggest that the homeodomain folds into three α helices, with the last two forming a motif similar to the helix-turn-helix motif found in prokaryotic DNA binding proteins (Pabo and Sauer, 1984; Scott et al., 1989). By analogy with these bacterial proteins, it is often supposed that the last helix, the so-called recognition helix, determines DNA specificity (Laughon and Scott, 1984; Desplan et al., 1988; Scott et al., 1989). By further analogy with the bacterial proteins, we might imagine that major groove contacts made by the first two amino acids of the recognition helix would be critical for DNA specificity (Hochschild et al., 1986; Wharton and Ptashne, 1987).

To test this idea, we created LexA-abdA*, a mutant in which the glutamic acid and arginine at positions 1 and 2 of the putative abd-A protein recognition helix were replaced by alanines. This substitution was chosen since the DNA contacts likely to be made by glutamic acid and arginine cannot be made by alanine, a smaller, chemically
dissimilar amino acid (Seeman et al., 1976; Hochschild et al., 1986; Wharton and Ptashne, 1987). Unexpectedly, LexA-abdA* activated gene expression of Ubx targets. This result suggests that amino acids 1 and 2 of the abd-A protein recognition helix are not essential for Ubx specificity, and that the specific DNA contacts made by LexA-abdA may differ from those made by the prokaryotic helix-turn-helix proteins.

Biological Significance

Our experiments suggest that Ubx and abd-A proteins regulate gene expression if and only if they are bound to DNA in the vicinity of the regulated genes. In yeast, in the absence of other Drosophila proteins, Ubx binding by Ubx protein and abd-A protein activates nearby genes. In Drosophila, Ubx protein has been shown both to activate and to diminish gene expression, whereas abd-A protein has thus far only been observed to diminish it (Hafen et al., 1984; Struhl and White, 1985; Carroll et al., 1986; Thali et al., 1988; Bienz and Tremml, 1988). Based on our results, we propose that negative gene regulation by these proteins, for example, the apparent repression of Ubx expression by abd-A protein in the visceral mesoderm (Bienz and Tremml, 1988), requires the interaction of DNA-bound Ubx and abd-A proteins with ancillary Drosophila-specific proteins.

Experimental Procedures

Strains and Transformants

Strains were propagated and transformations were performed according to standard procedures (Miller, 1972; Sherman et al., 1986; Ausubel et al., 1987). E. coli strains were MM294, endA1 thi-1 hsdR17 (Backman et al., 1976), used for plasmid DNA propagation and for most constructions, and CJ382, dh5a ung-1 thi-1 relA1 F- thr (pCJ105 Cmr) and SC5-1, F- endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1, used to construct LexA-abdA*. Saccharomyces cerevisiae strains were MGLD-4 4a, a leu2 ura3 his3 trpl lys2, the standard host (a gift from Doug Treco), and DJ511-5-3, a ura3 bar1 ade2 his4 leu2 lys2 trp7 tyr1 SUP4-3 cry1, a gift from Duane Jennes, which contains a temperature-sensitive tyrosine amber suppressor and was used to test suppressibility of amber mutations.

Plasmids

Target plasmids, shown in Figure 3, carry a 2 μm replicator, a URA3 gene, a CYCl-lacZ or GAL1-lacZ fusion gene, and various sequences upstream. Both pLG6702 and LR1a208 have been previously described (West et al., 1984; Brent and Ptashne, 1985). Plasmid 1107 is a pLG6702 derivative containing a LexA operator, which is a binding site for a LexA dimer, 178 bp upstream of the CYCl-lacZ transcription start (Brent and Ptashne, 1985; see Figure 3). We constructed JK101int from JK101 (Kamens, unpublished data), which is an integration of the resulting proteins (data not shown). All of the proteins, except LexA-abdA72, were stably synthesized in MGLD-4 4a (data not shown).

LexA fusion proteins

Differential splicing of the Ubx transcripts gives rise to a family of related proteins (O'Connor et al., 1988, Kornfeld et al., 1989). Plasmid E6-9#7 contains a cDNA that encodes the largest Ubx product (499 amino acids) (U'Connor et al., 1988; Kornfeld et al., 1989). We constructed a lexA-Ubx fusion gene by inserting a 1.9 kb PstI fragment from E6-9#7 into the PstI site of pKA1035 (Lech et al., 1988) to create pU01 (Figure 1). Plasmid FNC211 encodes a 330 amino acid form of the abd-A protein (Karch and Bender, unpublished data). We constructed a lexA-abdA fusion gene by inserting the 1.1 kb BsaBI-Xmal fragment from this plasmid into the Xmal site of pKA1035 (Lech et al., 1988) to create pA36 (Figure 1). The HindIII fragment that contained each fusion gene was inserted into the unique HindIII site of pAA115 to create pU02, the LexA-Ubx maker plasmid, and pA43, the LexA-abdA maker plasmid.

Fusion protein derivatives

Mutant proteins were generated by deleting portions of the corresponding fusion genes or by inserting oligonucleotides specifying termination codons into restriction sites within the genes. The oligonucleotides used were a Nhel linker (5'-TCAGTGCGTAGCT-3') and an XmaI linker (5'-CTAGTCTAGACTAG-3') from New England Biolabs. Restriction analysis of the resulting DNAs, and size determination of the resulting proteins (data not shown) were used to verify the mutations. The number in the mutant designation indicates the most C-terminal amino acid derived from wild-type Ubx or abd-A proteins. The C-terminal five amino acids at the U-terminus of each mutant that derive from the native protein are listed below, together with the newly added amino acids resulting from the manipulations that created the mutation. All of the proteins, except LexA-abdA72, were stably synthesized in MGLD-4 4a (data not shown).

LexA-Ubx183, AALVSAE, was generated by cutting pU01 with Styl, making the ends flush with Klenow, and inserting a single Xmal linker at the filled-in site. LexA-Ubx308, RYQFLD, was generated by cutting pU01 with Xhol, making the ends flush with Klenow, and inserting a Nhel linker in the filled-in site. LexA-Ubx260, SIKRGSQRTGE, was generated by cutting pU01 at two internal Bglll sites, one of which cuts in the coding sequence, the other of which cuts downstream of the coding sequence, and ligating the cut DNA. LexA-Ubx193, RGGGALS, and LexA-Ubx130, GDDLAALS, were generated by cutting pU01 with NheI within the coding sequence, and inserting Nhel linkers into these sites. Since an amber codon normally terminates Ubx translation, a stretch of 37 new amino acids should be added to the C-terminus of Ubx derivative proteins when the amber codon is suppressed. The new amino acids are YRLRILPDYRVRVHLGEMTRIVIQSNIVVTNNLFT, where Y indicates a tyrosine inserted at an amber codon.

To create LexA-abdA*, the HindIII fragment from pA36 was inserted into mini-cut blueescue plasmids: this plasmid was introduced into Escherichia coli DJ226 and DNA was isolated by alkaline lysis. Amplification of the denatured DNA to the oligonucleotide 5'-CTTCATGCGCTTCGAC- CCGGCGACAGATACAGTGCAG-3' elongation, and ligation were performed with the Mutagenex kit (Biorad) according to the manufacturer's instructions. The CGCC stretch differed from the AGCG present in the wild-type abd-A sequence, and altered it so that it contained a new SacI site. E. coli SC31-1, transformed with mutated DNA and plasmid DNA minipreps, isolated from transformed colonies by the boiling technique (Ausubel et al., 1987), were screened to identify one that had incorporated a new SacI site. Digestion sequencing of the portion of the gene that encoded amino acids 103-107 (which includes the homeodomain) revealed that the sequence was identical to that of wild-type abd-A except for the oligonucleotide-directed point mutations, which changed the glutamine and arginine codons at positions 179 and 180 into alanine codons. The mutant gene was inserted into
pAAH to generate pA68. LexA-abdA57-179 was generated by cutting pA68 at two SacI sites, one at the 5' end of the abdA coding sequence, the other the new SacI site introduced by mutation, then religating the cut plasmid backbone. LexA-abdA285, LKGLASL0, and LexA-abdA184, RQKIVL, were generated by cutting pA6 at StyI and BglII sites, filling in those ends with Klenow, and inserting an XbaI linker in each site. LexA-abdA72, SAGASALAS, was created by cutting pA6 at a Nael site, and inserting a Nhel linker.

**Determination of β-Galactosidase Activity**

Assays were as described by Harshman et al. (1988), except that we used between 5 × 10⁹ and 1 OD₅₇₀ cell equivalent for our determinations, and continued the reactions for up to 5 hr. β-galactosidase units were calculated by the equation Units = 1000 x OD₄₂₀/ sample vol x time of reaction (min) x OD₅₇₀. We sometimes observed colonies containing LexA-Ubx and LexA-abdA and derivatives gave heterogeneous colony color when patched onto X-gal plates. Colonies that contained fusion proteins grew more slowly than colonies that did not, and we attributed the heterogeneity in colony color to the selective growth of cells that did not express the fusion proteins. For this reason, a sample of the culture used for each assay was plated on X-gal medium. If the blue color from the assayed cells did not match the blue color from the cells used to inoculate the liquid culture, that value was discarded. The final values were the result of at least three independent determinations; variation was less than 20%.

**Immunoblot Analysis**

To prepare protein extracts, 2 ml of yeast culture in late exponential growth phase were centrifuged. Acid-washed glass beads were added to the pellet in a 1:1 ratio (vol:vol). Laemmli loading buffer (200 μl) was then added to the samples (Laemmli, 1970) which were vortexed for 30 s. The samples were loaded on 6%, 10%, or 12% SDS-polyacrylamide gels, and the gels were run until the tracking dye had reached the bottom (Laemmli, 1970). Transfer to nitrocellulose membrane was at 4°C in 25 mM Tris base, 192 mM glycine, and 20% methanol for 2 hr at 37°C. Incubation with the anti-LexA antibody was performed overnight at 4°C in 1 ml of 1% BSA, 20% FCS, 1 mg/ml bovine gamma globulin (BGG), 0.05% Tween 20, and 1 to 5 μl/ml of rabbit anti-LexA antisera (Brent and Ptashne, 1984). The filter was washed six times for 10 min in 1 x PBS, 0.05% Tween 20, and then incubated for 1 hr at room temperature in 1 x PBS, 20% FCS, 1 mg/ml BGG, 0.05% Tween 20 that contained a 1:500 dilution of an alkaline phosphatase-conjugated, affinity-purified, goat anti-rabbit IgG IgG (H+L) (Sigma). The filter was washed at room temperature six times for 10 min in 1 x PBS, 1 mg/ml BGG, 0.05% Tween 20. It was then incubated at room temperature in 10 ml 100 mM Tris (pH 8.0), 2 μM NaCl, 0.5 μM MgCl₂ containing 6 μl nitro blue tetrazolium and 33 μl 5-bromo-4-chloro-3-indolyl phosphate for 15–25 min until LexA protein derivatives were visualized (these reagents were supplied with the Protoblot reagent system, Promega).

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