thin-layer chromatography of EtOH extracts from seeds (*12*). Second, the saponin antagonist, cholesterol (*13*), added to the chick diet at 1%, reversed the growth inhibition of *S. bigelovii* meal. Third, soaking the seeds in 1% NaOH before meal extraction deactivated the anti-growth factor or factors, similar to the finding of saponin detoxification by NaOH treatment in Kochia seeds (*14*). The unamended meal may be suitable for swine or ruminants that are less sensitive to saponins than poultry (*15*).

The areas of greatest demand for oilseed imports (*16*) coincide with some of the greatest expanses of subtropical coastal desert (*17*). *Salicornia bigelovii* appears to be a potentially valuable new high-yielding oilseed crop for these regions, yielding a vegetable oil high in unsaturated fatty acids, which is amenable to commercial oilseed extraction methods.

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Characterization of a Dimerization Motif in AP-2 and Its Function in Heterologous DNA-Binding Proteins

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The mammalian transcription factor AP-2 is a retinoic acid inducible sequence-specific DNA-binding protein that is developmentally regulated. In this report, the functional domains necessary for AP-2 DNA binding were studied. AP-2 required a dimerization domain and an adjacent region of net basic charge to achieve a sequence-specific protein:DNA interaction. The sequences responsible for dimerization consisted of two putative amphipathic alpha helices separated by a large intervening span region. This helix-span-helix (HSH) domain was unable to bind DNA when separated from the basic region, but was still capable of dimerization. The ability of the HSH domain to function as a module that promotes DNA binding through dimerization was further demonstrated by attaching it to the heterologous basic region of the c-Jun proto-oncogene product. The resulting chimeric protein specifically recognized an AP-1 DNA-binding site in the absence of an intact c-Jun leucine repeat and in a manner that was dependent on the presence of a functional AP-2 dimerization domain.

SEQUENCE-SPECIFIC DNA-BINDING proteins provide a fundamental mechanism for the regulation of transcription. Analysis of these proteins has indicated that they are often modular in nature, containing separate domains for DNA binding and transcriptional activation (*1*). Several transcription factors also possess dimerization domains, which are essential for DNA binding and may modulate the activity of the protein via complex formation (*2*). Recent studies have indicated the importance of amphipathic α-helices for these protein: protein interactions. Members of the bZIP family of proteins, including c-Jun, c-Fos, and C/EBP, dimerize via a single amphipathic α-helix (*2–7*) that has a heptad repeat of leucine residues on one face of the helix ("leucine zipper"). An alternative arrangement that promotes dimerization consists of two putative amphipathic helices separated by a short loop of 10 to 25 amino acids. This helix-loop-helix (HLH) motif is found in a number of DNA-binding proteins, including myoD, E12, TFE3, achaete scute, daughterless, and AP-4 (*8*).

The mammalian transcription factor AP-2 binds to a cyclic adenosine monophosphate (cAMP) and phorbol ester inducible sequence motif found in the cis-regulatory regions of several viral and cellular genes (*9*). The concentration of AP-2 is regulated temporally and spatially during development and is induced by retinoic acid treatment of teratocarcinoma cells (*10, 11*). AP-2 contains a COOH-terminal region of ~200 amino acids that is responsible for both DNA binding and dimerization of the protein in solution (*12*). The AP-2 DNA-binding region is organized in a manner similar to basic region–leucine zipper (bZIP) and HLH proteins, with a stretch of net basic charge adjacent to an area that is predicted to have an α-helical structure (Fig. 1). Examination of this potentially helical region reveals the presence of two amphipathic α-helices separated by a span of ~80 amino acids (Fig. 2).

To determine the contribution of this predicted helix-span-helix (HSH) structure to protein:protein interaction, we constructed a series of internal deletion mutants within the DNA-binding domain. The ability of

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these mutants to recognize an AP-2 binding site was then compared to the wild-type (wt) AP-2 protein with an electrophoretic mobility shift assay (EMSA). The wt protein produced a protein:DNA complex of slow mobility, while an NH_2-terminal deletion (ΔN165), which retained the ability to bind to DNA and dimerize (*12*), produced a faster migrating species (Fig. 1A). In contrast, none of the internal deletions in the basic and α-helical regions were able to generate a protein:DNA complex, confirming the importance of the entire region for DNA binding. We also assessed whether these proteins could interact with DNA in conjunction with ΔN165. When the wt and the ΔN165 mutant proteins were cotranslated, a band of intermediate mobility was produced upon addition of DNA (Fig. 1A), indicating the presence of a heteromeric complex. In contrast, a deletion of five amino acids in the first potential amphipathic α-helix eliminated the ability of the protein to bind to DNA either alone or with ΔN165 (INT 292/298; Fig. 1A). An identical result was produced by a deletion of four amino acids in the second helix (INT 390/395). However, a protein that contained a deletion of 19 amino acids in the span region retained the ability to produce a heteromeric DNA-binding complex with ΔN165 protein, but was incapable of DNA binding on its own (INT 363IR385). This observation suggests that the span deletion mutant is unstable as a homodimer, but is complemented by a normal AP-2 DNA-binding domain.

To determine directly if the mutants in the HSH region affected the ability of the proteins to dimerize, we performed crosslinking experiments in the absence of DNA. Proteins were translated in vitro and crosslinked with glutaraldehyde. The wt and ΔN165 mutant proteins both yielded dimeric products upon crosslinking, indicating that these proteins exist as dimers in solution (Fig. 1B) (*12*). In contrast, none of the internal deletions in the HSH domain formed a stable homodimer. We also tested whether the mutant proteins could associate with the normal dimerization domain present in the ΔN165 protein. The wt and ΔN165 proteins produced a complex that was intermediate in size (80 kD) when they were cotranslated, but not when mixed after translation. However, the corresponding heteromeric complex was not produced by proteins that contained deletions in the α-helical regions, indicating that they were not able to dimerize with ΔN165. In contrast, cotranslation of ΔN165 with INT 363IR385, which contained a deletion in the span region, generated a heteromeric complex, demonstrating that this mutant retained the amino acid sequences necessary for dimerization in solution. This conclusion is in agreement with the results obtained with EMSA, which indicated that the span deletion formed a heteromeric complex with ΔN165 in association with DNA (Fig. 1A). The INT 165/216 mutant, which contained a deletion in the basic region, did not produce a heteromeric DNA-binding complex with ΔN165 (Fig. 1A) but dimerized with ΔN165 in solution (*13*). These findings indicate that dimerization with a functional ΔN165 monomer is not sufficient to form a DNA-binding complex. Therefore, although both INT 363IR385 and INT 165/216 were capable of dimerization, only the former is likely to retain the sequences pertinent to sequence-specific DNA recognition.

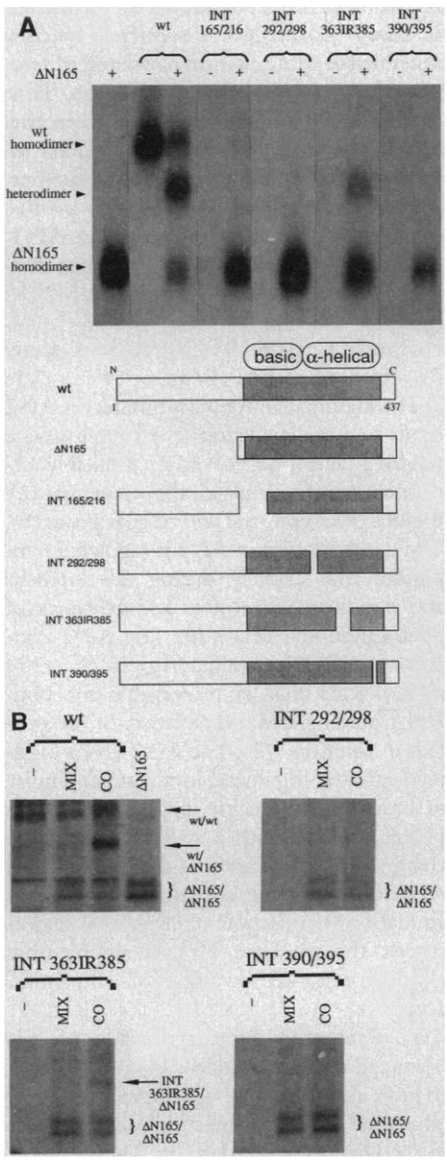


Fig. 1. Sequence analysis of the AP-2 dimerization domain. (**A**) EMSAs performed with mutant proteins. Bottom: schematic representation of wt AP-2 and the deletion mutants. AP-2 is represented by a box, with the shaded area indicating the sequences necessary for DNA binding. The designation ΔN165 indicates that the NH_2-terminal 165 amino acids have been deleted. For the internal deletion mutants (INT), the first and second number refer to the last amino acid present before the deletion and the first amino acid present after the deletion, respectively. The IR present in 363IR385 indicates that an isoleucine (I) and arginine (R) were introduced at the site of deletion. The "/" means that no extraneous amino acids were introduced upon deletion. Top: EMSA performed with AP-2 deletion mutants, as indicated above each lane. Proteins were translated in vitro either in the presence (+) or absence (−) of the ΔN165 mutant and incubated with a ^{32}P-labeled AP-2 DNA-binding site oligonucleotide. The positions of homodimers and heterodimers are indicated. The free probe runs a considerable distance from the shifted products and is not shown. (**B**) Glutaraldehyde cross-linking of the AP-2 HSH mutant proteins. Proteins were translated in vitro in the presence of [^{35}S]Met, cross-linked with glutaraldehyde, separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by autoradiography. The constructs analyzed are indicated above each panel. −, Protein cross-linked on its own; CO, the two proteins were cotranslated; MIX, the protein was mixed with ΔN165 after separate translation (*19*); ΔN165, results obtained by crosslinking ΔN165 itself. The cross-linked homodimers and heterodimers are marked by arrows, except for the ΔN165 homodimers, which are bracketed. To obtain better resolution of the higher molecular size forms, the monomers are not shown on these gels. Duplicate samples indicated that all proteins were present in equivalent amounts (*13*). Plasmid constructs and methods were as described (*20*). The ability of ΔN165 to dimerize with the wt protein was reported (*12*) and serves as a positive control in these experiments.

These results suggest that the AP-2 dimerization domain has a bipartite HSH structure (Fig. 2). The ability of this HSH domain to dimerize was disrupted by the mutations in INT 292/298 and ΔN292, which impinged upon the NH_2-terminal α-helix, and by the mutations in INT 390/395 and ΔC390, which altered the COOH-terminal α-helix (Fig. 2A) (*12*). Furthermore, the substitution of a proline in either of these potential helices greatly reduced DNA binding (*13*). These two helices are separated by a large span of amino acids that may affect stability of the dimer, but which may not be essential for dimer formation. Indeed, the finding that a span deletion mutant can still heterodimerize with an intact HSH motif indicates that this region must be quite flexible to enable correct alignment of surfaces responsible for protein:protein interactions.

The AP-2 HSH motif does not contain any sequence similarity to the leucine repeat or HLH dimerization domains (Fig. 2B). Moreover, the AP-2 HSH domain encompasses more than 120 amino acid residues and is approximately twice as large as these other structures. In particular, the HLH

proteins have an intervening region between the two predicted helices of only about 10 to 25 amino acids, but AP-2 contains over 80 amino acids in the span region. However, AP-2 has an overall organization that is similar to bZIP and HLH proteins, possessing an NH_2-terminal basic region adjacent to a dimerization motif, which together mediate sequence-specific DNA binding [this report and (*12*)]. Analysis of both bZIP and HLH proteins indicates that adjacent regions of net basic charge are the major determinants of DNA binding specificity (*4–6, 14–17*). If the dimerization and basic regions present in the AP-2, bZIP, and HLH DNA-binding domains are independent functional modules, we should be able to exchange the dimerization domain from one of these proteins with the basic region of another to generate a novel DNA-binding molecule. This experiment would provide a stringent test of the ability of the HSH motif to act as a dimerization domain independent of other AP-2 sequences. Therefore, the AP-2 HSH dimerization motif was fused at the COOH-terminal end of

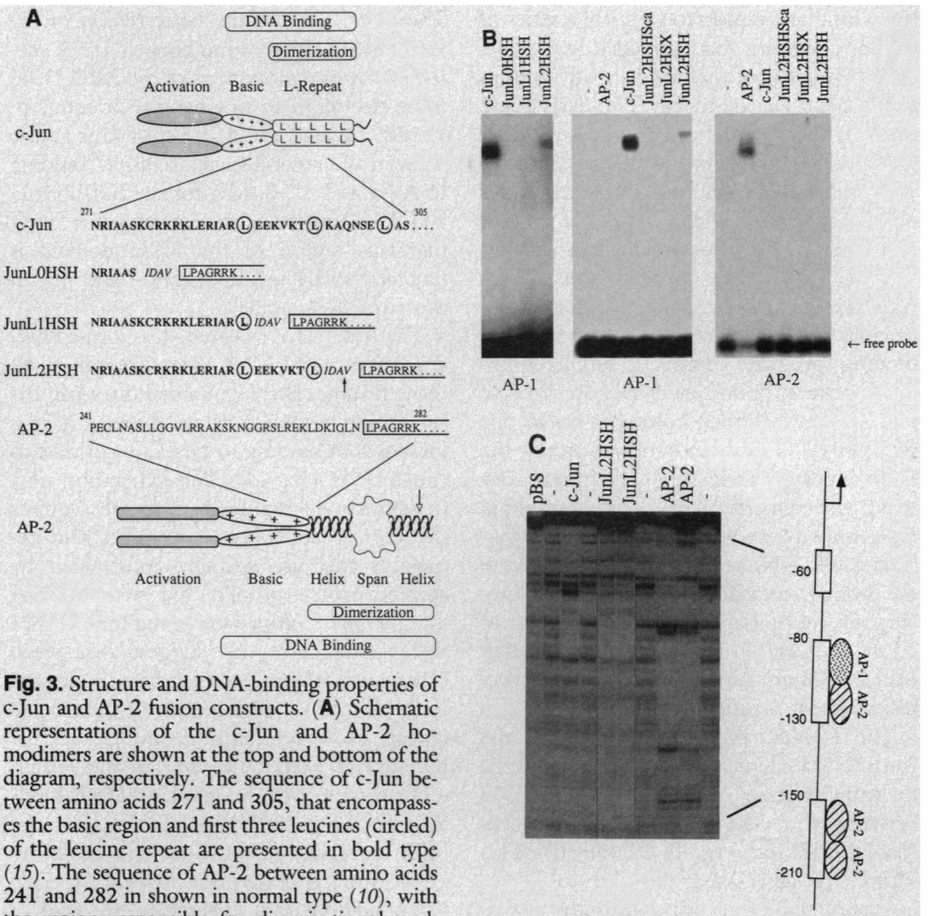
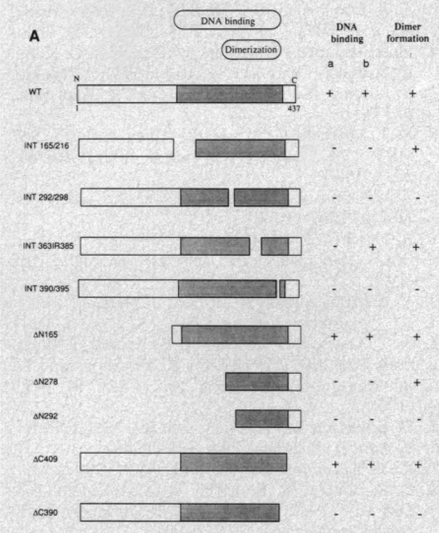


Fig. 3. Structure and DNA-binding properties of c-Jun and AP-2 fusion constructs. (**A**) Schematic representations of the c-Jun and AP-2 homodimers are shown at the top and bottom of the diagram, respectively. The sequence of c-Jun between amino acids 271 and 305, that encompasses the basic region and first three leucines (circled) of the leucine repeat are presented in bold type (*15*). The sequence of AP-2 between amino acids 241 and 282 in shown in normal type (*10*), with the region responsible for dimerization boxed. The amino acid sequence of the fusion constructs JunL0HSH, JunL1HSH, and JunL2HSH, which contain none, one, or two leucines from the repeat, respectively, are also given. The sequence in italics represents amino acids encoded by linker sequence used to join the two coding regions. The black arrow indicates the position of the truncation of JunL2HSHSca. The open arrow shows the position of the mutation in JunL2HSX. (**B**) Mobility shift assays performed with the fusion proteins. Proteins expressed in vitro from the constructs indicated above the lanes were incubated with either an AP-1 or AP-2 oligonucleotide as shown beneath the gels. −, No RNA added to the in vitro translation system. Plasmid constructs and methods are as described (*23*). (**C**) The DNA binding ability of bacterially expressed JunL2HSH was compared to c-Jun, AP-2, and an extract made from bacteria that contained the parental pBluescript (pBS) expression vector by DNase I footprinting (*24*). The pBS, JunL2HSH, and c-Jun tracks contain 25, 5, and 0.25 μl of bacterial extract, respectively. Titration of these proteins indicates that JunL2HSH required ~5-fold more protein to achieve the same AP-1 site occupancy as c-Jun (*13*). Control DNase reactions that lacked protein are also presented (−). A schematic diagram of the human metallothionein IIa promoter is shown to the right. Numbering is relative to the transcriptional initiation site at +1 indicated by the arrow. The GC box (at −60) proximal (−80 to −130) and distal (−150 to −210) basal level elements, and the AP-1 (dotted oval) and AP-2 (striped oval) binding sites are also shown.



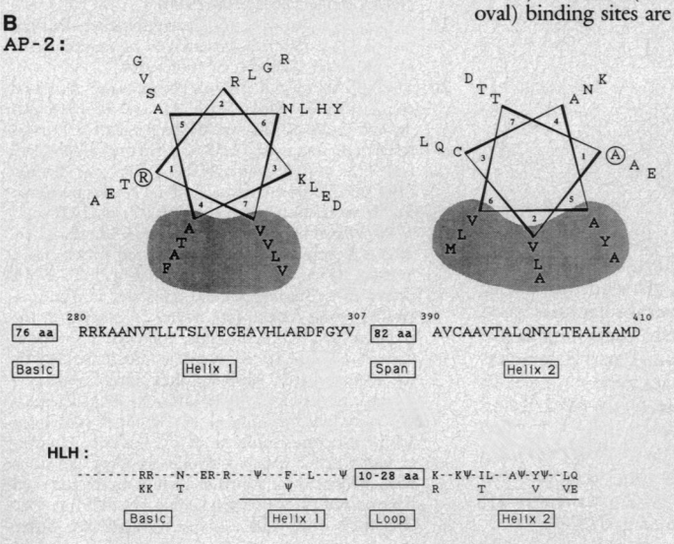


Fig. 2. Structure of the AP-2 HSH dimerization domain. (**A**) Summary of the DNA-binding and dimerization characteristics of AP-2 deletion mutants. The AP-2 open reading frame is as in Fig. 1A. The DNA-binding and dimerization domains are shown by the ovals at the top of the diagram (*21*). Dimer formation refers to the ability of the proteins to dimerize with a full-length DNA-binding domain (wt or ΔN165) as determined by cross-linking or coimmunoprecipitation. Column a illustrates the ability of the proteins to bind DNA on their own in EMSA; column b indicates that the proteins generated a complex consistent with a heterodimeric product after cotranslation with a full-length DNA-binding domain (wt or ΔN165). (**B**) The proposed structure of the AP-2 DNA binding domain, which consists of a region of net basic charge adjacent to the HSH dimerization domain. The sequence of each amphipathic helix is also shown in a helical wheel format with the hydrophobic surface indicated by shading. The first amino acid of each potential helix is circled. The consensus sequence of the HLH structure is shown beneath for comparison (*22*); aa, amino acid; Ψ, hydrophobic residues. Abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln, R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

the c-Jun basic region to generate a series of chimeric proteins that contained none, one, or two leucines of the five normally present in the c-Jun leucine repeat (Fig. 3A). These proteins were then tested with the EMSA for their ability to bind to DNA. Chimeras with the AP-2 HSH that contained either no leucines (JunL0HSH) or one leucine (JunL1HSH) from c-Jun failed to bind to DNA (Fig. 3B). In contrast, the JunL2HSH protein, which possesses two leucines from the c-Jun repeat, had the binding specificity of intact c-Jun. This chimeric protein bound to an oligonucleotide that contained the AP-1 site, but not to one with an AP-2 site. In order to verify the DNA binding specificity of the hybrid construct, the chimeric protein was expressed in *Escherichia coli* and tested in a deoxyribonucleasease I (DNAse I) footprinting assay on the human metallothionein IIa promoter (Fig. 3C). Comparison of the footprints of c-Jun, JunL2HSH and AP-2 indicated that JunL2HSH exhibited a DNA binding specificity almost identical to c-Jun.

The DNA binding ability of the JunL2HSH chimera was further analyzed by truncating the protein at the junction between the c-Jun and AP-2 sequences (JunL2HSHSca; Fig. 3, A and B). This shorter protein failed to bind to DNA, indicating that two leucines from the repeat, combined with adjacent linker sequences, were not sufficient for DNA binding. Furthermore, the presence of a proline and glycine in the AP-2 sequence immediately adjacent to the junction makes it highly unlikely that a helical region that extended through the joint was responsible for dimerization independent of the AP-2 HSH motif.

Direct evidence that the JunL2HSH protein was forming dimers via the AP-2 HSH sequences was obtained by mutating the HSH dimerization motif. The deletion of four amino acids in the second helix of the HSH destroyed the ability of AP-2 to dimerize and bind to DNA (Fig. 1; INT390/395). In a similar manner when this mutation is introduced into JunL2HSH, it also precluded binding of the fusion protein to DNA (JunL2HSX; Fig. 3). Coimmunoprecipitation experiments confirmed that the JunL2HSX protein was incapable of dimerizing through the AP-2 HSH motif, while the JunL2HSH chimera could form a heterodimer with AP-2 (*13*). These results are summarized schematically (Fig. 4). The data indicate that dimerization of the JunL2HSH chimera through the AP-2 HSH domain is essential for the protein to bind the AP-1 site. Furthermore, in the wild-type c-Jun protein, the two monomers of the leucine repeat are aligned in a parallel orientation upon dimerization (*4*). Therefore, because the basic region of the JunL2HSH chimera can contact DNA correctly, the two monomers of the AP-2 HSH must also align in this parallel orientation. Finally, because the deletion of four amino acids in the second helix abolished binding to AP-1 and AP-2 sites, for the JunL2HSH and AP-2 proteins, respectively, it is likely that this region of the HSH domain is involved solely in dimerization and not in protein:DNA contact.

The JunL1HSH protein also formed heterodimers with AP-2 (*13*). Therefore, although JunL1HSH contained the c-Jun basic region and can form dimers, it was incapable of binding to DNA, in contrast to JunL2HSH (Fig. 4). The generation of a fully functional molecule apparently requires the correct apposition of amino acid sequences that are spatially constrained by specific protein:protein and protein:DNA interactions. Comparison of the JunL1HSH and JunL2HSH may suggest that some component of DNA recognition is present in the first seven amino acids of the leucine repeat. However, these sequences can be mutated in both c-Jun and in the related c-Fos protein with no effect on DNA binding specificity (*5–7*). In addition, for the bZIP protein GCN4 only the basic sequences are necessary for sequence-specific DNA binding (*17*). Therefore, we favor a model in which the presence of two leucines from the c-Jun repeat allows the dimerization domain to align the basic regions correctly with respect to the AP-1 binding site. In contrast, a single leucine is incapable of correctly forming the hinge region between the basic region and dimerization domain, possibly because it cannot maintain the necessary protein:protein interaction (Fig. 4). This hypothesis is consistent with the scissors grip model of DNA binding proposed for leucine repeat proteins, which predicts that sequences that correspond to the hinge region must maintain the correct helical register for the basic region to contact DNA effectively (*16, 18*).

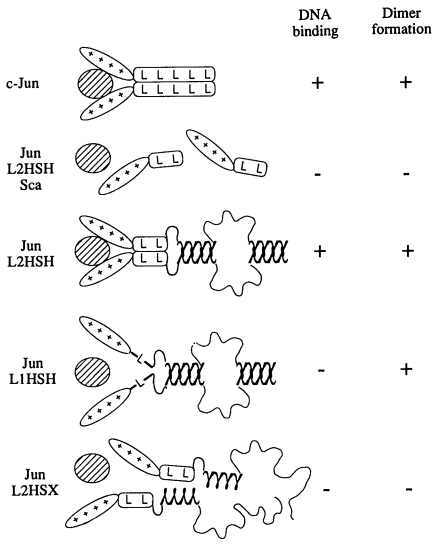


Fig. 4. Summary of the DNA-binding and dimerization properties of the c-Jun–AP-2 fusion proteins. DNA binding refers to sequence-specific binding to an AP-1 site. Dimer formation is based on evidence that bZIP proteins containing all five leucines from the repeat can dimerize, whereas truncations with two leucines cannot (*4–6*). In addition, with respect to the c-Jun–AP-2 fusion proteins, the capability of homodimer formation is inferred from the ability or inability to form heterodimers with AP-2. The DNA is represented by the striped oval. The other structures are equivalent to those shown in Fig. 3A.

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19. Mixing serves as a control to determine whether the two proteins might be crosslinked by random association rather than specific interaction.
20. The pβSAL wt and ΔN165 plasmids are described elsewhere (*12*). Plasmid pβSAL INT165/216 was derived from pβSAL wt by removal of a Hpa II restriction fragment [AP2-9, nucleotides (nt) 555 and 705; *10*]. Plasmid INT 390/395 was derived from pβSAL wt by removal of a 12–base pair (bp) Sac II restriction fragment (AP2-9, nt 1228 and 1240). Plasmids pβSAL INT 363IR385 and INT 292/298 were made by site-directed mutagenisis. Constructs were transcribed in vitro with T7 RNA polymerase (Stratagene) and translated in nuclease-treated rabbit reticulocyte lysates (Promega) in the presence of [^{35}S]methionine, followed by ribonucleaseA (RNaseA) treatment as recommended by the manufacturer. Mobility shift assays were performed as described (*12*). DNA-binding reactions were incubated on ice with 833 ng of poly(dIdC) for 10 min before addition of ^{32}P-labeled, double-stranded AP-2 oligonucleotide. Binding reactions were incubated for a further 15 min on ice and then fractionated on a polyacrylamide gel (4%) at 4°C. Gels were dried and exposed through two blank

x-ray films to cut out the ^{35}S label and record only the ^{32}P signal. The AP-2 oligonucleotide was derived from the human-metallothionein IIa upstream region (*10*).

Cross-linking was performed in crude reticulocyte lysate, which enables proteins that do not bind to DNA to be analyzed. In this method, 2 M KCl (6.6 μl) was added to reticulocyte lysate (10 μl). A portion of this mixture (3.1 μl) was taken out and left untreated at room temperature for 60 min as a control. Glutaraldehyde (1.5 μl of 0.1%) was added to the rest (13.5 μl) of the mixture and it was incubated at room temperature for 60 min. Reactions were stopped by the addition of nine volumes of RIPA buffer (150 mM NaCl, 10 mM tris-Cl, pH 7.4, 1% Triton X-100, 1% sodium deoxycholate, 1 mM EDTA, 1 mM PMSF and 2 μg/ml aprotinin). Proteins were separated by SDS-PAGE and subsequently visualized by fluorography, following treatment of the gel with Amplify (Amersham).

21. The data concerning the dimerization and DNA binding (column a) of the wt, and NH_2 and COOH-terminal deletion mutants was reported in (*12*). Data on the internal deletions was obtained in this report. The DNA-binding data reported in column b for the NH_2- and COOH-terminal deletions is from (*12*) for ΔN165, or otherwise from (*13*).
22. Members of the HLH family of proteins are characterized and defined by a degree of sequence similarity, which enabled a consensus motif to be derived (*8*). The AP-2 HSH does not fit this consensus and is also much larger. However, both the HLH and HSH motifs are defined only by primary sequence and it is therefore possible that these proteins may share similarities on a tertiary structural level.
23. Three c-Jun–AP-2 fusions were constructed by cleaving pHJ19-MUT14, pHJ19-MUT16, and pHJ19-MUT17 (*15*) with Cla I and Kpn I and inserting an ~750-bp fragment of AP-2 that spanned from the Pst I site at nt 892 of AP2-9 to the Kpn I site in the 3′ polylinker sequence (*10*) with a Cla I–Pst I adaptor oligonucleotide. The sequence of the adaptor, which also contains a Sca I site, is ATCGATGCAGTACTGCCTGCAG. These fusions were then transferred into Pst I–Eco RI digested full-length c-Jun expression vector pHJ40 (*15*) with a restriction fragment that spanned from the Pst I site in c-Jun to the Eco RI site at the 3′ end of the AP-2 sequences, to generate JunL0HSH, JunL1HSH, and JunL2HSH, respectively. JunL2HSX is identical to JunL2HSH except that it lacks 12 bp between nt 1231 and 1242 of AP2-9. All constructs were verified by sequencing and Western blotting. The constructs were transcribed in vitro with T3 RNA polymerase (Stratagene) and then translated and assayed as in (*20*). The AP-1 oligonucleotide was derived from the human metallothionein IIa upstream region (*5*). Autoradiography of SDS-PAGE gels indicated that equivalent amounts of each protein were synthesized (*13*).
24. The JunL2HSH, HJ40, (*6*) and pBluescript SK$^+$ (Stratagene) plasmids were transformed into the *Escherichia coli* TG1 strain, and protein was prepared by the inclusion body method (*15*). AP-2 is not efficiently made with this procedure and was therefore obtained with the urea method (*10*). DNase I footprinting reactions (*25*) were performed with a ^{32}P 5′ end-labeled Ava I–Pst I fragment from LS-ES-TK (*26*).
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27. We are indebted to R. Turner, V. Baichwal, G. Gill, S. Jackson, and N. Mermod for discussion, encouragement, and criticism throughout the course of this work; and to D. Bohmann both for the c-Jun plasmids and helpful advice at the outset of the project. Supported in part by an NIH grant (R.T.) and a Howard Hughes Medical Institute Postdoctoral Fellowship (T.W.).

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Molecular Nature of the *Drosophila* Sex Determination Signal and Its Link to Neurogenesis

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In 1921 it was discovered that the sexual fate of *Drosophila* is determined by the ratio of X chromosomes to sets of autosomes. Only recently has it been found that the X chromosome to autosome (X:A) ratio is communicated in part by the dose of *sisterless-b (sis-b)*, an X-linked genetic element located within the *achaete-scute* complex of genes involved in neurogenesis. In this report, the molecular nature of the primary sex determination signal and its relation to these proneural genes was determined by analysis of *sis-b*$^+$ germline transformants. The *sis-b*$^+$ function is confered by protein T4, a member of the helix-loop-helix family of transcription factors. Although T4 is shared by *sis-b* and *scute-α*, the regulatory regions of *sis-b*, which control T4 expression in sex determination, are both separable from and simpler than those of *scute-α*, which control T4 expression in neurogenesis. Dose-sensitive cooperative interactions in the assembly or binding of *sis*-dependent transcription factors may directly determine the activity of the female-specific promoter of *Sex-lethal*, the master regulator of sexual development. In this model there is no need to invoke the existence of analogous autosomal negative regulators of *Sex-lethal*.

THE SOMATIC SEXUAL FATE OF THE fruit fly is determined by the dose of at least two X-linked *sisterless* loci (*sis-a* and *sis-b*) and by the interactions of these genes with their regulatory target *Sex-lethal (Sxl)* (*1*). *Sxl* is a female-specific gene that coordinates all aspects of sexual differentiation including the vital process of X chromosome dosage compensation. Increases in *sis* gene dose increase the probability that a cell will express the female-specific sex determination and dosage compensation activities of *Sxl*, while decreases have the converse effect. What feature of the *sis* genes allows them to serve as indicators of the X chromosome to autosome (X:A) ratio? How does *Sxl* reliably distinguish the twofold difference in *sis* gene dose that defines the chromosomal sex of the fly? Is the role of the *sis* genes in development limited to sex determination? The answers to these questions are fundamental to understanding how sexual dimorphism is controlled in *Drosophila* and how that control has evolved.

The location of *sis-b* within the extensively studied *achaete-scute* complex (AS-C) (*1, 2*) has facilitated analysis of the sex determination signal. At the same time, it has raised the question of how a sex determination signal element might be functionally related to a gene complex that is directly involved in the development of the peripheral nervous system (*3*). The AS-C is composed of a few small transcription units flanked by an enormous amount of regulatory DNA (*4–7*) (Fig. 1). The initial characterization of *sis-b* indicated that most, but not all, *sis-b* activity mapped to a 22-kilobase (kb) portion of the complex that contains the transcription unit (T4) of the *scute-α(sc-α)* gene (*1*). Subsequent genetic analyses of point mutations in the protein coding region of T4 suggested that the protein product of this gene participates in the sex determination functions of *sis-b* (*2*), although the picture was clouded by the variable, and sometimes surprisingly weak, *sis* phenotypes of lesions that eliminated T4 protein activity. Here we use *P* element mediated transformation with genomic *sis-b* DNA to address the question of how the sex determination signal in *Drosophila* is related to neurogenesis and whether a *sis-b* encoded protein is part of that signal.

Three constructs that contained the intact T4 transcription unit with varying amounts of flanking genomic DNA (Fig. 1) were found to have *sis-b*$^+$ activity by the criteria listed in Fig. 2 (*8*). The T4$^+$ transgenes complemented the female-specific, temperature-sensitive lethal allele *sis-b*$^{sc3\text{-}1}$ (Table 1A). Of the 14 lines tested, only *P*[B5]21 failed to fully complement the *sis-b* mutation, and even it rescued most of the mutant females. The ability of extra copies of one *sis* locus to partially compensate in females for decreases in the activity of another *sis* locus is a distinguishing feature of these genes. The *P*[B5]T4$^+$ transgenes displayed this additive behavior, rescuing females that carried a partial loss-of-function *sis-a* point mutation over a deletion of the *sis-a* locus (Table 1B). This test demands much higher *sis-b*$^+$ activity than the *sis-b*$^{sc3\text{-}1}$ complemen-

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