MOLECULAR AND CELLULAR BIOLOGY, May 1997, p. 2920–2932 Vol. 17, No. 5
0270-7306/97/$04.00+0
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Heterodimerization of Msx and Dlx Homeoproteins Results in Functional Antagonism

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Received 12 November 1996/Returned for modification 14 January 1997/Accepted 19 February 1997

Protein-protein interactions are known to be essential for specifying the transcriptional activities of homeoproteins. Here we show that representative members of the Msx and Dlx homeoprotein families form homo- and heterodimeric complexes. We demonstrate that dimerization by Msx and Dlx proteins is mediated through their homeodomains and that the residues required for this interaction correspond to those necessary for DNA binding. Unlike most other known examples of homeoprotein interactions, association of Msx and Dlx proteins does not promote cooperative DNA binding; instead, dimerization and DNA binding are mutually exclusive activities. In particular, we show that Msx and Dlx proteins interact independently and noncooperatively with homeodomain DNA binding sites and that dimerization is specifically blocked by the presence of such DNA sites. We further demonstrate that the transcriptional properties of Msx and Dlx proteins display reciprocal inhibition. Specifically, Msx proteins act as transcriptional repressors and Dlx proteins act as activators, while in combination, Msx and Dlx proteins counteract each other's transcriptional activities. Finally, we show that the expression patterns of representative *Msx* and *Dlx* genes (*Msx1*, *Msx2*, *Dlx2*, and *Dlx5*) overlap in mouse embryogenesis during limb bud and craniofacial development, consistent with the potential for their protein products to interact in vivo. Based on these observations, we propose that functional antagonism through heterodimer formation provides a mechanism for regulating the transcriptional actions of Msx and Dlx homeoproteins in vivo.

It is widely accepted that the specific actions of transcriptional regulatory proteins are mediated through their selective association with other protein factors. Such interactions allow transcription factors to distinguish relevant target sequences from the many fortuitous binding sites in the genome and confer highly precise transcriptional regulatory properties. Selective protein-protein interactions are thought to be particularly important for specifying the actions of homeodomain-containing transcriptional regulatory proteins. Homeoproteins are notorious for their promiscuous DNA binding specificities, which contrast with their highly selective biological functions. It is therefore presumed that specificity is achieved through their interactions with other protein factors. Protein-protein interactions are likely to be particularly important for specifying the transcriptional activities of Msx homeoproteins. The murine Msx family includes three members, two of which (Msx1 and Msx2) have been well characterized with respect to their DNA binding and transcriptional properties (3–5, 40, 43) and one of which (Msx3) has been recently described (14, 33). The homeodomain sequences of Msx proteins are highly conserved (>90%), and Msx proteins also share several other conserved features, including nearly identical sequences that flank the homeodomain (the extended homeodomain [EHD]) and three other regions of similarity located N terminal and C terminal of the homeodomain (Msx homology regions) (see Fig. 1). In addition, the DNA binding specificities of Msx1 and Msx2 are virtually identical, and both proteins function as transcriptional repressors (4, 40). Moreover, Msx1 and Msx2 share an unusual feature in which repression is mediated through interactions with other protein factors rather than binding to homeodomain DNA sites (4, 5, 43). Therefore, the Msx1 homeodomain interacts directly with the TATA-binding protein (TBP), and the residues in the homeodomain that mediate this interaction are also required for repression by Msx1 (43). However, the ability of Msx proteins to regulate specific target genes undoubtedly requires additional, as yet undefined, interactions with protein factors that exhibit tissue-restricted expression and promoter-specific activities.

The embryonic expression patterns of *Msx* genes, as well as the phenotypic consequences of targeted disruption of *Msx1*, are consistent with a role for Msx proteins in inductive signaling between epithelial and mesenchymal tissues. *Msx* genes are expressed primarily in regions of epithelial-mesenchymal interactions, such as the limb bud, tooth, heart, and neural tube (2, 4, 6, 9, 13, 18, 22, 23, 25, 31), and targeted disruption of *Msx1* leads to significant defects in the development of craniofacial structures (32). Moreover, a role for Msx proteins in active morphogenesis is further suggested by the lack of *Msx1* expression in cells undergoing terminal differentiation (35, 40) and by the restricted expression of *Msx1* transcripts during periods of rapid cellular proliferation (38) in tissues that are maintained in a developmentally plastic state (26) and during tissue regeneration (29, 34).

These features of their expression patterns and their biochemical activities as transcriptional repressors suggest that Msx proteins might function as negative regulators of cellular differentiation through repression of differentiation-specific target genes. Given that repression by Msx proteins is mediated through interactions with other protein factors, we have

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sought to identify partners that modulate the transcriptional actions of Msx proteins. In this report, we demonstrate that members of the Dlx family of homeoproteins form dimeric complexes with Msx homeoproteins in vitro and in vivo. Interestingly, dimerization blocks, rather than facilitates, DNA binding by Msx and Dlx proteins and results in reciprocal inhibition of their transcriptional activities. Based on these biochemical data and on their overlapping spatiotemporal expression patterns during murine embryogenesis, we propose that functional antagonism provides a mechanism by which Msx and Dlx proteins mutually regulate their transcriptional actions in vivo.

MATERIALS AND METHODS

Plasmids. Descriptions of all of the plasmids used in this study are provided in Table 1. The full-length coding sequence of *Dlx2* was isolated by PCR from the corresponding cDNA (27) by using oligonucleotides that contained the appropriate restriction sites for cloning, as indicated in Table 1. A cDNA encoding murine *Dlx5* was isolated from a 12.5-day postcoital total embryonic cDNA library (45). The full-length coding sequence and various truncated derivatives of *Dlx5* were isolated by PCR with the appropriate oligonucleotides for cloning, as indicated in Table 1. The plasmids used for yeast two-hybrid analysis, pGADGH and pGBT9 (Clontech), were modified to alter the coding frame of the *Bam*HI site in the polylinker such that the coding sequences of the *Msx* or *Dlx* gene could be subcloned directly from the pGEX constructs. The *MyoD* promoter-enhancer–luciferase plasmid was constructed by subcloning a *Sac*I-*Xho*I fragment containing the F3/-2.5 *MyoD* promoter-enhancer fragment (40) into the corresponding sites of pGL2-basic (Promega).

Protein preparation. Proteins were produced by in vitro transcription-translation as previously described (43). Recombinant proteins were hexahistidine fusion polypeptides (the pDS56 series) or glutathione *S*-transferase (GST) fusion proteins (the pGEX series). Procedures for production and purification of hexahistidine fusion proteins have already been described (3, 4). Production of GST fusion proteins was described in reference 43. As noted previously, the GST fusion proteins used in these experiments were immobilized on GST-agarose beads. The concentrations and purities of the GST-fusion proteins were estimated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, followed by Coomassie blue staining.

In vitro assays. GST interaction assays were performed as described in reference 43, and gel retardation assays were performed as described in references 3 and 4. The double-stranded oligonucleotide DNA site was the Msx1 consensus DNA site (site 6) (3) or the *Wnt-1* genomic site (the *WIP* series) (16). The sequences of the *WIP* oligonucleotides are as follows (top strand shown): *WIP*, 5′-CACTAATTGAGGTAATTATCT-3′; WIP5, 5′-CACTAATTGAGGGTAATTATCT-3′; WIP10, 5′-CACTAATTGAGGGAGAGGTAATTATCT-3′. Assays were performed a minimum of four times; representative results are shown. Unless otherwise indicated, reciprocal experiments performed with other members of the Msx and Dlx families (besides the ones shown) were entirely consistent with the data shown.

Yeast two-hybrid analysis. Yeast strain YGH1 (**a** *ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3* Can^r *gal4-542 gal80-538 LYS2::gal1$_{uas}$-gal1$_{tata}$-his3 URA3::gal1-lacZ*) was grown in YEPD (yeast extract-peptone-dextrose) medium and transformed by a lithium acetate method (1). Individual transformant colonies were cultured in liquid SD medium (2% dextrose; 1× Leu$^-$, Trp$^-$, His$^-$ dropout solution; 1× yeast nitrogen base) and assayed for β-galactosidase activity as recommended by the manufacturer (Clontech). Assays were performed three times in triplicate; the data shown are averages of three experiments, and standard deviations are indicated by error bars.

Transient transfection assays. Procedures for maintenance of NIH 3T3 and C2C12 cells were described in references 4 and 5. NIH 3T3 cells were transfected by using calcium phosphate as previously described (5). C2C12 cells were transfected by using Lipofectamine as described by the manufacturer (GIBCO Bethesda Research Laboratories). A plasmid encoding β-galactosidase was included as an internal control in all transfection assays, and transfection efficiency was monitored by measuring β-galactosidase activity. Data are expressed as the fold difference between the luciferase activity obtained with the indicated expression plasmid (e.g., pCB6$^+$ Msx or pCB6$^+$ Dlx) and that obtained with the expression plasmid lacking exogenous sequences (e.g., pCB6$^+$). For clarity of presentation, data are represented as fold luciferase activity relative to a baseline of 0 (rather than 1). All transfection assays were repeated a minimum of four times in duplicate; representative assays are shown. We verified that the Msx and Dlx proteins were expressed at similar levels in mammalian cells by Western blot analysis (15a). To obtain equivalent amounts of the Dlx2 protein relative to the Msx1 protein, we used twice the amount of the corresponding expression plasmid.

Whole-mount in situ hybridization. Whole-mount in situ hybridization of mouse embryos was performed exactly as previously described (4). Digoxigenin-labeled antisense riboprobes were prepared by using T7 or SP6 RNA polymerase as previously described (4). The probes corresponded to the full-length coding sequences of Msx1, Msx2, Dlx2, and Dlx5, as described in Table 1. Mouse embryos from days 8.5 to 11.5 post coitum were analyzed in parallel. Although these four genes have not been directly compared previously, the expression patterns of the individual genes have been described (4, 8, 12, 23, 27, 30, 45).

RESULTS

Formation of dimeric complexes by Msx and Dlx proteins in vitro and in vivo. Since many transcription factors are known to dimerize through conserved domains, we predicted that Msx proteins might interact with other, related homeoproteins. The most similar homeoproteins are members of the Dlx family, which share 58% identity with Msx proteins within the homeodomain (Fig. 1). Outside of the homeodomain, the Dlx and Msx proteins are completely divergent, although members of the Dlx family have certain sequence features in common (Fig. 1). In particular, the regions flanking the homeodomain (the EHD) are virtually identical among all Dlx proteins and two other regions, Dlx homology regions 1 and 2 (DHR1 and DHR2) are also well conserved (Fig. 1). To test whether the Msx and Dlx proteins form dimeric complexes in vitro, we performed GST interaction assays (Fig. 2A). These and subsequent experiments were performed with two Msx proteins (Msx1 and Msx2) and two Dlx proteins (Dlx2 and Dlx5) that we consider to be representative of these homeoprotein families. As shown in Fig. 2A, ^{35}S-labeled Msx1 and Msx2 interacted with GST-Msx1, GST-Msx2, GST-Dlx2, and GST-Dlx5 but not with GST alone. Conversely, ^{35}S-labeled Dlx2 and Dlx5 interacted with GST-Msx1, GST-Msx2, GST-Dlx2, and GST-Dlx5 but not with GST alone. In contrast, none of these Msx and Dlx proteins interacted efficiently with a more divergent homeoprotein, HoxC8 (Fig. 2A). Three types of Msx-Dlx dimeric complexes were formed: (i) homodimeric complexes between individual Msx and Dlx proteins (e.g., Msx1-Msx1), (ii) heterodimeric complexes between members of the same family (e.g., Msx1-Msx2), and (iii) heterodimeric complexes between members of the Msx and Dlx families (e.g., Msx1-Dlx2).

To test whether the Msx and Dlx proteins also interact in vivo, we performed yeast two-hybrid analysis by using Msx1 as the "bait" (DNA binding domain [DB] fusion) and Msx1, Msx2, or Dlx2 as the test protein (activation domain [AD] fusion) (Fig. 2B). (We were unable to include Dlx5 in this assay, because it produced high background levels of transcriptional activity in yeast cells [14a].) As shown in Fig. 2B, coexpression of DB-Msx1 along with AD-Msx1, -Msx2, or -Dlx2 resulted in transcriptional activation of the reporter gene (depicted as β-galactosidase activity), whereas expression of DB-Msx1 or the AD fusion proteins alone was unable to support transcription. Furthermore, the levels of transcriptional activation were significantly greater for the Msx-Dlx heterodimeric complex (DB-Msx1–AD-Dlx2) than for the homodimeric (DB-Msx1–AD-Msx1) or Msx-Msx heterodimeric (DB-Msx1–AD-Msx2) complex (Fig. 2B). These findings demonstrate that the Msx and Dlx proteins (i) form dimeric complexes in vitro, (ii) have the potential to dimerize in vivo, and (iii) may exhibit a preference for formation of heterodimeric complexes.

Mediation of dimerization of Msx and Dlx proteins by specific residues in their homeodomains. We next examined the regions of the Msx and Dlx proteins that mediate their interactions in vitro by using a series of truncated GST-Msx and GST-Dlx fusion proteins (Fig. 3). The GST-Msx1 proteins contained various combinations of the N-terminal region, the homeodomain, or the C-terminal region (Fig. 3A). Since the biochemical properties of Dlx proteins had not been well described, we produced a more extensive series of truncated

TABLE 1. Probes and plasmids used in this study

Purpose and name of protein or probe	Plasmid and characteristic(s)[a]	Source or reference
In vitro transcription-translation		
Msx1	pGEM7zf(+)-Msx1(1–297)	43
Msx1-A	pGEM7zf(+)-Msx1-A (1–297): K168A, R170A, F173A	43
Msx1-B	pGEM7zf(+)-Msx1-B (1–297): L181A, F185A	43
Msx1-C	pGEM7zf(+)-Msx1-C (1–297): R183A, K184A, R186A, Q187A	43
Msx1-D	pGEM7zf(+)-Msx1-D (1–297): R196A	43
Msx1-E	pGEM7zf(+)-Msx1-E (1–297): I212A, Q215A, N216A	43
Msx2	pGEM7zf(+)-Msx2 (1–267): Msx2 ORF inserted into *Kpn*I-*Hin*dIII sites	
Dlx2	pGEM11zf(−)-Dlx2 (1–332): Dlx2 ORF inserted into *Xba*I-*Hin*dIII sites	
Dlx5	pGEM11zf(−)-Dlx5 (1–289): Dlx5 ORF inserted into *Bam*HI-*Hin*dIII sites	
HoxC8	pGEM7zf(+)-Myc-HoxC8 (1–242): 5′ Myc-HoxC8 ORF inserted into *Kpn*I-*Hin*dIII sites	
Myc-Msx1	pGEM11zf(−)-Myc-Msx1 (1–297): 5′ Myc-Msx1 ORF inserted into *Kpn*I-*Hin*dIII sites	
HA-Msx1	pGEM11zf(+)-HA-Msx1 (1–297): Msx1 ORF-3′ hemagglutinin inserted into *Xba*I site	
In situ hybridization		
Msx1	BluescriptII SK(−)-Msx1 (1–297): Msx1 ORF inserted into *Bam*HI-*Hin*dIII sites	
Msx2	BluescriptII SK(−)-Msx2 (1–267): Msx2 ORF inserted into *Bam*HI-*Hin*dIII sites	
Dlx2	BluescriptII SK(−)-Dlx2 (1–332): Dlx2 ORF inserted into *Bam*HI-*Hin*dIII sites	
Dlx5	BluescriptII SK(−)-Dlx5 (1–289): Dlx5 ORF inserted into *Bam*HI-*Hin*dIII sites	
GST fusions		
GST-Msx1	pGEX2T-Msx1 (1–297)	43
GST-Msx1Δ1	pGEX2T-Msx1 (1–165)	43
GST-Msx1Δ2	pGEX2T-Msx1 (1–225)	43
GST-Msx1Δ3	pGEX2T-Msx1 (166–225)	43
GST-Msx1Δ4	pGEX2T-Msx1 (166–297)	43
GST-Msx1Δ5	pGEX2T-Msx1 (226–297)	43
GST-Msx2	pGEX2T-Msx2 (1–267): Msx2 ORF inserted into *Bam*HI-*Eco*RI sites	
GST-Dlx2	pGEX2T-Dlx2 (1–332): Dlx2 ORF inserted into *Bam*HI-*Eco*RI sites	
GST-Dlx5	pGEX2T-Dlx5 (1–289): Dlx5 ORF inserted into *Bam*HI-*Eco*RI sites	
GST-Dlx5Δ1	pGEX2T-Dlx5 (1–136): Dlx5 (1–136) inserted into *Bam*HI-*Eco*RI sites	
GST-Dlx5Δ2	pGEX2T-Dlx5 (1–53): Dlx5 (1–53) inserted into *Bam*HI-*Eco*RI sites	
GST-Dlx5Δ3	pGEX2T-Dlx5 (54–112): Dlx5 (54–112) inserted into *Bam*HI-*Eco*RI sites	
GST-Dlx5Δ4	pGEX2T-Dlx5 (1–197): Dlx5 (1–197) inserted into *Bam*HI-*Eco*RI sites	
GST-Dlx5Δ5	pGEX2T-Dlx5 (137–197): Dlx5 (137–197) inserted into *Bam*HI-*Eco*RI sites	
GST-Dlx5Δ6	pGEX2T-Dlx5 (137–289): Dlx5 (137–289) inserted into *Bam*HI-*Eco*RI sites	
GST-Dlx5Δ7	pGEX2T-Dlx5 (198–289): Dlx5 (198–289) inserted into *Bam*HI-*Eco*RI sites	
Yeast two-hybrid system		
DB	pGBT9: modified to make *Bam*HI site in frame	Clontech
DB-Msx1	pGBT9-Msx1 (1–297): Gal4 DNA binding domain-Msx1 ORF inserted into *Bam*HI-*Sal*I sites	
AD	pGAD GH: modified to make *Bam*HI in frame	Clontech
AD-Msx1	pGAD GH-Msx1 (1–297): Gal4 activation domain-Msx1 ORF inserted into *Bam*HI-*Sal*I sites	
AD-Msx2	pGAD GH-Msx2 (1–267): Gal4 activation domain-Msx2 ORF inserted into *Bam*HI-*Eco*RI sites	
AD-Dlx2	pGAD GH-Dlx2 (1–332): Gal4 activation domain-Dlx2 ORF inserted into *Bam*HI-*Sal*I sites	
Transient transfection		
Expression plasmids		
Msx1	pCB6$^+$-Msx1 (1–297)	5
Dlx2	pCB6$^+$-Dlx2 (1–332): Dlx2 ORF inserted into *Hin*dIII-*Xba*I sites	
Dlx5	pCB6$^+$-Dlx5 (1–289): Dlx5 ORF inserted into *Eco*RI-*Hin*dIII sites	
Gal4-Msx1	pM2-Msx1 (1–297)	5
Gal4-Dlx5	pM2-Dlx5 (1–289): Dlx5 ORF inserted into *Bam*HI-*Hin*dIII sites	
Reporter plasmids		
MyoD reporter	pGL2-MyoD-F3/-2.5Luc: MyoD enhancer-promoter fragment inserted into *Sac*I-*Xho*I sites of pGL2-basic	40
WIP reporter	pGL2-WIP	16
mWIP reporter	pGL2-WIP-mHBS1+2	16
Gal4 reporter	5XGal4-pGL2 promoter mutant	5
Bacterial expression		
Msx1	pDS56-Msx1 (1–297)	5
MsxHD	pDS56-Msx1 (157–233)	3
Dlx2	pDS56-Myc-Dlx2 (1–332): 5′-Myc-Dlx2 ORF inserted into *Bam*HI-*Hin*dIII sites	
Dlx5	pDS56-Dlx5 (1–289): Dlx5 ORF inserted into *Bam*HI-*Hin*dIII sites	

[a] ORF, open reading frame.

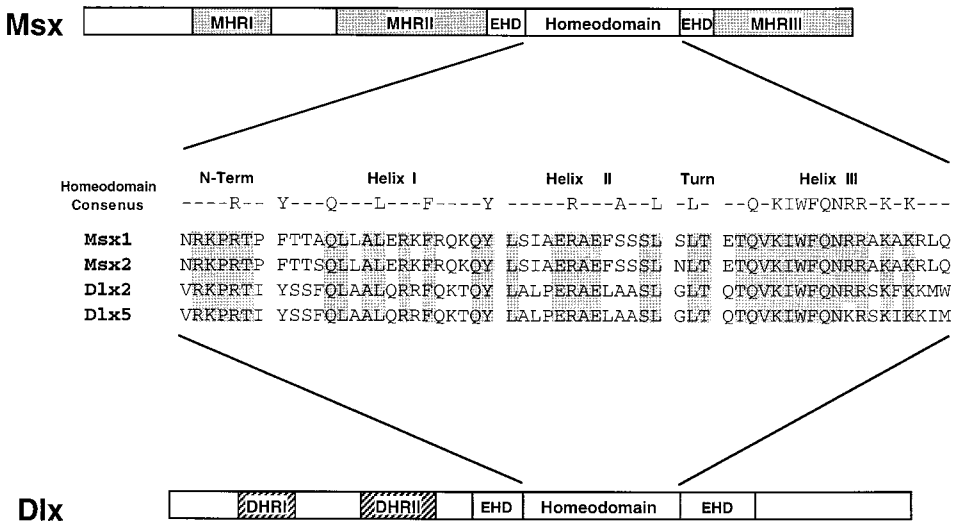


FIG. 1. Comparison of Msx and Dlx proteins. The schematic diagrams show the general organization of Msx (top) and Dlx (bottom) proteins. The locations of the regions conserved among members of the Msx family (MHR for Msx homology region) and the DHRs conserved among members of the Dlx family are shown. Also indicated are the positions of the homeodomains and the conserved regions directly flanking these domains that correspond to the EHD. The amino acid sequences of the Msx1, Msx2, Dlx2, and Dlx5 homeodomains are compared with the consensus sequence; identical residues are highlighted by the shaded boxes. Also indicated are the positions of the N-terminal arm (N-Term) and helices I, II, and III.

GST-Dlx5 fusion proteins containing various combinations of the conserved regions (Fig. 3B). Only those GST-Msx or GST-Dlx proteins that contained the homeodomain (i.e., GST-Msx1, GST-Msx1Δ2, GST-Msx1Δ3, GST-Msx1Δ4, GST-Dlx5, GST-Dlx5Δ4, GST-Dlx5Δ5, and GST-Dlx5Δ6) interacted with Dlx5 or Msx1, respectively (Fig. 3). No such interaction was observed when the GST-Msx or GST-Dlx proteins that lacked the homeodomain (i.e., GST-Msx1Δ1, GST-Msx1Δ5, GST-Dlx5Δ1, GST-Dlx5Δ2, GST-Dlx5Δ3, and GST-Dlx5Δ7) were used (Fig. 3). Similar results were obtained in reciprocal experiments performed by using a similar series of truncated GST-Dlx2 proteins and with various combinations of input ^{35}S-labeled Msx or Dlx proteins (42a). We therefore concluded that the primary dimerization domain is the homeodomain.

To define specific residues within the homeodomain required for dimerization, we used a series of mutated Msx1 proteins containing multiple alanine substitutions clustered in the N-terminal arm or helix I, II, or III (Fig. 4) (43). (Note that most of the residues that were substituted are also conserved in Dlx homeodomains [Fig. 1].) Substitutions of certain residues in the N-terminal arm, helix I, or helix III (Msx1-A, Msx1-B, or Msx1-E, respectively) abrogated or significantly reduced dimerization, whereas substitutions of other residues in helix I or II (Msx1-C and Msx1-D) had no such effect (Fig. 4). It is noteworthy that the association of Msx1 with TBP required only the residues in the N-terminal arm (43), suggesting that dimerization between the Msx and Dlx proteins differs from the interaction of Msx1 with this general transcription factor.

Mutual exclusiveness of dimerization and DNA binding activities of Msx and Dlx proteins. Since the residues required for dimerization correspond to those required for DNA binding (43; shown for comparison in Fig. 4), we next investigated the relationship between dimerization and DNA binding by Msx and Dlx proteins. To test whether these proteins form dimeric complexes on DNA, full-length Msx1 or Dlx2 was mixed with a truncated Msx protein containing the homeodomain (MsxHD) (Fig. 5A). Formation of a protein-DNA complex with intermediate mobility upon the mixing of MsxHD with Msx1 or Dlx2 would suggest their dimeric interaction on DNA. However, no such intermediate-mobility complex was detected when MsxHD was mixed with either Msx1 or Dlx2 (Fig. 5A). The experiment whose results are shown in Fig. 5A was performed with recombinant proteins and the consensus DNA site; similar results were obtained with in vitro-translated Msx and Dlx proteins and with variations of the consensus site (44).

The previous experiments were performed by using a single copy of the consensus DNA site, which may preclude detection of cooperative interactions of Msx and Dlx proteins on DNA. We therefore performed additional gel retardation assays by using a natural genomic DNA element, termed *WIP*, that contains two tandem copies of the homeodomain DNA binding site (HBSI and HBSII) separated by four nucleotides (16). Since appropriate spacing is important for cooperativity among homeodomain proteins (17, 39), we produced two variants of *WIP* that contained 5- and 10-nucleotide spacers (*WIP5* and *WIP10*, respectively). Cooperativity of Msx and Dlx proteins would be evident as increased overall DNA binding activity when the two proteins were assayed together compared with their individual activities and might also depend upon the spacing between the homeodomain DNA sites. However, no notable difference in DNA binding activity was observed when Msx1 and Dlx2 were assayed alone or together on the *WIP* element (Fig. 5B). Moreover, although Msx1 and Dlx2 interacted somewhat more efficiently with *WIP5* and *WIP10*, they did not exhibit enhanced DNA binding activity when assayed in combination (Fig. 5B). These DNA binding experiments indicate that Msx and Dlx proteins bind to homeodomain DNA sites as monomers and interact noncooperatively with multiple homeodomain DNA sites.

Given that the DNA binding residues in the homeodomain are required for dimerization but we detected no evidence of dimerization or cooperativity on DNA, we next examined whether homeodomain DNA binding sites affect the interaction of Msx and Dlx proteins in solution. We performed GST interaction assays in the presence of the consensus DNA site from the *WIP* element (HBS) or a mutated version of this site to which Msx1 does not bind (mHBS) (16). As shown in Fig.

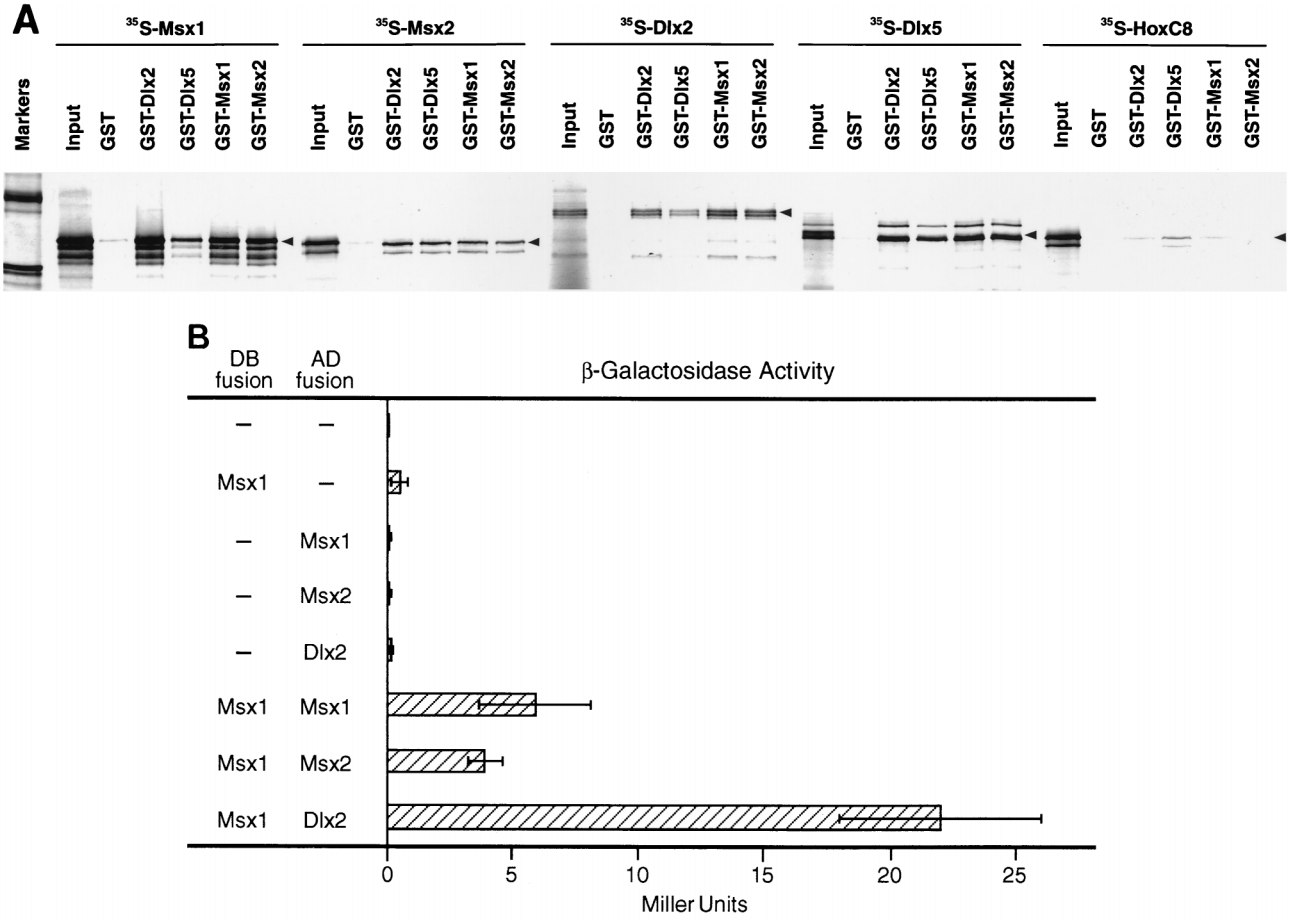
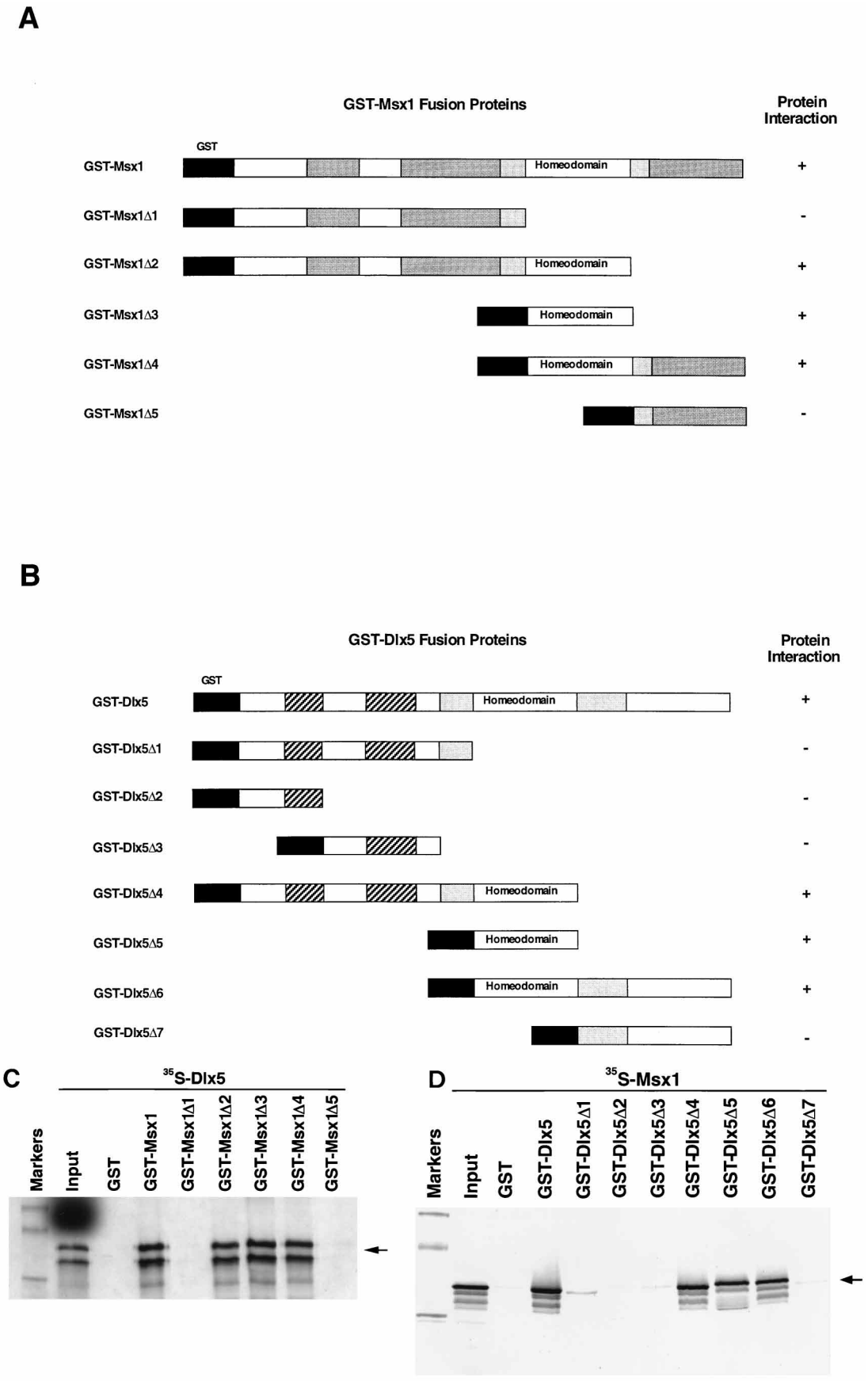


FIG. 2. Formation of dimeric complexes by Msx and Dlx proteins in vitro and in vivo. (A) GST interaction assays performed with 5 μg of GST or the indicated GST fusion protein (GST-Dlx2, GST-Dlx5, GST-Msx1, or GST-Dlx2) and ^{35}S-labeled Msx1, Msx2, Dlx2, Dlx5, or HoxC8, as shown. Immobilized proteins were resolved by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. The positions of precipitated proteins are indicated by the arrowheads. The input lanes contained 20% (1 μl) of the total ^{35}S-labeled protein (5 μl) used in the interaction assays, and the marker lane shows the positions of ^{14}C-labeled protein molecular size standards (bovine serum albumin, 68 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 31 kDa). (B) Interaction of Msx and Dlx proteins in a yeast two-hybrid assay. Plasmids expressing fusion proteins containing the GAL4 DB and full-length Msx1 or the GAL4 AD with Msx1, Msx2, or Dlx2 were transformed into yeast strain YGH1 with the parental plasmid alone or in the combinations shown. β-Galactosidase activity was measured in yeast cell extracts of individual transformants. The data shown are averages of three independent experiments performed in triplicate; each error bar represents 1 standard deviation.

5C, the interaction of GST-Msx1 with ^{35}S-labeled Dlx5 was abolished by inclusion of an equimolar amount of HBS but not by inclusion of the mutated version (mHBS). In contrast, neither HBS nor mHBS inhibited the association of Msx1 with TBP (Fig. 5C), which is, as noted above, mediated through homeodomain residues that are different from those required for the Msx-Dlx interaction (43). Consistent with these observations, the efficiency of the Msx-Dlx interaction was not reduced by the addition of ethidium bromide (Fig. 5D) or micrococcal nuclease (42a), which are used to ensure that in vitro interaction mixtures are free of contaminating DNA (20). Together with the previous DNA binding experiments, these findings demonstrate that the dimerization and DNA binding activities of Msx and Dlx proteins are mutually exclusive.

Reciprocal inhibition of transcriptional activities of Msx and Dlx proteins. We next explored the functional consequences of the Msx-Dlx interaction for the respective transcriptional activities of these homeoproteins (Fig. 6). Since the transcriptional properties of Dlx proteins had not been described, we first compared the activities of Dlx2 and Msx1 in transient cotransfection assays (Fig. 6A). Assays were performed with NIH 3T3 cells by using a reporter plasmid containing the *WIP* element or a mutated *WIP* element (*mWIP*) upstream of the simian virus 40 promoter and a luciferase reporter gene (16). As shown in Fig. 6A, transfection of the *Dlx2* expression plasmid resulted in significant transcriptional activation of the *WIP* reporter plasmid (~15-fold), whereas the activity of the *mWIP* reporter plasmid was minimally increased

FIG. 3. Mediation of Msx and Dlx protein dimerization through their homeodomains. (A and B) Diagrams of the GST-Msx1 and GST-Dlx5 fusion proteins showing the regions contained in each of the truncated polypeptides (designated GST-Msx1Δ1-5 and GST-Dlx5Δ1-7). The amino acid sequences included in the truncated polypeptides are listed in Table 1. Experiments were performed a minimum of three times, and the summary was derived from these experiments. (C and D) GST interaction assays performed with ^{35}S-labeled Dlx5 or Msx1 (5 μl) and GST or the indicated GST-Msx1 or GST-Dlx5 fusion protein (5 μg). Immobilized proteins were resolved by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. The positions of the precipitated proteins are indicated by the arrows. The input lanes contained 20% (1 μl) of the total ^{35}S-labeled protein (5 μl) used in the interaction assays, and the marker lanes show the positions of ^{14}C-labeled protein molecular size standards.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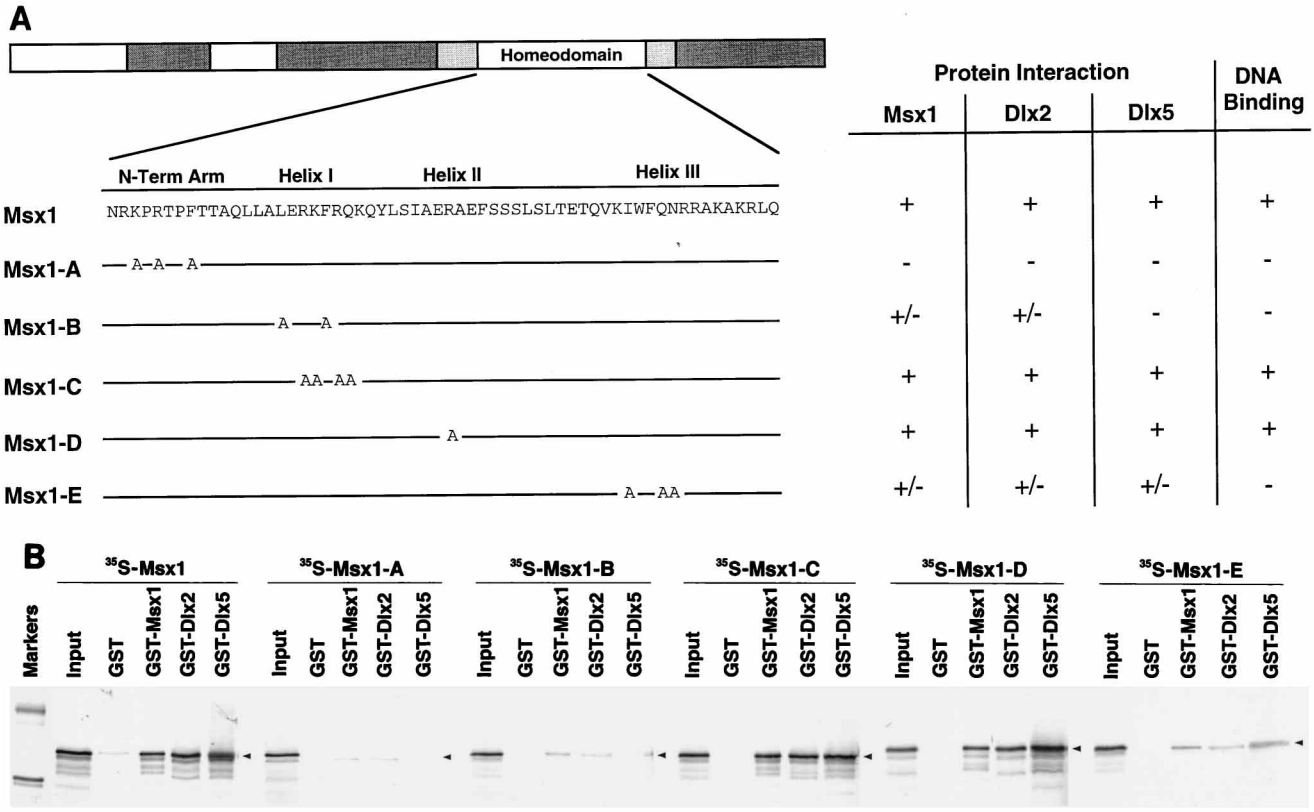

FIG. 4. Requirement of DNA binding residues in the homeodomain for dimerization of Msx and Dlx. (A) Schematic diagram showing the position of the homeodomain in Msx1. Shown below is the amino acid sequence of the homeodomain indicating the relative positions of the N-terminal arm (N-Term Arm) and helices I, II, and III. Msx1-A to -E contain the alanine substitutions of the residues represented by the letter A (the exact amino acid substitutions are listed in Table 1). The summary to the right shows the relative interaction of Msx1-A to -E with Msx1, Dlx2, and Dlx5. DNA binding refers to the corresponding binding activities of the mutated Msx1 proteins which were published previously (43). Protein interaction experiments were performed a minimum of three times, and the summary is derived from these experiments. (B) GST interaction assays performed with ^{35}S-labeled Msx1 or Msx1-A to -E (5 μl) and with GST, GST-Msx1, GST-Dlx2, or GST-Dlx5 (5 μg), as indicated. Immobilized proteins were resolved by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. The positions of the precipitated proteins are indicated by the arrowheads. The input lanes contained 20% (1 μl) of the total ^{35}S-labeled protein (5 μl) used in the interaction assays, and the marker lane shows the positions of ^{14}C-labeled protein molecular size standards.

(~2-fold). In comparable experiments, the *Dlx5* expression plasmid also activated the *WIP* reporter plasmid to a similar extent (14a). In contrast, transfection of the *Msx1* expression plasmid resulted in transcriptional repression of both the *WIP* and *mWIP* reporter plasmids (Fig. 6A), as has been shown previously for Msx1 and Msx2 (4). Therefore, the transcriptional properties of Dlx proteins differ from those of Msx proteins in at least two respects: (i) Dlx proteins are transcriptional activators, whereas Msx proteins are repressors; and (ii) Dlx proteins mediate transcription preferentially through homeodomain DNA sites, while repression by Msx proteins is independent of these sites.

To examine the combined action of Msx1 and Dlx2 proteins, we cotransfected a constant amount of the *Dlx2* expression plasmid with increasing amounts of the *Msx1* plasmid and vice versa. As shown in Fig. 6A, activation by Dlx2 was reduced by inclusion of increasing amounts of Msx1 and, conversely, repression by Msx1 was alleviated by increasing amounts of Dlx2. One interpretation is that repression by Msx1 is "canceled out" due to activation by Dlx2 and vice versa. However, an alternative possibility is that interaction of Msx and Dlx proteins in solution renders them transcriptionally inactive. To distinguish between these possibilities, we performed additional cotransfection assays by using reporter plasmids that are repressed by Msx proteins but are minimally affected by Dlx proteins.

Since *MyoD* is a known target gene for Msx1 (35, 40), we performed transfection assays with C2C12 myoblast cells by using a reporter plasmid containing the *MyoD* enhancer and promoter sequences driving expression of the luciferase reporter gene (*MyoD*/luciferase). As shown in Fig. 6B, transfection of the *Msx1* expression plasmid alone resulted in significant repression (40- to 60-fold) of the *MyoD*/luciferase reporter plasmid, whereas transfection of either the *Dlx2* or *Dlx5* expression plasmid alone had a minimal effect. Cotransfection of a constant amount of the *Msx1* expression plasmid with increasing amounts of the *Dlx2* or *Dlx5* plasmid resulted in alleviation of repression by Msx1 (Fig. 6B). The effect of the Dlx proteins was concentration dependent, such that complete inhibition of repression by Msx1 required equivalent amounts of Dlx2 or Dlx5 protein.

Msx1 also functions as a potent transcriptional repressor when directed to a heterologous DNA element (i.e., the *GAL4* DNA binding site) as a fusion protein with the corresponding heterologous DNA binding domain (i.e., the GAL4 DNA binding domain) (4, 43). Thus, we performed transfection assays by using a *GAL4-Msx1* expression plasmid, a *Dlx2* expression plasmid (which did not contain the GAL4 DNA binding domain) and a *GAL4*/luciferase reporter plasmid (Fig. 6C). As shown in Fig. 6C, transfection of the *GAL4-Msx1* expression plasmid resulted in significant repression (~20-fold) of the

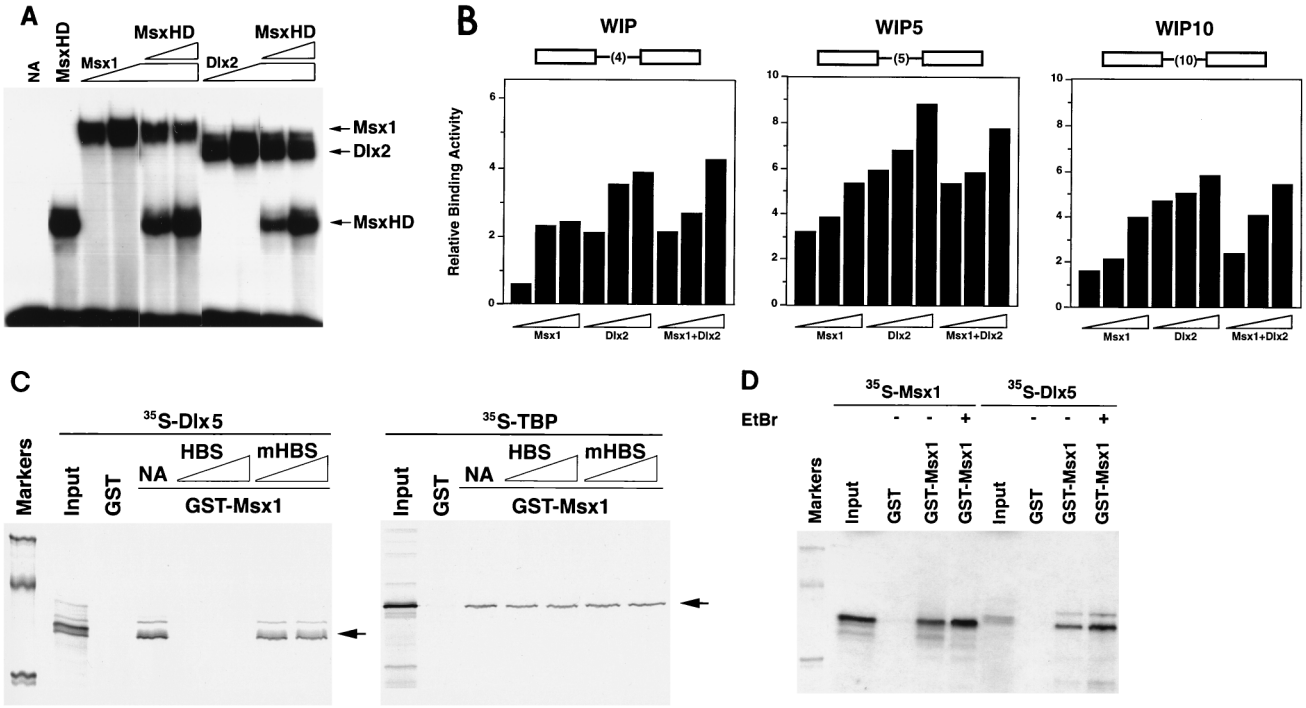

FIG. 5. Mutual exclusiveness of dimerization and DNA binding. (A) Gel retardation assay performed with the Msx1 consensus DNA site (3) and recombinant MsxHD (37.5 or 75 ng), Msx1 (150 or 300 ng), or Dlx2 (150 or 300 ng) (as indicated by the symbols) alone or in combination, as indicated. Note that due to MsxHD's smaller size, this represents an equimolar amount of MsxHD relative to Msx1 and Dlx2. The positions of the protein-DNA complexes are shown at the right; note that no complexes with intermediate mobility are evident. NA, no added protein. (B) Gel retardation assay performed with recombinant Msx and Myc-Dlx2 proteins alone or in combination; each lane contained 75, 150, or 300 ng of total protein (indicated by the symbols at the bottom). The DNA sites (*WIP*, *WIP5*, and *WIP10*) containing two homeodomain DNA binding sites (open boxes) are separated by 4 (*WIP*), 5 (*WIP5*), and 10 (*WIP10*) nucleotides. The total DNA binding activity in each lane was quantitated with a PhosphorImager. Shown is a summary of the relative DNA binding activities; the results are representative of four independent assays. (C) GST interaction assays performed with GST or GST-Msx1 (5 μg; 0.34 μM) and ^{35}S-labeled Dlx5 or TBP. Where indicated, reaction mixtures also contained a DNA fragment corresponding to homeodomain DNA binding site I (HBS) of the *WIP* element or a mutated version of this site (mHBS) (16). Immobilized proteins were resolved by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. The positions of the precipitated proteins are indicated by the arrows. NA, no added DNA site. (D) GST interaction assays performed with GST or GST-Msx1 (5 μg; 0.34 μM) and ^{35}S-labeled Msx1 or Dlx5. A plus sign indicates that the reaction mixture also contained ethidium bromide (EtBr; 0.4 mg/ml). Immobilized proteins were resolved by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography.

GAL4/luciferase reporter plasmid, whereas transfection of the *Dlx2* expression plasmid had a minimal effect (~2- to 3-fold activation). Cotransfection of a constant amount of the *GAL4-Msx1* expression plasmid with increasing amounts of the *Dlx2* expression plasmid resulted in concentration-dependent alleviation of repression by GAL4-Msx1. Similar results were obtained with the Dlx5 expression plasmid (14a). These observations indicate that Dlx proteins antagonize transcriptional repression by Msx1. Combining these with the previous results, we concluded that Msx and Dlx homeoproteins are reciprocal inhibitors of each other's transcriptional actions.

Spatial and temporal overlap of expression patterns of *Msx* and *Dlx* genes during murine embryogenesis. Our preceding results indicate that Msx and Dlx proteins can function antagonistically through their ability to form heterodimeric complexes, which are incapable of interacting with DNA and are transcriptionally inactive. However, for Msx and Dlx to be bona fide coregulators in vivo, they must be expressed in overlapping spatiotemporal domains during embryogenesis. Previously, it has been noted that the expression patterns of *Msx* and *Dlx* genes partially overlap during embryogenesis in mice, chicks, and zebra fish (10–12). In this study, we directly compared the expression of *Msx1*, *Msx2*, *Dlx2*, and *Dlx5* in mouse embryos by whole-mount in situ hybridization analysis. Shown in Fig. 7 are representative embryos at similar stages from days 8.5 through 12.5 of gestation. During this period of embryogenesis, dynamic expression patterns for all four genes are observed in two primary overlapping domains. These domains correspond to derivatives of the cranial neural crest that contribute to the mesenchyme of the branchial arches and of the ectodermal and mesodermal components of the developing limb buds.

Thus, in the first two branchial arches, which will give rise to craniofacial structures, *Msx1*, *Msx2*, *Dlx2*, and *Dlx5* display overlapping domains of expression (Fig. 7A to D). At day 8.5 of embryogenesis, all four genes are expressed in neural crest-derived mesenchymal cells in the distal region of the first branchial arch (Fig. 7A to D). However, unlike the other three genes, *Dlx2* is expressed throughout the first arch and is also found in neural crest cells migrating into the first and second arches (Fig. 7C). By day 9.5 of embryogenesis, all four genes are expressed in the distal regions of the first two branchial arches, although the *Dlx* genes are expressed more proximally (Fig. 7E to H). Note that at these developmental stages, spatial differences in expression between the *Msx* and *Dlx* genes are also evident, in that *Msx1* and *Msx2* are expressed in the neural tube (Fig. 7A, B, E, and F) but *Dlx2* and *Dlx5* are not (Fig. 7C, D, G, and H).

Another example of the overlapping expression of *Msx* and *Dlx* genes is provided during limb bud outgrowth, in both the apical ectodermal ridge (AER) and the underlying mesenchyme (progress zone). At day 9.5, coincident with initial outgrowth of the forelimb bud, the expression patterns of the four genes are as follows: *Msx1* is expressed throughout the limb

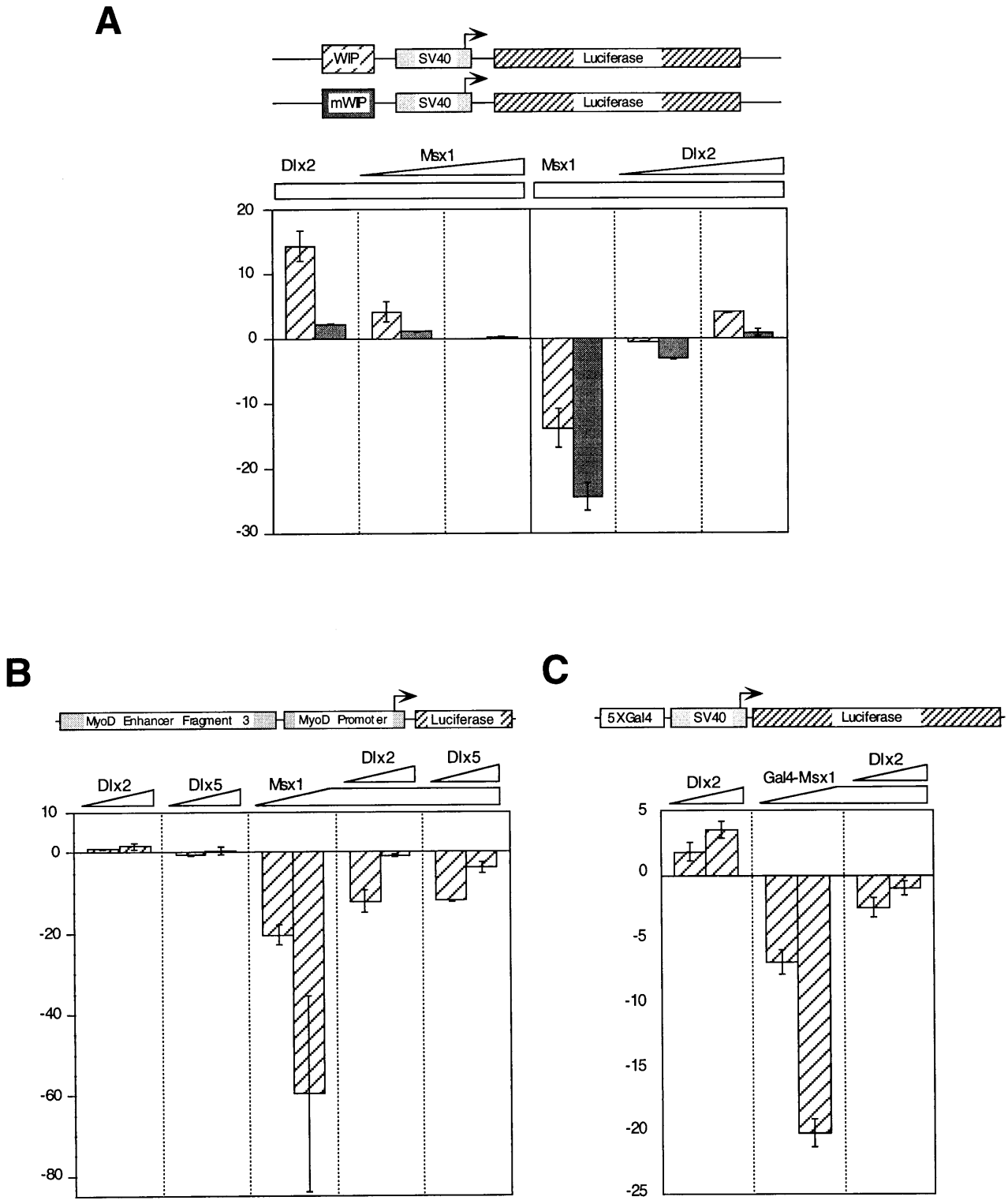

FIG. 6. Mutually antagonistic transcriptional properties of Msx and Dlx proteins. (A to C) Transient-transfection assays performed with NIH 3T3 cells (A) or C2C12 cells (B and C) by using the reporter and expression plasmids shown (described in Table 1). The amount of reporter plasmid in each case was 1 μg. The amounts of expression plasmids were as follows: A, 500 ng of pCB6$^+$Myc-Dlx2 alone or with 125 or 250 ng of pCB6$^+$Myc-Msx1 and 250 ng of pCB6$^+$Myc-Msx1 alone or with 250 or 500 ng of pCB6$^+$Myc-Dlx2; B, 250 and 500 ng of pCB6$^+$Myc-Dlx2 or pCB6$^+$Myc-Dlx5 and 125 or 250 ng of pCB6$^+$Myc-Msx1 with or without 250 or 500 ng of pCB6$^+$Myc-Dlx2 or pCB6$^+$Myc-Dlx5; C, 250 and 500 ng of pCB6$^+$Myc-Dlx2 and 125 or 250 ng of pM2-Msx1 with or without 250 or 500 ng of pCB6$^+$Myc-Dlx2. Data are expressed as fold luciferase activity (as detailed in Materials and Methods); representative assays are shown with bars indicating the error between duplicates. SV40, simian virus 40.

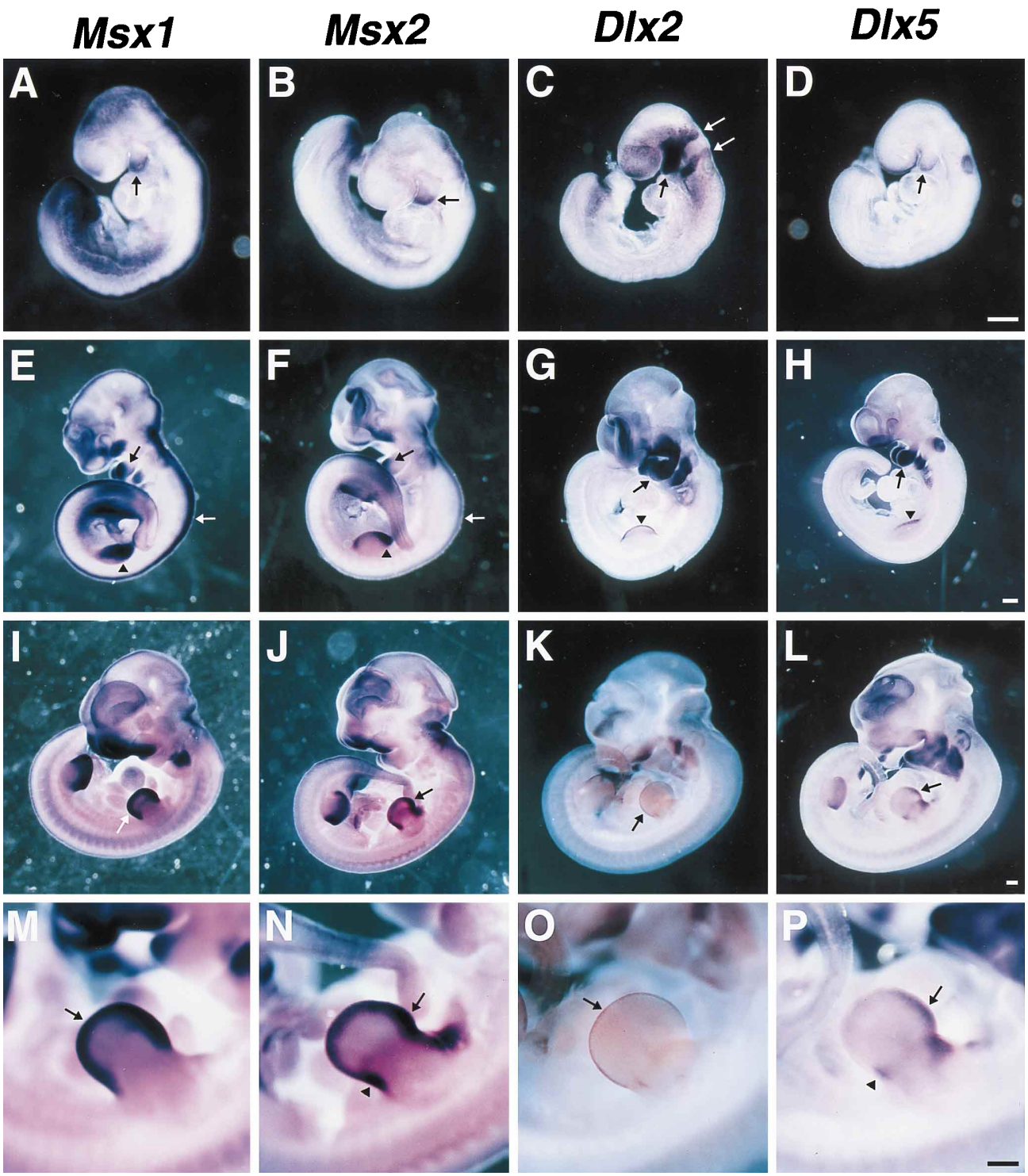

FIG. 7. Whole-mount in situ hybridization analysis of *Msx1*, *Msx2*, *Dlx2*, and *Dlx5*. (A to D) Mouse embryos at day 8.5 post coitum. *Msx1*, *Msx2*, and *Dlx5* are expressed in the distal first branchial arch (arrows in panels A, B, and D), but *Dlx2*, in contrast, is expressed throughout the first arch (black arrow in panel C). Also, note the expression of *Dlx2* in the migrating cranial neural crest populating the first two branchial arches (white arrows in panel C). (E to H) Embryos at day 9.5 post coitum. In the first two branchial arches, *Msx1* and *Msx2* are expressed distally (black arrows in panels E and F), while *Dlx2* and *Dlx5* are both expressed distally and more proximally (arrows in panels G and H). In the emerging forelimb bud, *Msx1* and *Msx2* are expressed in the mesenchyme, while *Dlx2* and *Dlx5* are expressed in the ectoderm (arrowheads); note the asymmetric expression of *Msx2* and *Dlx5* along the anterior-posterior axis. In addition, *Msx1* and *Msx2* are expressed in the neural tube (white arrows in panels E and F), where *Dlx2* and *Dlx5* are not expressed. (I to L) Embryos at day 11.5 post coitum. In the forelimb bud, *Msx1* and *Msx2* are expressed in the mesenchymal progress zone (arrows in panels I and J), while *Dlx2* is found in the apical ectodermal ridge (arrow in panel K). By this stage, expression of *Dlx5* has shifted from the ectoderm to the underlying mesenchyme (arrow in panel L). Expression of each gene persists in craniofacial structures, although with notably distinct distributions. (M to P) Higher-power views of forelimb buds from embryos depicted in panels I through L, respectively. While expression of *Msx1* is symmetric in the limb bud mesenchyme (arrow in panel M), expression of *Msx2* is more prominent along the anterior margin panel (arrow in panel N) and in a localized region of the posterior margin (arrowhead), as well as in the AER (data not shown). *Dlx2* expression is restricted to the AER (arrow in panel O), while *Dlx5* is expressed asymmetrically in the mesenchyme at the anterior margin (arrow in panel P) and in a region along the posterior edge (arrowhead). Scale bars, 0.2 mm.

bud mesenchyme (Fig. 7E); *Msx2* is expressed predominantly at the anterior distal mesenchyme and the ectoderm (Fig. 7F and data not shown), *Dlx2* is expressed only in the ectoderm (Fig. 7G), and *Dlx5* transcripts are found primarily in the anterior ectoderm (Fig. 7H and data not shown). By day 11.5, a notable transition in the expression pattern of *Dlx5* has occurred, such that it is now in the distal mesenchyme of the anterior margin and in a posterior proximal region (Fig. 7P). Thus, during early limb bud outgrowth, coexpression of *Msx* and *Dlx* genes occurs in the ectoderm, while in later stages, coexpression is also observed in restricted regions of the limb bud mesenchyme. Therefore, our results demonstrate that *Msx1*, *Msx2*, *Dlx2*, and *Dlx5* are expressed in overlapping spatiotemporal domains in the developing limb buds and branchial arches, consistent with the potential for their protein products to function in a mutually regulatory capacity in vivo.

DISCUSSION

Selective protein-protein interactions are known to play an essential role in specifying the actions of homeoproteins. In most previously described examples, such interactions promote cooperative DNA binding by homeoproteins and/or facilitate their transcriptional activities. Here we demonstrate that protein-protein interactions may also provide a mechanism for negatively regulating the DNA binding and transcriptional activities of homeoproteins. We show that members of the Msx and Dlx families form dimeric complexes through their homeodomains, mediated by residues that are required for their DNA binding activities. Consequently, these findings indicate that dimerization and DNA binding are mutually exclusive activities. Furthermore, the transcriptional properties of Msx and Dlx proteins display reciprocal inhibition, such that repression by Msx proteins is blocked by Dlx proteins and activation by Dlx proteins is inhibited by Msx proteins. Finally, the overlapping expression patterns of *Msx* and *Dlx* genes during murine embryogenesis suggest that their protein products may form heterodimeric complexes in vivo. These observations indicate that functional antagonism through heterodimer formation may provide a mechanism for regulating homeoprotein function.

Three categories of homeoprotein interactions. In the several known examples of homeoprotein interactions, residues within the homeodomain of one or both partners mediate their association. These interactions can be classified into three distinct categories: (i) interactions of homeoproteins with nonhomeoprotein partners, (ii) interactions of dissimilar homeoproteins (e.g., from unrelated families), and (iii) interactions of homeodomain proteins from similar, closely related families. Notably, within each category, these associations result in similar functional outcomes.

The first type of interaction involves a homeoprotein and a heterologous partner, as exemplified by association of the Oct-1 homeoprotein with the viral transactivator VP16 (7, 15, 19). A notable feature of this category of interaction is that the specificity for the association resides within the homeodomain and that dimerization facilitates the transcriptional activity and/or specificity of the homeoprotein.

In the second category, dimerization is mediated by the homeodomain of one partner and a region adjacent to the dissimilar homeodomain of the second partner. Examples of such interactions between dissimilar homeoproteins include the association of Hox and Pbx proteins (24), the interaction between yeast homeoproteins Mata1 and Matα2 (17), and the interaction between two *Caenorhabditis elegans* homeoproteins MEC-3 and UNC-86 (21, 41). In each of these cases, dimerization results in a similar functional outcome, promoting cooperative DNA binding and/or synergistic transcriptional activity (17, 21, 41).

In contrast, the third category of homeoprotein interactions is mediated by the similar homeodomains of both partners. In some cases, dimerization may result in cooperative DNA binding activity (39), whereas in other cases it may result in the inhibition of DNA binding and/or transcriptional activity. The latter category is composed of two examples, the association between Msx and Dlx proteins that we have described and the previously described interaction between HoxD8 and HoxD9 (42). In both cases, dimerization occurs in solution, is independent of DNA binding, is mediated by DNA binding residues within the homeodomain, and results in inhibition of transcriptional activity.

From this survey of homeoprotein interactions, it is apparent how different categories of interaction might operate together to provide regulatory complexity. For example, the negative regulation imposed by Hox-Hox interactions could be counterbalanced by the positive regulation afforded by Hox-Pbx interactions. Similarly, we might anticipate that the negative Msx-Dlx association could be balanced by an as yet uncharacterized protein partner analogous to Pbx proteins. Undoubtedly, the transcriptional outcome for particular homeoproteins in vivo represents the cumulative effect of both positive and negative protein-protein interactions.

Functional antagonism: a model for reciprocal inhibition by Msx and Dlx. Based on the observations described herein, we can envision three general models to describe the functional consequences of Msx and Dlx protein interactions (Fig. 8A). First, dimerization may prevent Msx proteins from acting as transcriptional repressors (Fig. 8A, part I). Second, dimerization may prevent Dlx proteins from binding to genomic DNA target sites, thereby inhibiting transcriptional activation (Fig. 8A, part II). In each of these two cases, Msx and Dlx proteins would be prevented from acting as transcriptional regulators through the formation of heteromeric complexes. Thus, monomeric proteins would be functionally active while heterodimeric complexes would be inactive. It is notable that in both scenarios, the actions of Msx proteins would invariably result in repression and the actions of Dlx proteins would result in activation. The difference between these two models is in the levels at which their transcriptional regulatory actions are achieved. Therefore, models I and II are not mutually exclusive but are, instead, complementary.

A third possibility is that Dlx-Msx heterodimers have a unique function(s), perhaps through binding to distinct target DNA sites (Fig. 8A, part III). While we cannot rule out this possibility, we have observed no evidence of cooperative DNA binding. Furthermore, we have been unable to detect the interaction of Msx-Dlx heterodimers with alternative DNA sites by using random DNA site selection strategies (24a).

Not illustrated in these models are various competing reactions that would necessarily accompany the regulatory interactions depicted. First, competition for formation of homodimers versus heterodimers, which is dependent upon the actual intracellular concentrations of individual protein factors, would be important for maintaining the appropriate balance of their respective repressing or activating functions. Second, all of the potential interactions of Msx and Dlx proteins would be dependent upon the availability of accessory protein factors, which would undoubtedly modulate their transcriptional activities. Finally, the accessibility and strength of DNA binding sites would also be critical for maintaining the appropriate balance of active monomers relative to inactive dimers.

Msx-Dlx heterodimers: a model for their coregulatory actions in vivo. The previous considerations provide a biochem-

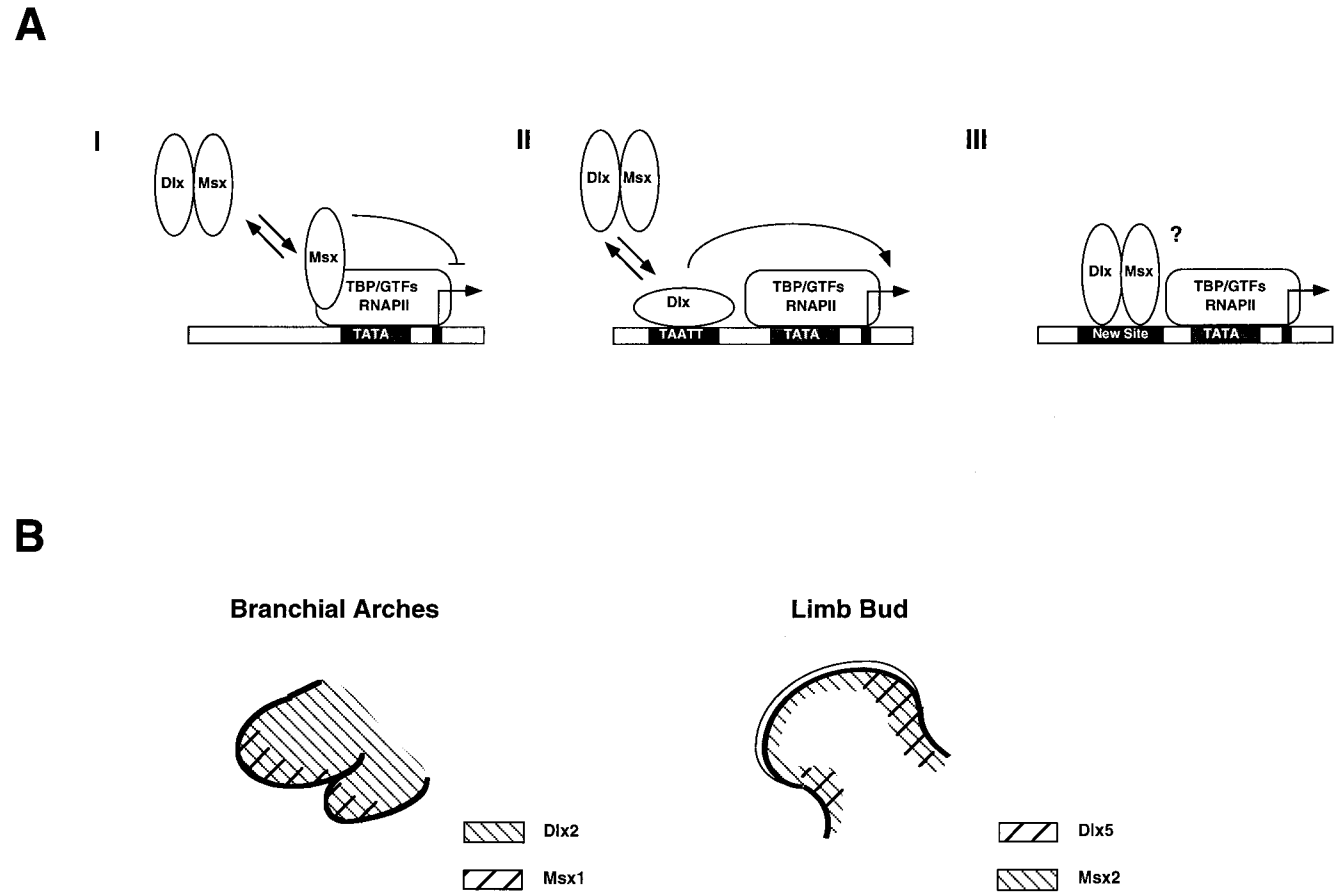

FIG. 8. Models of biochemical and biological actions of Msx and Dlx proteins. The models are described in the text.

ical basis for understanding the antagonistic functions of Msx and Dlx proteins in transcriptional regulation. Our studies also provide insights into the potential roles of *Msx* and *Dlx* genes during mouse embryonic development, particularly in light of recent loss-of-function studies. As illustrated in Fig. 7 and schematically summarized in Fig. 8B, the expression patterns of *Dlx2* and *Msx1* partially overlap in the first two branchial arches, with *Dlx2* expressed throughout the arches while *Msx1* (and *Msx2*) is restricted to the distal regions. Based on our biochemical observations, we predict that the primary zone of *Dlx2* activity resides in the proximal arches, since *Msx1* activity is expected to counteract *Dlx2* activity in the distal regions. Indeed, phenotypic consequences of loss-of-function mutations for *Dlx2* are observed only in the proximal branchial arches (28).

Another region of spatiotemporal overlap between *Msx* and *Dlx* genes is found during limb bud development. Notably, the expression patterns of *Msx2* and *Dlx5* are similar in the anterior distal mesenchyme and posterior margin (Fig. 7N and P and Fig. 8B), as previously noted for their chick homologs (12). A previous interpretation of this coincident expression pattern was that it might reflect regulatory interactions among *Msx1*, *Msx2*, and *Dlx5* in specifying the anterior and posterior limits of the mesenchymal progress zone (12). Our results provide a biochemical foundation for these observations and suggest that such regulatory interactions occur at the level of protein-protein interactions.

In other embryonic regions, however, *Msx* genes are expressed without corresponding expression of *Dlx* genes and vice versa. For example, *Msx* genes are expressed in the neural tube while *Dlx* genes are expressed in regions of the developing brain (Fig. 7). We propose that other factors may have analogous modulatory roles in these spatial domains. Indeed, we have identified a homeoprotein whose expression overlaps that of *Msx* genes in the neural tube and which interacts with Msx1 in vitro (1a).

In summary, these biochemical and biological observations support a model for functional antagonism of Msx and Dlx homeoproteins through their ability to form dimeric complexes that are transcriptionally inactive. We propose that this type of regulatory control may be shared by other homeoproteins, in addition to HoxD8 and HoxD9 (42). Indeed, many homeoproteins are expressed in overlapping patterns during embryogenesis, which may reflect their potential functions as coregulators. Interestingly, Spicer et al. have recently shown that Twist, a member of the basic-helix-loop-helix (bHLH) family of transcription factors, can antagonize the actions of the bHLH and MEF myogenic transcription factors by forming dimers that are functionally inactive (36). Moreover, Taggart and Pugh have shown that dimerization of TBP (and TFIID) inhibits DNA binding and have suggested that dimerization provides a mode of regulating TBP-DNA interaction (37). The similarity of these observations and our findings raises the possibility that functional antagonism through control of the ratio of inactive dimer to active, DNA-bound monomers is a more general mode of regulating transcription factor action.

ACKNOWLEDGMENTS

We thank J. Rubenstein (UCSF) for the murine *Dlx2* cDNA, Mathew Thayer (Oregon Health Sciences University) for the plasmid containing the *MyoD* enhancer-promoter sequences, Peter Lobel (CABM) for the HA antibody, and Lakshmi Rao and Eileen White (CABM) for the yeast two-hybrid plasmids and advice. We thank Martin Nemeroff (CABM) and Lenore Neigeborn (Waksman Institute) for advice in establishing the yeast two-hybrid assay. We thank Arnold Rabson and Celine Gelinas for critical comments on the manuscript.

This work was supported by a grant to C.A.-S. from the NIH (HD29446) and a grant to M.M.S. from the NSF (96-30903). H.Z. was supported by a predoctoral fellowship from the American Heart Association, New Jersey Affiliate (95-FS-07). P.S. was supported by a postdoctoral fellowship from the New Jersey Cancer Commission. N.I. was supported by a predoctoral fellowship from the NIH Biotechnology Training Program (2-T32-GM08339). M.M.S. is a recipient of a Leukemia Society Special Fellowship, and C.A.-S. is a recipient of an NSF Young Investigator Award.

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