Ssdp proteins interact with the LIM-domain-binding protein Ldb1 to regulate development

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The LIM-domain-binding protein Ldb1 is a key factor in the assembly of transcriptional complexes involving LIM-homeodomain proteins and other transcription factors that regulate animal development. We identified Ssdp proteins (previously described as sequence-specific, single-stranded-DNA-binding proteins) as components of Ldb1-associated nuclear complexes in HeLa cells. Ssdp proteins are associated with Ldb1 in a variety of additional mammalian cell types. This association is specific, does not depend on the presence of nucleic acids, and is functionally significant. Genes encoding Ssdp proteins are well conserved in evolution from *Drosophila* to humans. Whereas the vertebrate *Ssdp* gene family has several closely related members, the *Drosophila Ssdp* gene is unique. In *Xenopus*, Ssdp encoded by *Drosophila Ssdp* or mouse *Ssdp1* mRNA enhances axis induction by *Ldb1* in conjunction with the LIM-homeobox gene *Xlim1*. Furthermore, we were able to demonstrate an interaction between *Ssdp* and *Chip* (the fly homolog of *Ldb1*) in *Drosophila* wing development. These findings indicate functional conservation of Ssdp as a cofactor of Ldb1 during invertebrate and vertebrate development.

LIM-homeodomain proteins, encoded by *Lhx* genes, are important transcriptional regulators of invertebrate and vertebrate embryonic development. Their involvement in early patterning events, the development of the nervous system, and organogenesis is well documented (1, 2). Their action is facilitated by cofactors that were identified by their ability to dimerize and bind to the LIM domain, a specialized zinc-finger structure present in *Lhx* gene products and in many other proteins. In vertebrates these LIM-binding cofactors are known as Ldb (3–5), Nli (6), or Clim (7), in *Drosophila* as Chip (8), and in *Caenorhabditis elegans* as Ldb-1 (9). Protein–protein interactions involving Ldb/Nli/Clim (henceforth referred to as Ldb) and Chip are not restricted to LIM domain-containing factors but can involve a host of other transcriptional regulators as well (for review, see ref. 10). Ample evidence supports the notion that the Ldb and Chip cofactors are essential components of developmental programs controlled by transcriptional regulators (3, 8, 9, 11–14). More recently, the Rlim cofactor was identified and shown to negatively control transcription factors by targeting Ldb proteins for degradation (15, 16). Furthermore, competition of transcription factors for binding to Chip or Ldb can also alter developmental cell fates (11, 17–19).

In an effort to identify additional components of Ldb/Chip-containing nuclear protein complexes, we generated HeLa cells that express FLAG and hemagglutinin (HA) epitope-tagged mouse Ldb1, purified nuclear complexes with the aid of the tags, and identified constituent proteins by mass spectrometry of tryptic peptides, by using a previously established approach (20, 21). We identified peptides corresponding to human Ssdp1 and Ssdp3, structural relatives of a chicken nuclear protein previously termed Ssdp, or ''sequence-specific single-stranded DNA-binding protein'' (22). Peptides from the chicken protein were originally detected on the basis of their high-affinity binding to a single-stranded, polypyrimidine sequence from the chicken α2(I) collagen promoter (22). On the basis of EST sequence analysis, a family of closely related genes exists that are well conserved in vertebrate evolution (ref. 23; BLAST searches described below). Here we report a study to address the functional significance of Ssdp/Ldb1 protein interactions. From analysis of interactions in *Xenopus* embryos and phenotypic examination of *Drosophila* mutants we conclude that Ssdp proteins share a role with Ldb/Chip as essential cofactors involved in the transcriptional control of embryonic development.

Materials and Methods

Cell Lines. All cell lines used in this study were obtained from the American Type Culture Collection and cultured in DMEM with 10% FBS. HeLa cell lines expressing FLAG/HA-tagged mouse Ldb1 (3) and Ssdp1 (gi:20452448) were generated by retroviral transduction by using the bicistronic retroviral vector pOZFHN that allows for coordinated expression of the protein of interest with an IL2R surface marker. The transduced cells were purified by repeated cycles of magnetic affinity cell sorting by using anti-IL2R antibodies (Upstate Biotechnology, Lake Placid, NY, no. 05-170) coupled to magnetic beads (Dynal, Great Neck, NY, no. 110.06). Details of these procedures have been described (20, 21).

Complex Purification and Immunoprecipitation. Complexes that contained tagged proteins were purified as described by Ikura *et al.* (21). In brief, 5 ml of nuclear extract from transduced HeLa cells was incubated for 5 h with 1 ml of anti-FLAG agarose (Sigma, no. A2220) and washed five times with 10 ml of 10% glycerol/0.2 mM EDTA/0.1% Tween 20/300 mM KCl/10 mM 2-mercaptoethanol/0.22 mM PMSF (Roche Diagnostics, no. 1359061)/20 mM Tris·HCl, pH 8.0. Bound material was eluted by 1 h incubation with the same buffer containing FLAG peptide (Sigma, no. F3290, 0.4 mg/ml). The eluates were subjected to further purification by using immobilized anti-HA mAb (Covance, Richmond, CA, no. 139050001). The bound proteins were eluted from the matrix by incubation with 100 mM glycine (pH 2.5) for 5 min at room temperature. Polypeptides were resolved by SDS/PAGE and visualized by silver staining as described by Shevchenko *et al.* (24).

In coimmunoprecipitation experiments, nuclear extracts from cells that expressed epitope-tagged Ssdp1 were incubated with anti-FLAG agarose for 5 h, washed (100 mM KCl/20% vol/vol glycerol/0.2 mM EDTA/10 mM 2-mercaptoethanol/0.22 mM PMSF/20 mM Tris·HCl, pH 7.3), and transferred directly into SDS sample buffer (Quality Biologicals, Gaithersburg, MD, no.

Abbreviation: HA, hemagglutinin.

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351082030). The presence of Ldb1 in the immunoprecipitates was determined by Western blot analysis (25), by using primary anti-Ldb1 polyclonal antibodies (L. W. Jurata and G. N. Gill, University of California, San Diego) and secondary peroxidase-conjugated antibodies (DAKO).

Mass Spectrometric Analysis and Database Searches. Material retrieved after gel fractionation was subjected to tryptic digestion, and samples were analyzed by nano-electrospray tandem mass spectrometry performed on a quadrupole time-of-flight instrument (QSTAR, Sciex, Toronto). Peptide sequence tags were assigned in the spectra to retrieve peptide sequences from nonredundant protein data (for details, see ref. 26). Our BLAST searches for Ssdp family members included GenBank CDS translations, Protein Data Bank, SwissProt, Protein Identification Resource, and Protein Research Foundation sequence databases comprising a total of $>10^6$ sequences. Amino-acid alignments were performed with the help of the GCG-LITE+ CLUSTALW MULTIPLE SEQUENCE ALIGNMENT program.

***Xenopus* Injections.** The ORFs of mouse Ssdp1 and *Drosophila Ssdp* were cloned into the *Eco*RI and *Xba*I sites of the pCS2+ expression vector (27, 28). For synthesis of mRNA, both constructs were linearized with *Not*I. Synthetic mRNAs were injected in the ventral equatorial zone of four-cell stage *Xenopus* embryos as described (3).

***Drosophila* Mutants.** Flies were raised on a cornmeal/molasses/yeast/agar/Tegosept medium at 25°C. Care was taken with all crosses to avoid overcrowding the cultures. Unless otherwise noted, the mutations and chromosome aberrations are described in Lindsley and Zimm (29) or in Flybase (ref. 30; http://flybase.bio.indiana.edu/). We identified five *Ssdp* alleles, including three *P* element insertional mutations ($Ssdp^{KG03600}$, $Ssdp^{BG01663}$, and $Ssdp^{neo48}$), one ethyl methanesulfonate-induced allele ($Ssdp^{31}$), and one allele from hybrid dysgenesis ($Ssdp^{11}$) (J.A.K., unpublished data). The *P* element insertion alleles were obtained from the *Drosophila* stock center (Bloomington, IN) and H. Bellen at the Baylor College of Medicine (Houston). The sites of insertion for the *P* elements are from GenBank (*neo48*, AQ034104; *KG03600*, BH25648) or http://flypush.imgen.bcm.tmc.edu/pscreen (*BG01663*). Both $Ssdp^{31}$ and $Ssdp^{11}$ were isolated as dominant suppressors of the *Moonrat* mutation (ref. 31; J.A.K., unpublished data). The molecular lesions in the $Ssdp^{31}$ and of $Ssdp^{11}$ alleles were not determined. *Df(3R)P14* (90C2; 91B1–2) is a large chromosomal deficiency that deletes *Ssdp*. Other mutations used include: $Chip^{e5.5}$ (32); ap^4, ap^{56f}; four *Bx* mutants, the severe alleles Bx^J and Bx^2 and the mild alleles Bx^3 and Bx^{17-3} (18, 30). Balancers used were: *In(2LR)CyO, Ts(Y;2Lt)B80, Kr*$^-$ *y*$^+$; *In(3LR)TM6B, Hu Sb e Tb ca*; *In(3LR)TM3, p*^{p} *sep Sb bx*^{34e} *e Ser*; and *In(3LR)TM3, P{w*$^{+mC}$ *= ActGFP}JMR2, p*^{p} *sep bx*^{34e} *e Ser*. Germ-line *Ssdp* clones were generated either by X-irradiation (20 Gy at 120 kVp from a Torrex 2800 x-ray cabinet) of first instar larvae of the genotype *P{ry*$^{+t7.2}$ *= neoFRT}82B Ssdp*^{x} *e/P{ry*$^{+t7.2}$ *= neoFRT}82B P{w*$^{+mC}$ *= ovo*$^{D1-18}$*}3R,* or by heat shocking (37°C, 1 h) first instar larvae of the genotype *y*^{1} *w*^{1118} *P{ry*$^{+t7.2}$ *= hsFLP}1/+; P{ry*$^{+t7.2}$ *= neoFRT}82B P{w*$^{+mC}$ *= Ubi-GFP}83 Ssdp*^{x} *e/P{ry*$^{+t7.2}$ *= neoFRT}82B P{w*$^{+mC}$ *= ovo*$^{D1-18}$*}3R* (33). Resulting females were crossed to *Df(3R)P14/TM3, P{w*$^{+mC}$ *= ActGFP}JMR2, p*^{p} *sep bx*^{34e} *e Ser* males. For the x-ray-generated clones, development of GFP-positive and GFP-negative offspring was monitored.

Results

Ssdp Proteins Are Associated with Ldb1. To search for new interaction partners of Ldb1, we generated a HeLa cell line that expresses Ldb1 proteins carrying an N-terminal FLAG/HA epitope tag. Nuclear extracts prepared from these cells (and from nontransduced control cells) were incubated with immobilized anti-FLAG antibodies and the specifically bound materials were eluted by competition with excess amounts of FLAG peptide. Thereafter, immobilized anti-HA antibodies were used in a second round of purification. SDS/PAGE separation and silver staining of the final eluate revealed at least six polypeptides that were specific for the epitope-tagged Ldb1 sample and were not observed in the mock control (Fig. 1*A*).

All specific bands were analyzed by mass spectrometry. As expected, the 56-kDa band corresponded to epitope-tagged Ldb1, which we confirmed by Western blot analysis (not shown). The 50-kDa doublet contained closely related proteins. Two peptides, SAQTFLSEIR and NSPNNISGISNPPGTPR, present in tryptic digests of the lower band of this doublet, correspond to human Ssdp1 (gi:13449489). The upper band contained several tryptic peptides (LALYVYEYLLHIGAQK, SAQTFLSEIR, and SSPGAVAGLSNAPGTPR) that correspond to Ssdp3 (gi:13400104), a structural relative of Ssdp1 (Fig. 1*B*). Our database searches revealed four human and three mouse Ssdp sequences that are closely related. In addition, a unique *Drosophila* Ssdp sequence was identified (Fig. 1*C*). Mouse Ssdp1 and *Drosophila* Ssdp were selected for functional studies reported below.

Ldb1 Interacts with Ssdp1 in a Variety of Cell Types. To confirm the relevance of interactions between Ldb1 and Ssdp proteins in HeLa cells, we generated several cell lines that expressed double-epitope-tagged mouse Ssdp1 proteins. The parental cell lines were derived from various mammalian organisms (mouse, human, rat, and monkey), differed in their tissue origin (cervix, pituitary, skeletal muscle, kidney, B lymphocyte, bone marrow, urinary bladder, and breast), and included HeLa, GH_3, C2C12, U2OS, VERO, NIH3T3, Sy5Y, T24, MCF-7, COS-1, C33-A, and 293 cells. The presence of Ldb1 in a complex with mouse Ssdp1 was assayed by coimmunoprecipitation with anti-FLAG antibodies, followed by Western blot analysis with anti-Ldb1 antibodies. Parental cell lines served as controls. In every case, endogenous (and therefore not epitope-tagged) Ldb1 was found as a single 50-kDa band in the Ssdp1 immunoprecipitates (Fig. 2 and data not shown). No Ldb1-specific signal was detected in the controls.

Ldb1 and Ssdp1 might be associated by nonspecific interaction with nucleic acids present in the nuclear extracts. To exclude this possibility, nuclear extracts from cells expressing epitope-tagged Ldb1 were treated with DNase or RNase and fractionated by centrifugation in a 10–35% glycerol gradient. Western blot analysis with HA antibodies revealed that the peak of epitope-tagged Ldb1 from the nuclease-treated and the control samples appeared in the same fractions (data not shown), which makes it unlikely that Ldb1 and Ssdp1 interact by means of nonspecific binding to nucleic acids.

Ssdp Enhances Axis Induction by Lim1 Plus Ldb1. The function of Lim1 in axis formation in *Xenopus* depends on cooperation with Ldb cofactors (3, 4, 34). We asked whether Ssdp might synergize with *Xenopus* Lim1 and Ldb1 in this system. *Xenopus* embryos were injected in the prospective ventral marginal zone with different combinations of synthetic mRNAs encoding Xlim1, Ldb1, and mouse or *Drosophila* Ssdp. Neither of the Ssdp proteins alone had any axis-inducing activity at the levels tested (Fig. 3, Table 1). As reported (3), high levels of *Xlim1* and *Ldb1* mRNAs (400 pg of each) induced incomplete secondary axes, whereas lower levels (40–80 pg of each) were ineffective. The lower levels of *Xlim1* and *Ldb1* mRNAs became highly effective, however, when coinjected with either mouse *Ssdp1* or *Drosophila Ssdp* mRNAs (100 pg per embryo) (Fig. 3, Table 1). Injection of low levels of *Ldb1* plus *Ssdp*, or of *Xlim1* plus *Ssdp* RNAs did not induce secondary axes (Table 1). All secondary axes generated

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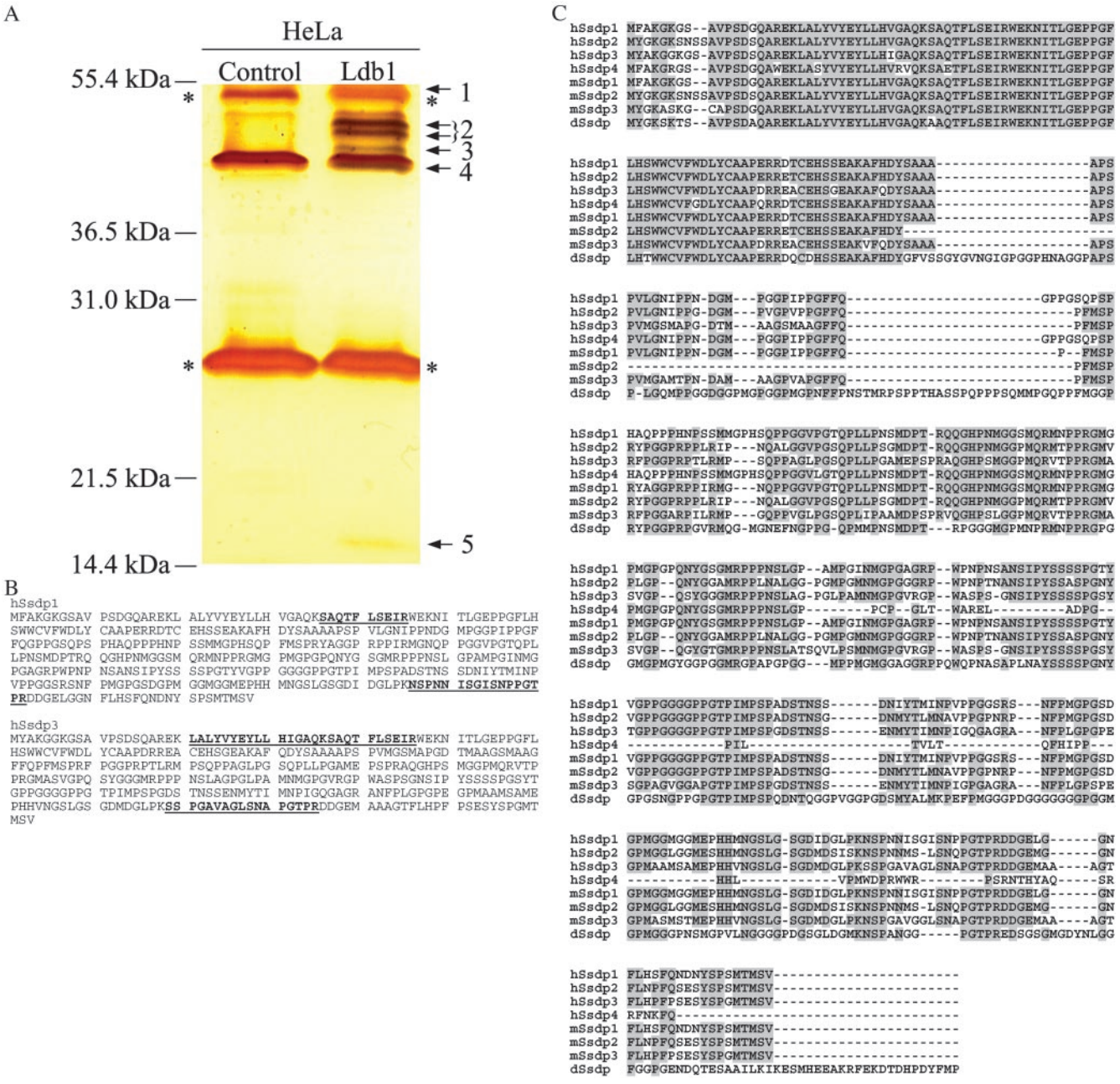

Fig. 1. Ssdp 1 and Ssdp3 peptides present in nuclear immunoprecipitates of Ldb1-transduced HeLa cells. (*A*) FLAG/HA epitope-tagged Ldb1 was purified from nuclear extracts of transduced HeLa cells by immunoprecipitation with antibodies specific for FLAG, followed by immunoprecipitation with antibodies specific for HA. As a control, purification was performed from nontransduced HeLa cells. Proteins were resolved by SDS/PAGE and peptide bands were visualized by silver staining. The major polypeptides specific for the epitope-tagged Ldb1 sample are indicated by arrows as 1, 2, 3, 4, and 5. Positions of the heavy and light chains of IgG are shown by asterisks. (*B*) Sequences corresponding to proteins encoded by human *Ssdp1* and *Ssdp3* genes, respectively. Highlighted are four tryptic peptides that were detected in the doublet band (labeled 2 in *A*). (*C*) Peptide sequence comparison of human, mouse, and *Drosophila melanogaster* Ssdp proteins.

in this manner were incomplete. It seems that injection of high levels of *Xlim1* plus *Ldb1* mRNAs or of the triple combination of mRNAs caused both secondary axis induction and an inhibition of gastrulation, because those embryos that did not display a secondary axis were abnormal, mostly because of an open blastopore (Table 1). This finding may explain the fact that injection of 80 pg of *Xlim1*, 80 pg of *Ldb1,* and 100 pg of mouse *Ssdp1* mRNAs led to a lower proportion of secondary axis induction than 40 pg of *Xlim1,* 40 pg of *Ldb1,* and 100 pg of mouse *Ssdp1* mRNAs. We believe that the higher *Xlim1*/*Ldb1* levels more effectively interfered with gastrulation, leading to a higher proportion of abnormal embryos rather than axis-duplicated embryos. *Drosophila Ssdp* mRNA was almost as effective as mouse *Ssdp1* mRNA in inducing secondary axes when coinjected with *Xlim1* and *Ldb1* mRNAs. Thus, mouse Ssdp1 and *Drosophila* Ssdp proteins are sufficiently similar in their functions as to be interchangeable in ectopic expression experiments. A deletion of amino acid residues 1 to 121 from mouse Ssdp1 (see Fig. 1*B*) yielded a protein that did not bind Ldb1 after cotransfection into cultured cells and was also unable

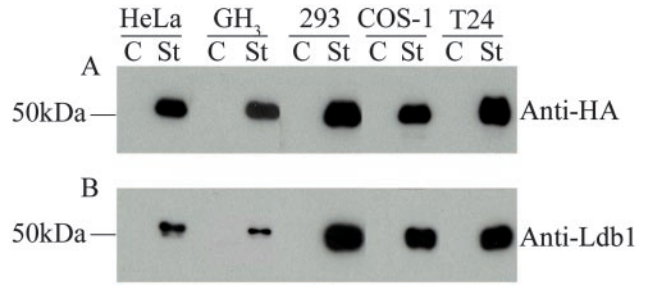

Fig. 2. Endogenous Ldb1 forms complexes with Ssdp1 in a variety of cell types. FLAG/HA epitope-tagged Ssdp1 was expressed in the cell lines shown, complexes were purified, and Ldb1 was detected by Western blot analysis in the nuclear immunoprecipitates. (*A*) Anti-HA detects epitope-tagged Ssdp1; (*B*) Anti-Ldb1 detects Ldb1. C lanes, controls; St lanes, Ssdp1-transduced cells.

to synergize with Xlim1 and Ldb1 in axis induction (data not shown). We conclude from these results that Ssdp synergizes with Xlim1 and Ldb1 *in vivo* during gastrulation in *Xenopus*, and that this synergy is likely to require interaction between Ssdp and Ldb1.

Ssdp Is an Essential Gene Product in *Drosophila*. The single *D. melanogaster* Ssdp protein (Fig. 1*C*) is encoded by CG7187 in polytene chromosome bands 90F1–2 in the right arm of the third chromosome. Searches of the EST databases identified 29 *Ssdp* ESTs, as well as ESTs for genes that flank *Ssdp* both proximally and

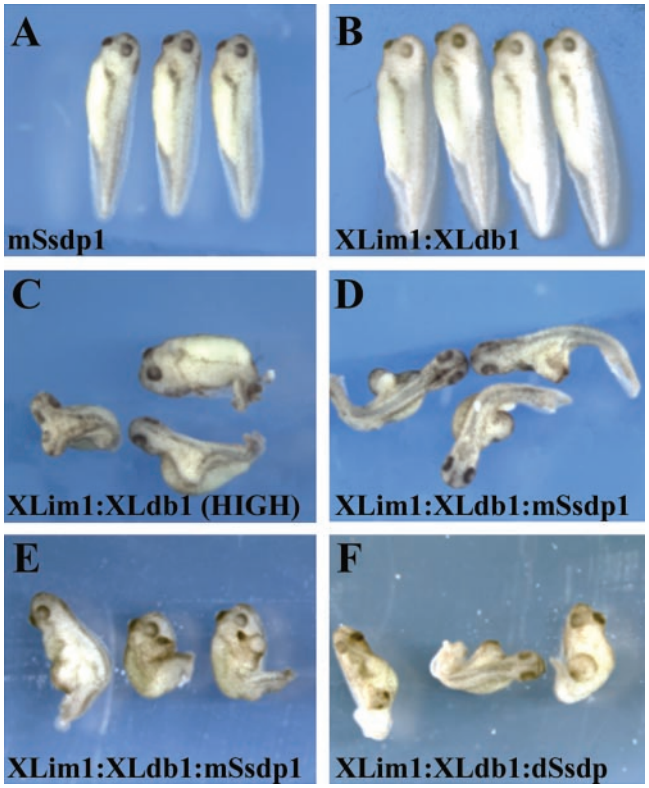

Fig. 3. Examples of *Xenopus* embryos injected at the four-cell stage and photographed at stage 35/36. Different combinations of mRNAs, in amounts listed individually below, were injected into the prospective ventral marginal zone. (*A*) 100 pg *mSsdp1*; (*B*) 40 pg *Xlim1*, 40 pg *Xldb1*; (*C*) 400 pg *Xlim1*, 400 pg *Xldb1*; (*D* and *E*) 40 pg *Xlim1*, 40 pg *Xldb1*, 100 pg *mSsdp1*; (*F*) 40 pg *Xlim1*, 40 pg *Xldb1*, 100 pg *dSsdp*. In *C–F*, examples of embryos that did show secondary axes are illustrated; the percentages of embryos showing this phenotype are listed in Table 1.

Table 1. Secondary axis induction

Injected RNA					Phenotype, %		
Xlim1	*Xldb1*	*mSsdp*	*dSsdp*	*N* (No. exps.)	Normal	Double axis	Other*
0	0	100	0	35 (2)	94	3†	3
0	0	0	100	35 (2)	97	0	3
40	40	0	0	44 (3)	95	0	5
80	80	0	0	34 (2)	100	0	0
40	0	100	0	44 (1)	80	0	20
0	40	100	0	41 (1)	90	0	10
400	400	0	0	69 (4)	0	46	54
40	40	100	0	73 (3)	0	86	14
80	80	100	0	35 (2)	0	69	31
40	40	0	100	50 (2)	0	78	22

*Various abnormalities without indication of secondary axis.
†Very slight ''bump'' in tail region in one embryo.

distally on the chromosome. One *Ssdp* EST (GM14473) was completely sequenced by the *Drosophila* Genome Project and corresponds to a transcription unit with a single intron of 1,581 bp. Alignments of the remaining ESTs show that *Ssdp* encodes at least two transcripts that derive by alternative splicing at the 3′ end of the first exon (both transcripts splice to the same second exon) (Fig. 4). Twenty-two ESTs match the GM14473 sequence; in four ESTs (GH23938, RE28366, GH18277, and RE64068) the first exon is 505 bp shorter than the first exon of GM14473. The predicted ORFs for both transcripts are entirely within the common second exons, suggesting that both transcripts encode identical proteins.

Five alleles of *Ssdp* were available for this study (see *Materials and Methods*). All of these are lethal when homozygous or hemizygous (heterozygous to a chromosomal deletion that includes *Ssdp*). Most of the homozygotes and hemizygotes die during the pupal stages. Likewise, most transheterozygotes of various combinations of *Ssdp* alleles die as pupae. A few $Ssdp^{11}/Ssdp^{BG01663}$ and $Ssdp^{11}/Ssdp^{KG03600}$ flies survive to eclose as adults with mild cuticular defects, including a slight distortion of the posterior scutellar bristles, often accompanied by dupli-

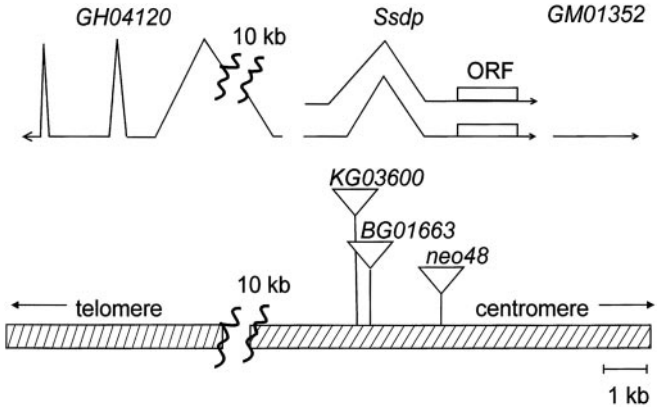

Fig. 4. The *Drosophila Ssdp* gene structure. The hatched bar at the bottom represents ≈23 kb of genomic DNA from polytene chromosome bands 90F1–2. The arrows above the genomic DNA indicate the orientation of the chromosome arm. The *Ssdp* transcription unit spans ≈4.7 kb. The two flanking transcription units, BcDNA:GH04120 (NM142432) and BcDNA:GM01352 (AY060829), are also shown. The arrows on the transcription units indicate 5′ to 3′ direction of transcription. Locations of three *P* element insertional *Ssdp* mutants (*KG03600*, *BG01663*, and *neo48*) are marked by the triangular balloons above the genomic DNA. Two alternatively spliced *Ssdp* transcripts exist, both giving rise to the same predicted protein (the ORF is depicted by boxes on the two *Ssdp* transcripts).

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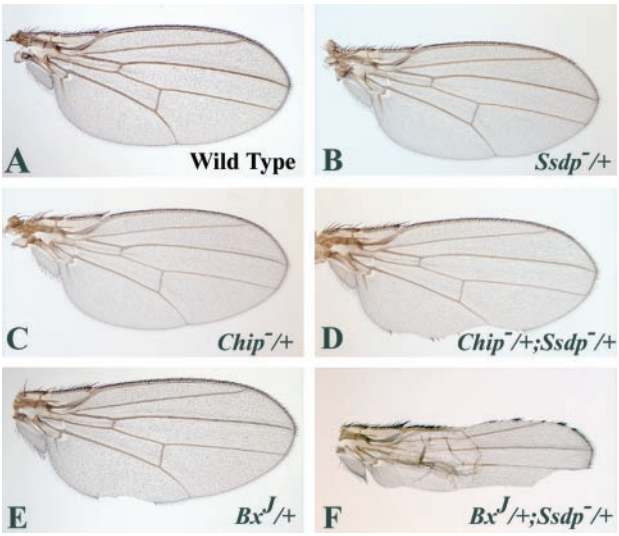

Fig. 5. Genetic interactions between *Ssdp*, *Chip*, and *dlmo* mutations. Wings of $Ssdp^{neo48}/+$ (*B*) and $Chip^{e5.5}/+$ (*C*) are morphologically indistinguishable from WT (*A*). Wings of $Ssdp^{neo48}/+$; $Chip^{e5.5}/+$ double heterozygotes (*D*) are scalloped. Similar results were obtained with other *Ssdp* alleles. Wing scalloping in flies heterozygous for the hypermorphic *dlmo* mutation Bx^J is dramatically enhanced in flies also heterozygous for *Ssdp* mutations, which is shown in *E* ($Bx^J/+$) and *F* ($Bx^J/+$; $Ssdp^{neo48}/+$). Similar results were obtained with other *Ssdp* and *Bx* mutations (see *Materials and Methods*).

cation of the anterior scutellar bristles. The survival of many homozygous *Ssdp* mutants to late pupal stages could be due to the maternal expression of *Ssdp*. We used mitotic recombination in the germ line to create oocytes that lack maternal contributions of either $Ssdp^{31}$ or $Ssdp^{neo48}$. When fertilized by a sperm that lacks the *Ssdp* gene, the zygotes that lack both maternal and zygotic *Ssdp* die at the beginning of the second larval instar. Survival of these animals through embryogenesis may conceivably be sustained by residual activity of the *Ssdp* alleles that we used in this study. Paternal rescue of *Ssdp* exists; when oocytes that lack *Ssdp* are fertilized by a wild-type sperm, the *Ssdp* heterozygotes often survive to eclose as normal adults. These observations show that *Ssdp* is an essential gene.

Ssdp Interacts with Chip. Given the interactions between the vertebrate Ssdp and Ldb1 proteins described above, we examined whether their *Drosophila* homologs might also interact in the context of the whole organism. Previous work has shown that Chip (the *Drosophila* homolog of Ldb1) forms a dimer capable of binding two molecules of the LIM-homeodomain transcription factor Apterous. The Chip-Apterous tetramer activates transcription of a reporter gene in cultured cells (35) and regulates the transcription of target genes involved in morphogenesis of the wing (11, 12). Dlmo, a LIM-only protein, competes with Apterous for binding to Chip, and elevated levels of Dlmo lead to the displacement of Apterous from the complex, which renders the complex transcriptionally inactive, causing scalloped wings (11, 12, 18, 35). Similar wing defects are displayed by homozygous *apterous* (*ap*) mutants and by double heterozygotes for mutations in *ap* and *Chip* (8, 18).

Double heterozygotes for a *Chip* mutation and any of the five *Ssdp* alleles have scalloped wings; all of the single heterozygous mutants have normal wings (Fig. 5 *A–D*). This genetic interaction is highly reminiscent of the genetic interaction displayed by double heterozygotes for *Chip* and *ap* (8, 18), and suggests that Chip and Ssdp interact *in vivo* and that Ssdp is a positive cofactor required for normal function of the Chip–Apterous complex. *Bx* mutations, hypermorphic alleles of *dlmo*, also cause scalloped wings (18). Double heterozygotes for *Bx* mutations and any of the five *Ssdp* alleles displayed marked enhancement of the wing scalloping characteristic of *Bx* flies (compare Fig. 5 *E* and *F*). Similar results were observed for several different *Bx* mutations. A similar enhancement of the wing scalloping of $Bx/+$ was reported in double heterozygotes for *Bx* and either *Chip* or *ap* mutations (18). No wing scalloping was observed, however, in double heterozygotes for any of the five *Ssdp* alleles and either ap^4 or ap^{56f}. These observations support the model that Ssdp interacts *in vivo* with the Chip complex to regulate normal wing development.

Discussion

This study describes the functional characterization of Ssdp proteins as essential cofactors in the transcriptional regulation of embryonic development of invertebrates and vertebrates. Their action is tied in with that of the Ldb/Chip cofactors with whom they can physically associate. In *Xenopus* embryos, Lim1 and Ldb1 can synergize to induce an ectopic axis (3, 34), but only if relatively high levels of mRNA are injected. Ssdp proteins markedly enhanced axis induction by Lim1/Ldb1 when these two components were injected at levels 10 times below a dose that is effective in the absence of exogenous Ssdp (Table 1). In *Drosophila*, *Ssdp* is an essential gene that interacts with *Chip* and *dlmo* in the regulation of wing patterning. Thus, Ssdp proteins play an active role in regulating transcriptional activity in the context of other nuclear factors, as had been suggested earlier (22). We propose that an interaction with Ldb/Chip and with LIM domain-containing nuclear regulators of transcription elicits positive patterning responses, whereas loss of Ssdp protein results in corresponding defects. The range of Ssdp associations with other nuclear regulators of transcription is almost certainly much broader than shown in the present study. For example, binding of Ssdp to Lhx9, a LIM homeodomain factor, has recently been observed in a random screen of mouse protein interactions (36). It is entirely possible that Ssdp is a component of Chip/Ldb-containing protein complexes that form during transcriptional activation of target genes (37).

It is not clear whether the *in vivo* functions of Ssdp are related to its ability to bind single-stranded DNA *in vitro* (22). Our results imply that protein–protein interactions are essential for Ssdp function, but this implication does not preclude the possible importance of single-stranded nucleic acid interactions.

Drosophila embryos defective in both maternal and zygotic *Ssdp* can develop into larvae. However, the alleles that we studied do not support subsequent development. Ssdp function in the fly may not be restricted to interactions with the Chip–Apterous complex, because the phenotype of the existing *Ssdp* mutants differs from that of known *Chip* and *ap* mutants. Furthermore, the facts that *Ssdp* genes are so well conserved throughout evolution, that they are expressed in a wide variety of cell types, and that they are indispensable for development seem to suggest that the encoded proteins function in many different transcriptional contexts.

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