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SpSoxB1 Serves an Essential Architectural Function in the Promoter *SpAN*, a *tolloid/BMP1*-Related Gene

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Transcription of *SpAN*, which encodes a secreted protease related to tolloid and BMP 1, is differentially regulated along the animal–vegetal axis of the sea urchin embryo by a maternally initiated mechanism. Regulatory sites that bind SpSoxB1 and CBF (CCAAT binding factor) are essential for strong transcriptional activity because mutations of these elements reduce promoter activity in vivo 20- and 10-fold, respectively. Here we show that multimerized SpSoxB1 elements cannot activate transcription from the *SpAN* basal promoter in vivo. However, like other factors containing HMG-class DNA binding domains, SpSoxB1 does induce strong bending of DNA. The CBF binding site lies abnormally far from the transcriptional start site at –200 bp. We show that the SpSoxB1 site is not required if the CCAAT element is moved 100 bp closer to the transcriptional start site, replacing the SpSoxB1 site. This supports a model in which the bending of *SpAN* promoter DNA by SpSoxB1 facilitates interactions between factors binding to upstream and downstream regulatory elements.

DNA bending HMG CCAAT

THE *SpAN* gene encodes a metalloprotease with similarity to tolloid and BMP1 that can function in a BMP-mediated pathway (22) and remodel the apical extracellular matrix of sea urchin blastulae (Howard et al., manuscript in preparation). *SpAN* transcription is transient and restricted to the nonvegetal domain of cleavage-stage embryos and very early blastulae. *SpAN* is activated cell autonomously, indicating that it is regulated by maternal activities that are spatially restricted along the animal–vegetal (AV) axis (18). Our laboratory identified two essential *cis* elements within the regulatory region that are sufficient to sponsor spatially correct, high-level transcription (14). One of these contains a motif found in the promoters of many genes that specifically interacts with CCAAT binding factors (CBF) that are abundant in early embryonic nuclear and whole-cell extracts [(14); Kenny, unpublished observations]. The second site contains Sox transcription factor binding motifs that confer strong positive activity to the *SpAN* promoter as shown by the fact that replacement of this binding site reduces promoter activity 20-fold (14).

Our studies identified SpSoxB1 as the major factor that binds at these essential Sox regulatory motifs in vivo (12), and we showed that the pattern of accumulation of SpSoxB1 protein in embryonic nuclei predicts the transcripitonal pattern of the *SpAN* target gene. In addition, recent work demonstrates that SpSoxB1 protein works in opposition to the Wnt signaling pathway to pattern cell fates along the animal–vegetal axis and that it is essential for endoderm differentiation (Kenny, Angerer, and Angerer, manuscript in preparation). Because of its important role in early fate specification, we are investigating the mechanism by which SpSoxB1 activates its known target gene, *SpAN*.

Sox factors contain a hallmark 70–80 amino acid segment called the HMG (high mobility group) do-

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main that mediates their binding in the minor groove of AT-rich *cis* elements. Sox factor binding typically induces a bend of 70–85 degrees (15,23), and several Sox proteins have been shown to function as architectural transcription factors, facilitating the assembly of stable multiprotein promoter complexes (6,17). Other Sox factors contain activation domains, providing them with the ability to serve as more classical activating transcription factors (9,10,21). In several cases, Sox factor function is mediated through association with partner transcription factors that bind nearby DNA sequences (11). Our previous promoter deletion analyses suggested that SpSoxB1 does not have the ability to activate transcription independently, because partial promoters containing Sox and only several other *cis* elements are not active in transgene assays in vivo (14).

Here we present evidence supporting a model in which SpSoxB1 functions primarily as an architectural factor that is required to facilitate the interaction of the *SpAN* basal promoter with transcription factors upstream in the regulatory region. We show that promoters containing multiple copies of the SpSoxB1 binding site linked to the *SpAN* basal promoter are unable to activate transcription of a reporter gene in sea urchin embryos. SpSoxB1 does induce dramatic DNA bending as observed for other HMG-box containing transcription factors (7), suggesting that a major function of this factor in the *SpAN* promoter is to facilitate the binding of other transcriptional activators and/or their interaction with downstream elements. Consistent with the latter idea, we find that wild-type levels of transcriptional activation can be achieved in the absence of Sox sites if the CBF binding site is positioned in its typical position closer to the transcription start site and in the correct orientation relative to the *SpAN* basal promoter.

MATERIALS AND METHODS

Embryo Culture

Strongylocentrotus purpuratus (Sp) adults were obtained from Marinus Co. (Westchester, CA) and eggs were fertilized and embryos cultured at 15°C as described previously (1).

In Vivo Transcription Activity Assays

Promoter activity measurements were made in vivo using chimeric constructs carrying wild-type or mutated *SpAN* promoters linked to a bacterial chloramphenicol acetyltransferase (CAT) reporter gene as described previously (24). Six new reporter constructs were made for the experiments described here. The first placed four copies of the higher affinity Sox motif in *SpAN* site V (−106 to −77) (14), upstream of a *SpAN* promoter that begins at −112 and contains sites V, VI, and the basal promoter (Fig. 1A2). The other five constructs contained replacements of the endogenous *SpAN* site II (CCAAT) with the same bases used in previous work (14), and also replaced 30 bp in *SpAN* site V with a wild-type *SpAN* site II in either the forward or reverse orientation, as shown in the Results section.

Circular Permutation Electrophoretic Mobility Shift Assay (EMSA)

EMSA probes containing the entire *SpAN* site V 36-mer (LongV) or just the upstream *SpAN* site V Sox motif 19-mer (5′V) were synthesized by PCR. PCR primers contained an upstream *Xba*I site as well as a downstream *Sal*I site. For the 5′V insert, the top primer used was 5′-CTA GTC TAG AGA GAA CAA TAA CAA TGA T-3′ and bottom primer was 5′ - GGA ATT CCG TCG ACC ATC ATT GTT ATT GTT CT-3′. For the LongV insert, the top primer used was 5′-CTA GTC TAG AGA GAA CAA TAA CAA TGA T-3′ and bottom primer was 5′-GGA ATT CCG TCG ACA GGA ACA AAG CAG TCG CC-3′. The amplified fragments were restricted with *Xba*I and *Sal*I and inserted in the middle of the dimerized multiple cloning site (MCS) present in the pBend2 vector (13). The recombinants were digested with *Bam*HI, *Eco*RI, *Mlu*I, *Nhe*I, *Nru*I, *Sma*I, or *Xho*I to release the set of circular permutation assay probes containing the LongV or 5′V binding sites located at different positions along the 160-bp or 143-bp fragments, respectively. The dephosphorylated inserts were labeled at their 5′ ends to a specific activity of 5,000–10,000 cpm/fmol.

Binding reactions were carried out in 20 mM HEPES adjusted to pH 7.9 with KOH, 5 mM $MgCl_2$, 10 mM KCl, 10 mM DTT, and 100 ng sonicated single-stranded salmon sperm DNA per microliter. For each reaction, 2–4 fmol of probe was incubated at 15°C for 15 min with either 2 μl of in vitro translated (IVT) SpSoxB1 protein prepared as described previously (12) or 0.5 μg of 9-h nuclear extract prepared as described elsewhere (3), excluding the dialysis step. Competition with unlabeled DNA fragments and antibody supershifts were carried out as reported previously (12). All reactions were then fractionated by electrophoresis through nondenaturing 8% polyacrylamide gels in 1× TBE buffer (100 mM Tris, 100 mM boric acid, 10 mM EDTA). Bending angles were determined as described previously (20).

RESULTS AND DISCUSSION

Multimerized SpSoxB1 Elements Do Not Activate the SpAN *Basal Promoter*

Some Sox factors have the capacity to behave as classical transcriptional activators at promoter target sites (8,21). Previous work demonstrated that ~300 bp upstream of the transcription start site of *SpAN* is sufficient for spatially correct, high-level transcription. Within this region, we identified two regulatory elements critical for activity. These included ~36 bp bearing Sox binding motifs between −103 and −75 (site V) and a CCAAT element centered at −203 (14). Site V contains 5′ and 3′ elements with high and low affinity, respectively, for SpSoxB1. To determine whether the factor(s) binding to the Sox motifs had independent ability to activate transcription, we constructed a promoter containing four high-affinity SpSoxB1 binding motifs placed upstream of *SpAN* site V, site VI (which binds the Sp1-like DNA looping factor, pGCF) (27), and the basal promoter (Fig. 1A). This promoter, fused to a CAT reporter, was injected into sea urchin one-cell embryos and blastulae were assayed for CAT activity. In four such experiments, one of which is illustrated in Figure 1B, no promoter activity was detectable, whereas control wild-type *SpAN* promoters gave high levels of activity. DNA slot blot hybridizations show that this difference in activity is not attributable to differences in transgene levels in these embryos. These results demonstrate that factors interacting with the Sox elements in sea urchin embryos are unable to activate a basal promoter and consequently suggest that SpSoxB1, which is the major factor binding at these elements (12), cannot independently activate transcription.

SpSoxB1 Induces a Sharp Bend in SpAN *Promoter DNA*

To test the possibility that SpSoxB1 serves an architectural role in the *SpAN* promoter by bending the DNA, we carried out circular permutation EMSAs. As probes we used either just the higher affinity upstream SpSoxB1 binding *cis* element from *SpAN* site V (5′V) or the entire site V (LongV), which contains both the upstream and the lower affinity downstream Sox binding motifs (Fig. 2A). With each probe from the 5′V circular permutation series prepared as described in Materials and Methods, SpSoxB1 translated in vitro (IVT) produced a single complex (indicated by the arrow in Fig. 2B) whose mobility increased when the Sox binding site was moved to more terminal positions in the probe. A DNA bending angle of ~60° was calculated from these measurements (see Fig. 2 legend). The same complex was seen for the LongV probe, as well as a much less abundant complex of lower mobility (Fig. 2B, arrowhead). This latter complex corresponds to occupancy of both upstream and downstream Sox elements. EMSAs show that in vitro translated SpSoxB1 can bind to either 5′ or 3′ Sox elements (Fig. 2C, left) and can occupy both sites simultaneously at higher protein concentration (Fig. 2C, right and also leftmost lane). Consistent with this, both sites are required for full promoter activity in vivo (12).

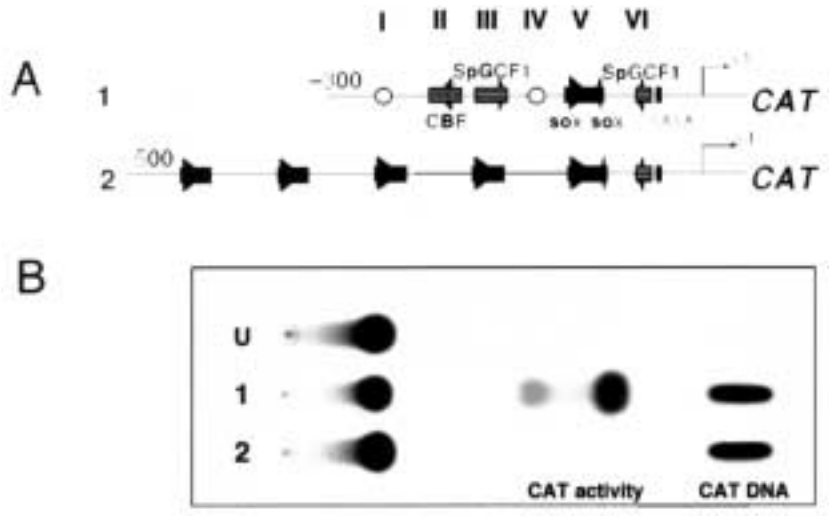

Figure 1. Multimerized Sox binding motifs do not mediate transcriptional activation. (A) Diagrams of wild-type (1) and mutant (2) *SpAN* promoter/CAT reporter transgenes. Identified sites of protein binding are indicated on the wild-type promoter. Open circles represent factors whose identity is not yet known. (B) *CAT* reporter assays of activities of these two constructs with corresponding quantitation of transgene DNA levels in embryos. U, uninjected embryos.

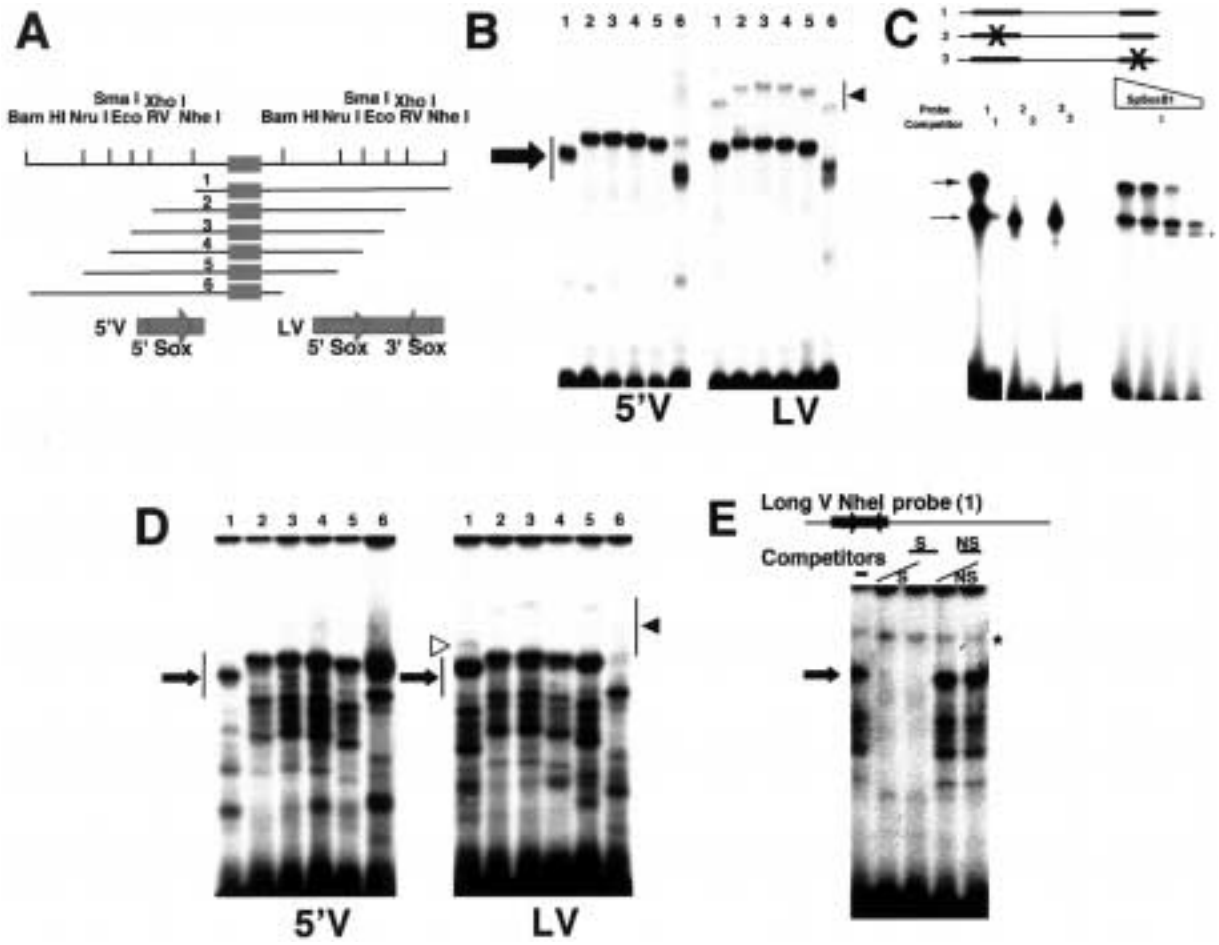

Figure 2. SpSoxB1 bends *SpAN* promoter DNA. (A) Bending assay probes used in EMSAs, which contain inserts of Sox binding sites at circularly permuted positions. Specific inserts used as binding sites are indicated for the long *SpAN* site V probe, LongV (LV), as well as for the upstream, high-affinity Sox motif (5′V). (B) EMSAs using SpSoxB1 protein translated in vitro and the 5′V or LongV probe sets (1–6) as indicated. The complex formed by single occupancy by SpSoxB1 is indicated by an arrow. A bending angle was computed as described previously (20). The complex formed on the LongV probe that results from binding at both the upstream and downstream Sox motifs is indicated with an arrowhead. (C) In vitro translated SpSoxB1 can bind simultaneously to both Sox elements in the LV *SpAN* sequence. Probe 1 contains both Sox motifs while in probes 2 and 3 the 5′ or 3′ elements, respectively, are mutated by sequence replacement. The heavy and light arrows mark complexes with double and single occupancy, respectively. In the experiment shown to the right, the concentration of SpSoxB1 protein is reduced by successive factors of 3. The asterisk indicates a complex formed with a protein other than SpSoxB1 present in the rabbit reticulocyte lysate and the LV probe. (D) Sox bending EMSAs using 9-h embryo nuclear extract and either 5′V or LongV probe sets. Lanes are arranged as in (B). Higher mobility complexes may be due to the binding of other Sox factors or degradation products of SpSoxB1, or the formation of multiprotein complexes. Single and double site occupancy SpSoxB1 complexes are indicated with arrow and solid arrowheads, respectively. The double occupancy complex formed with LongV, probe 1, is indicated by an open arrowhead. (E) Complexes formed with LongV, probe I (arrow) are specifically competed with the upstream Sox binding motif of *SpAN* site V (S) but not with heterologous DNA competitor (NS). A nonspecific, low mobility complex formed in these reactions is indicated by an asterisk.

To test whether DNA bending also occurs in the context of other nuclear proteins that might associate either with SpSoxB1 and/or with the probe, we carried out similar bending assays with proteins in 9-h sea urchin embryo nuclear extracts. Both 5′V and LongV probes produced a predominant band (indicated by the arrow in Fig. 2D) that has the same mobility as the complex produced by IVT SpSoxB1 as previously shown (12) (Fig. 2B, C). These complexes form specifically with the Sox motifs in the probe, as shown by competitions with unlabeled fragments (Fig. 2E). A minor complex with lower mobility that is observed in the competition experiment (Fig. 2E, asterisk) is nonspecific because it is not competed by the homologous competitor. It does not correspond to the minor complex formed by double occupancy (Fig. 2D, lane 1, open arrowhead), which has higher mobility. The migration shifts indicate that both 5′V and

LongV probe DNAs are also bent to approximately the same degree by proteins present in nuclear extracts as by IVT proteins.

Moving the CBF Binding Site Closer to TATA Can Substitute for SpSoxB1 Function in the SpAN *Promoter*

The results described above suggest a model in which SpSoxB1 promotes transcription of the *SpAN* gene primarily through DNA bending, rather than through transcriptional activation. Of the several *cis*-acting elements identified upstream in the *SpAN* promoter, the quantitatively most important is CCAAT, which binds CBF. Mutation of this element causes a significantly greater reduction (10-fold) in *SpAN* promoter activity (14) than does replacement of other binding sites: similar mutation by replacement of either site I or IV results in ~1.5-fold increase or decrease, respectively, in promoter activity in vivo, while site III replacement reduces promoter activity about threefold (14). A second reason to suspect that Sox-mediated DNA bending enhances the activity of CBF is that the position of the CBF binding site at −203 in the *SpAN* promoter is atypical: a survey of 178 CCAAT motifs in 96 unrelated promoters showed a strong preference for location between −80 and −100 (mean position −89) (16). CBF can function as an independent transcriptional activator. For example, two copies of the CBF binding motif are located at −136 and −96 in the *SpHE* promoter, which shares the same temporal and spatial pattern of expression as *SpAN*. These elements are sufficient to mediate transcription from the *SpHE* basal promoter in the correct spatial pattern (25).

For these reasons, a plausible hypothesis is that SpSoxB1 provides an appropriate structural context in the *SpAN* promoter that facilitates the interaction of upstream factors, particularly CBF, with elements downstream of the Sox site. If that were the case, then the Sox motifs and SpSoxB1 might be dispensable if the CBF binding site were relocated to its more typical proximal position. To test this possibility, several alterations were made in the −300 *SpAN* regulatory region, as illustrated in Figure 3: the sequence between −77 and −106 that contains the Sox elements was removed and the CBF binding site was inserted its place. The exact position and orientation of this motif relative to the basal promoter were varied in five separate constructs, labeled A–E, to allow for possible effects of helical rotation and orientation. With respect to sequences downstream of the inserted CBF binding site at −100, these constructs are identical to those used for testing SpSoxB1 transcriptional activating activity (Fig. 1). In constructs B, C, and E, the CCAAT motif is on one side of the helix while in A and D it is on the opposite side, as diagrammed in Figure 3 (top panel). In two separate experiments, high levels of transcription relative to wild-type activity were observed from constructs B (44%), C (46%), and E (96%), while much weaker or no detectable transcription was observed for constructs A (0%) and D (17%) (Fig. 3, lower panel). The fact that wild-type levels of transcription can be achieved with promoter construct E is consistent with the proposal that SpSoxB1 facilitates transcriptional activation, mediated by CBF and perhaps other upstream factors, through strong bending of *SpAN* promoter DNA at ~−100. For the *SpAN* promoter, this is an essential function because mutation of the bending site reduces promoter activity to nearly basal levels.

The relative activities of constructs A–E show that the −300 promoter is insensitive to the orientation of the CBF binding site but very sensitive to its position along the helix. Constructs B and C, which contain the CBF element at the same position relative to the basal promoter but in opposite orientations, are equally active. In contrast, constructs A and C, which have the Sox motif in the same orientation but on opposite sides of the helix, differ more than 10-fold in promoter activity (Fig. 3). The sensitivity of this shortened promoter to the exact positioning of CCAAT contrasts with the relative insensitivity of the wild-type promoter to similar alterations of CCAAT positioning around −200 (Kenny, unpublished observations). This difference probably reflects the greater flexibility of the longer stretch of DNA between the CBF binding site and the basal transcriptional machinery.

The possibility that the positive activity of Sox factors in the *SpAN* promoter is mediated, in part, through direct interaction with other factor(s) cannot be excluded. However, based on current evidence, we do not favor this view. Mutation of DNA sequences adjacent to the Sox motifs does not affect promoter activity (12,14) and complexes formed with SpSoxB1 translated in vitro and with nuclear extract proteins are indistinguishable in EMSAs. Most significantly, the fact that wild-type levels of *SpAN* promoter activity can be achieved in the absence of SpSoxB1 demonstrates that it does not contribute any essential protein–protein interactions.

A model for how the Sox *cis* elements could mediate *SpAN* transcriptional activation is presented in Figure 4. The Sox elements are located midway between the CBF binding sites and the basal promoter, a position that is appropriate for SpSoxB1 bending at these sites to act as a hinge. In addition, positioned

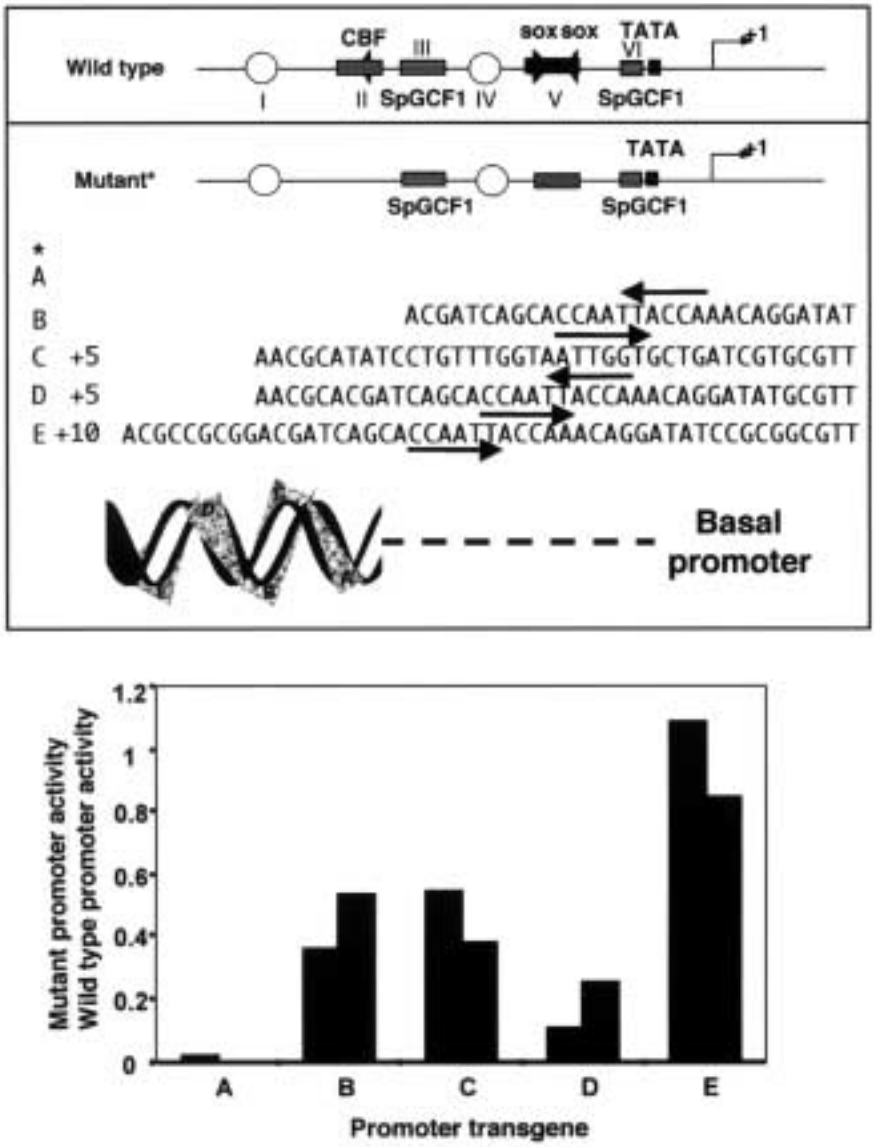

Figure 3. SpSoxB1 function can be substituted by altering the position of the CBF binding site. (Top) Diagrams of *SpAN* wild-type and mutant (A–E) promoters. The wild-type Sox, site (soxsox) was replaced with the CBF site, which was tested in both orientations and on opposite sides of the helix. Sequences containing the CBF site (indicated by arrows) are aligned in their relative positions with respect to the transcription start site. The drawing in the bottom of the top panel illustrates that constructs B, C, and E place SpSoxB1 on one side of the helix and A and D place it on the opposite side. Arrowheads indicate the orientation of the *cis* element (CCAATTA) for each construct. In promoters A and C, this orientation is the same as in the wild-type promoter. (Bottom) The activities of mutant promoters were measured in two separate experiments as described in the legend to Figure 1B and in Materials and Methods and are compared after normalization to the activity of the wild-type promoter.

at similar distances on either side of the Sox binding sites are elements that bind pGCF1 (14), a factor that has been shown to form homodimers with the capacity to loop out intervening DNA (27). Such an interaction might further stabilize the promoter architecture produced by SpSoxB1. This model proposes that transcriptional activity is mediated primarily through the CBF site and components of the basal transcription complex, based on previous in vivo assays showing the importance of this element and on the direct tests of repositioning the CBF element reported here. However, it does not exclude the possibility that bending by SpSoxB1 also facilitates interactions between factors bound at other upstream and downstream elements.

Many transcription factors that contain HMG domains have been shown to serve architectural functions through their ability to enhance the binding and/

or activity of closely associated transcription factors. For example, in the δ*1-crystallin* gene, a 30-bp-long fragment contains a Sox2 binding element and another element, both of which are required to elicit lens-specific expression (9). In the *Fgf-4* promoter, Sox-2 and Oct-3 are binding partners that require one another to strongly activate transcription (26). At the human interferon β enhancer, a short (57 bp) regulatory region, HMG-I(Y), cooperatively establishes protein–protein and DNA–protein interactions in a stereospecific manner to form a transcription–activating complex (5,19). However, other studies show that functional promoter architecture relies not only on close-range interactions between protein–DNA complexes, but also on interactions among proteins whose DNA binding sites may be more distant from one another (2,4). The results presented here suggest that a major function of SpSoxB1 in activating the *SpAN* promoter is to facilitate such interactions through DNA bending. However, it is likely that regions of the SpSoxB1 protein other than the DNA binding domain may play a role in the regulation of other genes. Because SpSoxB1 is an important developmental regulator of specification of germ layers in the sea urchin embryo being essential for both for endoderm specification and normal differentiation of ectoderm, it will be important to understand how it functions in other target genes.

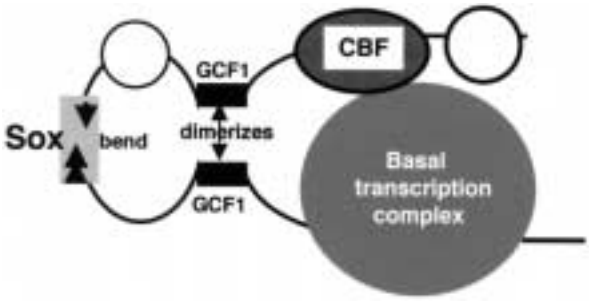

Figure 4. Model for the role of DNA bending by SpSoxB1 in activation of the *SpAN* promoter. Binding of SpSoxB1 to the Sox motifs of the *SpAN* promoter bends the DNA, facilitating the interaction of upstream and downstream factors. This conformation may be stabilized by pGCF homodimerization. Open circles represent other known sites of DNA–protein interaction (14).

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REFERENCES

1. Angerer, L. M.; Angerer, R. C. Detection of polyA+ RNA in sea urchin eggs and embryos by quantitative in situ hybridization. Nucleic Acids Res. 9:2819–2840; 1981.
2. Bewley, C. A.; Gronenbom, A. M.; Clore, G. M. Minor groove-binding architectural proteins: Structure, function, and DNA recognition. Annu. Rev. Biophys. Biomol. Struct. 27:105–131; 1998.
3. Calzone, F. J.; Theze, N.; Thiebaud, P.; Hill, R. L.; Britten, R. J.; Davidson, E. H. Developmental appearance of factors that bind specifically to cis-regulatory sequences of a gene expressed in the sea urchin embryo. Genes Dev. 2:1074–1088; 1988.
4. Dyer, M. A.; Hayes, P. J.; Baron, M. H. The HMG domain protein SSRP1/PREIIBF is involved in activation of the human embryonic beta-like globin gene. Mol. Cell. Biol. 18:2617–2628; 1998.
5. Falvo, J. V.; Thanos, D.; Maniatis, T. Reversal of intrinsic DNA bends in the IFN beta gene enhancer by transcription factors and the architectural protein HMG I(Y). Cell 83:1101–1111; 1995.
6. Ferrari, S.; Harley, V. R.; Pontiggia, A.; Goodfellow, P. N.; Lovell-Badge, R.; Bianchi, M. E. SRY, like HMG1, recognizes sharp angles in DNA. EMBO J. 11: 4497–4506; 1992.
7. Grossehedl, R.; Giese, K.; Pagel, J. HMG domain proteins: Architectural elements in the assembly of nucleoprotein structures. Trends Genet. 10:94–100; 1994.
8. Kamachi, Y.; Cheah, K. S.; Kondoh, H. Mechanism of regulatory target selection by the SOX high-mobility-group domain proteins as revealed by comparison of SOX 1/2/3 and SOX9. Mol. Cell. Biol. 19:107–120; 1999.
9. Kamachi, Y.; Sockanathan, S.; Liu, Q.; Breitman, M.; Lovell-Badge, R.; Kondoh, H. Involvement of SOX proteins in lens-specific activation of crystanin genes. EMBO J. 14:3510–3519; 1995.
10. Kamachi, Y.; Uchikawa, M.; Collignon, J.; Lovell-Badge, R.; Kondoh, H. Involvement of Sox1, 2 and 3 in the early and subsequent molecular events of lens induction. Development 125:2521–2532; 1998.
11. Kamachi, Y.; Uchikawa, M.; Kondoh, H. Pairing SOX off: With partners in the regulation of embryonic development. Trends Genet. 16:182–187; 2000.
12. Kenny, A. P.; Kozlowski, D.; Oleksyn, D. W.; Angerer, L. M.; Angerer, R. C. SpSoxB1, a maternally encoded transcription factor asymmetrically distributed among early sea urchin blastomeres. Development 126:5473–5483; 1999.
13. Kim, J.; Zwieb, C.; Wu, C.; Adhya, S. Bending of DNA by gene-regulatory proteins: Construction and use of a DNA bending vector. Gene 85:15–23; 1989.

14. Kozlowski, D. J.; Gagnon, M. L.; Marchant, J. K.; Reynolds, S. D.; Angerer, L. M.; Angerer, R. C. Characterization of a SPAN promoter sufficient to mediate correct spatial regulation along the animal-vegetal axis of the sea urchin embryo. Dev. Biol. 176:95–107; 1996.
15. Laudet, V.; Stehelin, D.; Clevers, H. Ancestry and diversity of the HMG box superfamily. Nucleic Acids Res. 21:2493–2501; 1993.
16. Mantovani, R. A survey of 178 NF-Y binding CCAAT boxes. Nucleic Acids Res. 26:1135–1143; 1998.
17. Pevny, L. H.; Lovell-Badge, R. Sox genes find their feet. Curr. Opin. Genet. Dev. 7:338–344; 1997.
18. Reynolds, S. D.; Angerer, L. M.; Palis, J.; Nasir, A.; Angerer, R. C. Early mRNAs, spatially restricted along the animal–vegetal axis of sea urchin embryos, include one encoding a protein related to tolloid and BMP-1. Development 114:769–786; 1992.
19. Thanos, D.; Maniatis, T. Virus induction of human IFN beta gene expression requires the assembly of an enhanceosome. Cell 83:1091–1100; 1995.
20. Thompson, J. F.; Landy, A. Empirical estimation of protein-induced DNA bending angles: Applications to lambda site-specific recombination complexes. Nucleic Acids Res. 16:9687–9705; 1988.
21. van de Wetering, M.; Oosterwegel, M.; van Norren, K.; Clevers, H. Sox-4, an Sry-like HMG box protein, is a transcriptional activator in lymphocytes. EMBO J. 12:3847–3854; 1993.
22. Wardle, F. C.; Angerer, L. M.; Angerer, R. C.; Dale, L. Regulation of BMP signaling by the BMP1/TLD-related metalloprotease, SpAN. Dev. Biol. 206:63–72; 1999.
23. Wegner, M. From head to toes: The multiple facets of Sox proteins. Nucleic Acids Res. 27:1409–1420; 1999.
24. Wei, Z.; Angerer, L. M.; Gagnon, M. L.; Angerer, R. C. Characterization of the SpHE promoter that is spatially regulated along the animal–vegetal axis of the sea urchin embryo. Dev. Biol. 171:195–211; 1995.
25. Wei, Z.; Angerer, L. M.; Angerer, R. C. Multiple positive *cis* elements regulate the asymmetric expression of the *SpHE* gene along the sea urchin embryo animal–vegetal axis. Dev. Biol. 187:71–78; 1997.
26. Yuan, H.; Corbi, N.; Basilico, C.; Dailey, L. Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. Genes Dev. 9:2635–2645; 1995.
27. Zeller, R. W.; Coffman, J. A.; Harrington, M. G.; Britten, R. J.; Davidson, E. H. SpGCF1, a sea urchin embryo DNA-binding protein, exists as five nested variants encoded by a single mRNA. Dev. Biol. 169: 713–727; 1995.