Nucleocytoplasmic localisation of extradenticle protein is spatially regulated throughout development in *Drosophila*

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**SUMMARY**

The extradenticle protein is a homeodomain transcription factor which has an important role regulating the DNA-binding specificity of homeotic selector proteins. We have made a monoclonal antibody against extradenticle and have studied the expression of the protein in the embryo and in imaginal discs. We find that extradenticle is initially uniformly distributed as expected but strikingly is excluded from nuclei until gastrulation. During the extended germ band stage the protein remains predominantly cytoplasmic and does not accumulate in nuclei until germ band retraction. Nuclear accumulation occurs in a highly spatially regulated pattern. In the imaginal discs the nuclear accumulation of extradenticle is also spatially regulated and, in the wing and leg discs, distal regions exhibit cytoplasmic extradenticle whereas proximally the protein is nuclear. We suggest that this regulation of the sub-cellular localisation of extradenticle is important for the interactions between extradenticle and the homeotic selector proteins and that extradenticle is not simply a ubiquitously available cofactor.

Key words: extradenticle, *Drosophila* development, homeotic gene, midgut development

**INTRODUCTION**

The extradenticle (exd) gene plays an important role as a partner to the homeotic genes in the generation of segmental identity in *Drosophila* (reviewed by Mann and Chan, 1996). Mutations in the exd gene produce a variety of segmental transformations but do not alter the patterns of expression of the homeotic genes themselves. Thus, it was proposed that exd acts at the same level as the homeotic genes and is required for the correct specificity of homeotic gene function (Peifer and Wieschaus, 1990). There is considerable evidence to support this and in vitro studies show that the homeodomain-containing EXD protein can enhance the specific DNA-binding of various homeotic gene products (Chan et al., 1994, 1996; van Dijk and Murre, 1994). Whereas homeotic gene products on their own bind to DNA in vitro with little specificity (Hoey and Levine, 1988), in combination with EXD they bind as heterodimers and different homeotic gene products reveal individual DNA-binding specificity when partnered with EXD (Chan and Mann, 1996). The vertebrate EXD homologues, the Pbx gene family proteins, also similarly have been shown to confer specificity of DNA-binding onto Hox gene products (Chang et al., 1996).

In addition to its role as a partner to homeotic gene products, exd also appears to regulate some genes independently of the homeotic genes (Chan et al., 1994; Rauskolb and Wieschaus, 1994) and exd mutations show defects not only in segmental identity but also in segmentation itself (Peifer and Wieschaus, 1990), in dorsoventral patterning and in the control of bristle patterning in the adult (González-Crespo and Morata, 1995; Rauskolb et al., 1995).

Consistent with its role as a partner for a variety of homeotic gene products, EXD is widely expressed (Flegel et al., 1993; Rauskolb et al., 1993). It has a maternal contribution which generates a uniform distribution of exd RNA in the early embryo followed by zygotic expression which shows a segmentally modulated distribution from mid-extended germ-band stage onwards (Flegel et al., 1993; Rauskolb et al., 1993). This modulated pattern appears, however, to be of little significance for exd’s role in the specification of segmental identities. Although zygotic gene expression is essential for viability, two maternal gene doses are sufficient for a wildtype larval cuticle pattern in the absence of a zygotic contribution (Wieschaus and Noell, 1986). Further support for the sufficiency of uniform exd expression is provided by experiments in which exd null mutants were rescued to almost normal morphology following a single pulse, at gastrulation, of ubiquitous exd expression driven from a heat-shock promoter (Rauskolb et al., 1995).

More recently, however, it has become clear that exd function is not simply regulated at the transcriptional level. In imaginal discs exd mRNA is uniformly distributed (Flegel et al., 1993; Rauskolb et al., 1995) but the EXD protein appears to be restricted to the proximal disc regions (González-Crespo and Morata, 1995). Here we extend the study of the distribution of EXD to the embryo and we demonstrate, both in the embryo and in the imaginal discs, a striking regulation of EXD at the level of nucleocytoplasmic localisation.

**MATERIALS AND METHODS**

Production of a monoclonal antibody against extradenticle

Antibodies were raised against the bacterially produced fusion protein
ExdHDec. ExdHDec is a truncated form of EXD containing the homeodomain plus 89 N-terminal and 75 C-terminal residues. It was produced in an Escherichia coli expression system using the methods outlined in Sun et al. (1995). The ExdHDec expression construct was very kindly provided by D. Jackson and P. Beachy.

Monoclonal antibodies were produced as described by White and Wilcox (1984). Balb/c mice were immunised intraperitoneally (i.p.) with purified and washed inclusion bodies containing 50 μg of fusion protein in complete Freund’s adjuvant. The mice were boosted with 50 μg i.p. in incomplete Freund’s adjuvant. Five days prior to fusion a further i.p. boost was given with 50 μg in PBS. Fusion supernatants were screened on blots of bacterial lysates. The line B11M was cloned by limiting dilution. The B11M antibody was shown to be specific for EXD as it gave no labelling on exd− embryos.

**Immunolabelling**

Embryos were fixed for 20 minutes in 4% paraformaldehyde in EM buffer (160 mM KCl, 40 mM NaCl, 4 mM Na2EDTA, 1 mM spermidine-HCl, 0.4 mM spermine-HCl, 0.2% β-mercaptoethanol and 30 mM Pipes, pH 7.4). Embryos were washed three times in PB0.1Tx0.1 (0.1% bovine serum albumin, 0.1% Triton X-100 in PBS), blocked in PB0.1Tx0.1 for 1 hour, incubated overnight in B11M (anti-EXD) hybridoma supernatant diluted 1:5 in PB0.1Tx0.1, washed in PB0.1Tx0.1, incubated with either HRP (horseradish peroxidase) coupled goat anti-mouse Ig, or with fluorescein coupled goat anti-mouse Ig for 4 hours and washed four times in PT (0.1% Tween-20 in PBS) over 30 minutes. Third instar larval imaginal discs were labelled in exactly the same way except PB0.3Tx0.3 (0.5% bovine serum albumin, 0.3% Triton X-100 in PBS) was used instead of PB0.1Tx0.1. HRP labelling was developed using Pierce metal enhanced DAB substrate kit. Nuclei were stained by adding 1 ng/ml DAPI (Sigma) at the same time as the secondary antibodies. Other antibodies used: FP3.38 anti-Ultrabithorax protein (UBX; White and Wilcox, 1984), anti-β-gal (Promega) and secondary antibodies from Jackson ImmunoResearch Laboratories.

For bright-field microscopy a Zeiss Axiophot microscope was used and fluorescence microscopy was carried out on a Leica TCS confocal microscope.

**Stocks**

The wild-type stock used was Oregon R. Other stocks used: wgcn1 dpp-c kindly provided by M. Bienz, heat shock-Ultrabithorax (Ubx) kindly provided by G. Struhl, heat shock-exd 4A (Rauskolb et al., 1995), Ubx msh e e / TM 1 (Ubx null). wgcn1 is a null allele and dpp-c is a hypomorph lacking DPP in the midgut. Ubx null embryos were identified using the monoclonal antibody FP3.38 (White and Wilcox, 1984). exd− embryos lacking both maternal and zygotic contributions were produced using y w exd100 f FRT 18D according to the method described by Rauskolb et al. (1993).

**Heat shock regime**

Two heat shock regimes have been used. In one, embryos were heat shocked for 1 hour at 37°C. In the other regime, embryos were heat shocked three times for 20 minutes at 37°C with a 40 minutes break at room temperature between each heat shock. In both regimes after the final heat shock the embryos were left to recover for a further hour at room temperature before fixation. The ectopic production of UBX or EXD was monitored using the monoclonal antibodies FP3.38 or B11M respectively.

**RESULTS**

**Overview of embryonic EXD localisation**

The exd mRNA is maternally transcribed and uniformly distributed throughout the egg (Flegel et al., 1993; Rauskolb et al., 1993). These uniform transcripts persist throughout the embryo until stage 9 at which point there is an increase in the levels of exd mRNA due to zygotic expression (Rauskolb et al., 1993). Maternal transcripts are uniformly translated throughout the embryo but we were surprised to find the homeodomain exd protein EXD not in the nucleus but in the cytoplasm (Fig. 1A,B). For cuticle morphology the exd function is believed to be required at about stages 8-10 and during this time the protein is less obviously restricted to the cytoplasm, particularly in the anterior ventral epidermis (Fig. 1C). However it is not until germ band retraction (stage 12) that EXD generally becomes strongly concentrated in nuclei. As is seen with the mRNA (Flegel et al., 1993; Rauskolb et al., 1993) from stage 9 onwards the EXD expression pattern shows segmental modulation and in the epidermis and central nervous system (CNS) a boundary develops between the high levels in the thoracic

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**Fig. 1. Overview of EXD expression.** Wild-type embryos labelled with B11M (anti-EXD), and stained using DAB. (A) Cellular blastoderm (stage 5), maternal expression produces uniform, cytoplasmic EXD. (B) Early gastrula (stage 6), maternal EXD clearly cytoplasmic throughout. (C) Germ band extended (stage 10), zygotic production of EXD starts uniformly throughout the embryo. EXD is less clearly excluded from nuclei. (D) Ventral view after germ band retraction (stage 15), the black line indicates the thoracic (t), abdominal (a) boundary. There are distinctly higher levels of EXD in the thoracic epidermis, where it is concentrated in the nucleus. (E) Ventral view of a slightly older embryo (stage 16) showing nuclear EXD in the epidermis. (F) Stage 17; there are high levels of EXD in the thoracic CNS and in constrictions in the midgut (mgc).
segments and the lower abdominal expression (Fig. 1D-F). This non-uniform expression is controlled, at least in part, by the homeotic selector genes of the Bithorax complex (BX-C). In mutations removing all of the genes of the BX-C, exd expression increases in the posterior end of the embryo (Rauskolb et al., 1993).

The cytoplasm to nuclear transition

We examined the development of the EXD expression pattern in more detail with confocal fluorescence microscopy. In the cellular blastoderm (Stage 5, Fig. 2) EXD is sharply concentrated in the cytoplasm and excluded from the nucleus in all cells. This exclusion breaks down locally in mitotic domains in cell cycle 14 and it appears that in cells undergoing mitosis EXD is distributed over the entire cell (Fig. 3). However during the extended germband stage the bulk of the EXD still appears cytoplasmic although it does not seem so sharply excluded from the nucleus as in the cellular blastoderm (Fig. 4A). The later nuclear accumulation is strongly spatially regulated and in the epidermis and CNS is seen most prominently in the thoracic segments (Fig. 4B,C). Even within the thoracic segments not all cells show nuclear accumulation and, for example, the cells of the developing Keilin’s organs have cytoplasmic EXD (Fig. 4C). The boundary between high levels of EXD in the thoracic CNS and lower levels in the abdominal CNS also becomes pronounced (Fig. 4C,D). This is not simply due to the levels of protein present but also its intracellular distribu-
tion (Fig. 4D). Throughout the length of the CNS there is a subset of cells which contain high levels of nuclear EXD (Fig. 4D). In the thorax the majority of cells in the CNS contain medium levels of EXD throughout both the nucleus and cytoplasm. This is in contrast to the majority of cells in the abdominal CNS which contain lower levels of cytoplasmically restricted EXD (Fig. 4D).

Localisation of EXD in the endoderm and visceral mesoderm

The visceral mesoderm and underlying endoderm together form the midgut. At stages 15 (Fig. 5A) and 16 (Fig. 5B) the midgut is partitioned by three constrictions: the first, second and third midgut constrictions. The gastric caeca form at the anterior end of the midgut. In exd maternal and zygotic null embryos, neither the gastric caeca nor any of the midgut constrictions form (Rauskolb and Wieschaus, 1994). In the
Fig. 4. EXD in the epidermis and CNS. Wild-type embryos fluorescently labelled with anti-EXD. (A) By stage 10, zygotic EXD is produced throughout the embryo. The majority of EXD is cytoplasmic, but it is not as strikingly excluded from the nucleus as at earlier stages (compare with Fig. 3B). (B) As the germ band retracts (stage 12), EXD is clearly concentrated in the nuclei of gnathal (mandibular (mb), maxillary (mx) and labial (lb)) segments as well as those of the thoracic segments (t1-3). (C) On closer examination of the CNS at stage 15, three levels of EXD are particularly high close to the segment boundaries. (C) After germ band retraction and head involution (stage 15) high levels of nuclear EXD are expressed throughout the epidermis of thoracic segments (t1-3) and along the ventral side of most of the embryo. The Keilin’s organs (ko) express low levels of cytoplasmic EXD. (D) In most of the thoracic CNS, cells (i) express intermediate levels of EXD in both the nucleus and cytoplasm. In the abdominal CNS, most cells (c) express predominantly cytoplasmic EXD.

absence of exd function in the visceral mesoderm the expression of several homeotic downstream target genes is disrupted (Chan et al., 1994; Manak et al., 1994; Rauskolb and Wieschaus, 1994; Sun et al., 1995) and exd mutations abolish expression of the homeotic gene labial in the endoderm (Chan et al., 1996).

EXD is present at high levels within the nucleus of visceral mesoderm cells at the positions where the gastric caeca and all the midgut constrictions will form (Fig. 5C-F). EXD is also present throughout the underlying endoderm, but it is cytoplasmic at both ends of the midgut and accumulates in nuclei in a central zone. At the anterior end of the endoderm, EXD is cytoplasmic under the gastric caeca but becomes nuclear anterior to the first midgut constriction. At the posterior end of the endoderm it is cytoplasmic again shortly posterior to the third midgut constriction (Fig. 5C-F).

Little is known about the patterning of the endoderm, however the expression of the labial gene in the endoderm underlying parasegment 7 (ps7) in the visceral mesoderm has been shown to be under the control of homeotic genes acting in the visceral mesoderm (Immerglück et al., 1990; Panganimal et al., 1990; Reuter et al., 1990). The homeotic gene Ubx is expressed in ps7 of the visceral mesoderm and activates the transcription of the decapentaplegic (dpp) gene which encodes a growth factor of the TGF-β family. The dpp gene product diffuses to the underlying endoderm and is required for the local activation of the labial gene (Panganiban et al., 1990). The zone of nuclear accumulation of EXD in the endoderm, although broader than the region of labial expression, is similarly centred around ps7, and we investigated whether it was also dependent on the same genetic cascade. In Ubx1 mutant embryos dpp expression in ps7 is eliminated, wingless (wg) expression in ps8 is severely reduced and the second midgut constriction fails to form (Immerglück et al., 1990). We found however that stage 14 Ubx1 homozygous embryos still exhibit a central zone of nuclear EXD accumulation in the endoderm (Fig. 6A). If Ubx is expressed ubiquitously under the control of the heat shock promoter the expression of dpp extends anteriorly in the visceral mesoderm, however we detected no dramatic shift in the pattern of sub-cellular localisation of EXD whether we used heat shocks to produce a high level of UBX for 1 hour or a lower level for 3 hours (Fig. 6B).

In Ubx1 homozygotes some wg expression in ps8 remains and to investigate whether this is responsible for the observed nuclear localisation we looked at wg<sup>cvs</sup>dpp<sup>s4</sup> mutant embryos, which lack both wg expression and the visceral mesoderm expression of dpp. EXD still shows a zone of nuclear localisation in the endoderm of wg<sup>cvs</sup>dpp<sup>s4</sup> mutant embryos which is most clearly seen in late stage embryos (Fig. 7A). The domain of nuclear localisation appears narrower than in wild-type embryos of approximately equivalent stages (Fig. 7B), suggesting that wg and/or dpp, although not essential for nuclear localisation of EXD in the endoderm, may still play some role in the process.

Localisation of EXD within the imaginal discs

exd mRNA is uniformly distributed throughout imaginal discs (Rauskolb et al., 1993). Clones of exd null cells have been used to examine the role of exd in imaginal development. The lack of exd affects imaginal development differently depending on the position of these clones within a disc (González-Crespo and Morata, 1995; Rauskolb et al., 1995). Whereas clones in proximal regions of leg and wing discs show a variety of transformations, clones in distal regions develop normally. In contrast to the mRNA, EXD has been reported to be non-uniformly distributed throughout imaginal discs being undetectable in distal regions (González-Crespo and Morata, 1995).

We find that in the wing disc EXD is expressed over the entire disc but is cytoplasmic throughout the wing pouch, and strongly nuclear in cells surrounding the wing pouch and in patches within the notum region (Fig. 8A). EXD is similarly localised throughout the haltere (Fig. 8B). In all leg discs EXD...
Fig 5. EXD in the visceral mesoderm and endoderm. Wildtype embryos were double labelled with anti-EXD (green) and with DAPI nuclear stain (red). Areas of overlap show up as yellow. Either whole embryos (A and B) or close-ups of the midgut (C-F) are shown. (A) From stage 14, EXD is cytoplasmic throughout the ectodermally derived foregut (fg) and hindgut (hg). This is in contrast to the midgut (mg), where EXD is nuclear in the middle and cytoplasmic at the ends of the endoderm, and nuclear throughout the visceral mesoderm with patches of high level expression. (B) By stage 16 the midgut is divided by the first (1), second (2) and third (3) midgut constrictions. The gastric caeca (gc) also form at the anterior end of the midgut. (C) At stage 14 EXD is nuclear in a region within the middle of the endoderm, (the anterior (a) and posterior (p) boundaries of this zone are labelled) flanked by endoderm with clearly cytoplasmic EXD. (D) In the overlying visceral mesoderm strong patches of nuclear EXD (vm) are expressed at the anterior of the midgut where the gastric caeca (gc) form, and at the site of the second (the first to form) midgut constriction (2). (E) Later, the first midgut constriction (1) forms, within the zone of endoderm with nuclear EXD. EXD is strongly nuclear in the visceral mesoderm at the position where the constriction forms. (F) This is also true for the third midgut constriction (3) which forms within the zone of nuclear endodermal EXD.

Fig 6. The nucleocytoplasmic distribution of EXD in the midgut is largely unaffected by over-expression or lack of UBX. Embryos were labelled with anti-EXD (green) or DAPI nuclear stain (red). (A) In Ubx null embryos the second midgut constriction fails to form. The first (1) and third (3) midgut constrictions still occur. Nuclear accumulation of EXD in the endoderm is still present in a broad central zone with an anterior boundary (a) lying anterior to the first midgut constriction. In these mutants the first midgut constriction may form slightly posterior relative to wild type, thus it is possible that the boundary of the zone of nuclear EXD may also be slightly displaced towards the posterior. The posterior (p) boundary is unaffected. Strong nuclear EXD is expressed in the visceral mesoderm above where the second midgut constriction (2) should have formed. (B) Stage 16 embryo expressing UBX under heat shock control; both the morphology of the gut and expression of EXD are normal.

Fig 7. EXD can still accumulate in the nuclei of endodermal cells in the absence of both WG and DPP. Embryos were labelled using anti-EXD and stained using DAB. (A) wg* dpp* mutant embryos still form endoderm, but lack the second and third gut constrictions. A region of the endoderm (e) still contains EXD localised in the nucleus. (B) In a wild-type embryo of a similar stage the region of nuclear EXD covers a larger part of the endoderm.
is cytoplasmic within the distal part of the disc and strongly nuclear in proximal parts (Fig. 8B,C). EXD is cytoplasmic throughout the ommatidial part of the eye-antennal imaginal disc but nuclear in surrounding cells (Fig. 8D).

**DISCUSSION**

In this paper we report a novel level of regulation acting on the *exd* protein product. The *exd* gene encodes a homeodomain-containing protein that has been shown to bind DNA, both on its own and in partnership with certain homeotic gene products (reviewed by Mann and Chan, 1996). We expect EXD protein to function in the nucleus but we demonstrate here that in early development it is exclusively in the cytoplasm, and that its accumulation in the nucleus at later stages is under tight spatial and cell-specific control. This result emphasises the heterogeneity of *exd* activity within the embryo and we suggest this is likely to be important for our understanding of the interaction between EXD and its homeotic gene product partners.

It has been known for some time that the zygotic expression of EXD is strongly modulated along the anteroposterior axis. As pointed out by (Rauskolb et al., 1993, 1995) the homeotic transformations produced by the loss of zygotic *exd* are most obvious in the anterior two-thirds of the embryo and this correlates with the regions of highest zygotic *exd* transcription. It has been convincingly argued, however, that the pattern of the zygotic expression is unlikely to be of much importance as uniform *exd* expression, delivered either maternally (Wieschaus and Noell, 1986) or via a heat-shock construct (Rauskolb et al., 1995), is capable of rescuing the cuticle phenotype of *exd* embryos. This has formed the prevailing view of the relationship between *exd* and the homeotic genes as a set of tightly patterned homeotic gene products interacting with the ubiquitous *exd* cofactor. However, our results challenge this view and suggest that the availability of EXD for interaction with homeotic gene product may be tightly regulated. We do not know how important this is for homeotic gene function in the embryo but we note that there is a correlation between distribution of nuclear EXD and the regions most affected by *exd* mutations. Also this posttranslation control of EXD subcellular localisation means that in the above experiments where *exd* was uniformly provided the active EXD may still have a patterned distribution through regulation of nuclear accumulation. Some evidence for this comes from the fact that uniform maternal EXD is still correctly localised even in the absence of zygotic EXD (unpublished results). In the imaginal discs the subcellular distribution of EXD correlates well with the requirement for *exd* function: for example, *exd* clones in the distal area of the leg disc, where EXD is cytoplasmic, develop normally (González-Crespo and Morata, 1995; Rauskolb et al., 1995).

The zone of nuclear EXD in the midgut endoderm is interesting but it is not immediately clear what role this would play in midgut development. Few functions defining specific areas of the midgut endoderm have been characterised. The expression of *labial* defines an area underlying *ps7* in the visceral mesoderm and studies on the regulation of *labial* expression have revealed a larger zone which is competent to express *labial* in response to *dpp* expression (Reuter et al., 1990; Staehling-Hampton and Hoffmann, 1994) or low level *wg* expression (Hoppler and Bienz, 1995). This larger region was demonstrated in three experiments, firstly ubiquitous expression of *Ubx* from a heat-shock construct resulted in ectopic *dpp* expression along the length of the visceral mesoderm covering the midgut (Reuter et al., 1990), secondly *dpp* expression was driven throughout the visceral mesoderm using a *UAS-dpp* construct (Staehling-Hampton and Hoffmann, 1994), and thirdly a series of short heat shocks were used to drive low levels of *wg* from a *wg* heat-shock construct (Hoppler and Bienz, 1995); all of which in turn induced *labial* expression in the underlying endoderm. Interestingly in all experiments, the induced *labial* expression did not extend the entire length of the endoderm but was restricted clear what role this would play in midgut development. Few functions defining specific areas of the midgut endoderm have been characterised. The expression of *labial* defines an area underlying *ps7* in the visceral mesoderm and studies on the regulation of *labial* expression have revealed a larger zone which is competent to express *labial* in response to *dpp* expression (Reuter et al., 1990; Staehling-Hampton and Hoffmann, 1994) or low level *wg* expression (Hoppler and Bienz, 1995). This larger region was demonstrated in three experiments, firstly ubiquitous expression of *Ubx* from a heat-shock construct resulted in ectopic *dpp* expression along the length of the visceral mesoderm covering the midgut (Reuter et al., 1990), secondly *dpp* expression was driven throughout the visceral mesoderm using a *UAS-dpp* construct (Staehling-Hampton and Hoffmann, 1994), and thirdly a series of short heat shocks were used to drive low levels of *wg* from a *wg* heat-shock construct (Hoppler and Bienz, 1995); all of which in turn induced *labial* expression in the underlying endoderm. Interestingly in all experiments, the induced *labial* expression did not extend the entire length of the endoderm but was restricted.

![Fig. 8. EXD expression in imaginal discs. Wild-type imaginal discs were labelled with anti-EXD. (A) The wing imaginal disc. There are high levels of nuclear EXD in a ring around where the wing blade (wb) will form. This corresponds to the hinge region. In the notum (nt) EXD is nuclear in patches. EXD is cytoplasmic in the wing blade (wb). Note, along the dorsoventral boundary the confocal section is not in the plane of the nuclei. (B) Haltere and third leg disc. The expression of EXD in the haltere (ht) disc closely resembles that of the wing. In the third leg (lg) disc, EXD is nuclear peripherally where proximal leg segments form, and cytoplasmic centrally where distal leg segments form. (C) A similar pattern is seen in all the leg discs as illustrated by this second leg disc. (D) A section of a portion of the eye disc showing that EXD is cytoplasmic in the photoreceptor clusters and immediately anterior to the morphogenetic furrow, but is nuclear in more peripheral cells.](image-url)
to a central zone with anterior and posterior boundaries reminiscent of the limits of the zone of nuclear EXD. As the accumulation of LABIAL appears to depend on an autoregulatory loop requiring exd (Pöpperl et al., 1995) then perhaps the zone of nuclear EXD sets the limits of the labial response.

We do not know how the nucleocytoplasmic localisation of EXD is controlled. In several systems the regulation of transcription factor localisation involves modulation of protein phosphorylation (reviewed by Vandromme et al., 1996). Like other homeodomain proteins EXD contains a basic region close to the N-terminal end of the homeodomain that may act as a nuclear localisation signal and EXD contains several potential phosphorylation sites. In the endoderm we investigated whether the zone of nuclear accumulation is dependent on expression of the homeotic gene Ubx in the visceral mesoderm. The precedent for this is the regulation of LABIAL in the endoderm by wg and dpp whose expression in the visceral mesoderm is dependent on Ubx (reviewed by Bienz, 1994). It is interesting to note that LABIAL expression is initially cytoplasmic in the endoderm, prior to stimulation of nuclear accumulation in response to wg and dpp (Immerglück et al., 1990). However, both in Ubx mutants and mutants directly removing wingless and dpp expression in the visceral mesoderm, EXD was still nuclear within a central zone in the endoderm by about stage 14. This reveals a mechanism, independent of the ps7 dpp and ps8 wg signalling centre, which is capable of patterning the endoderm.

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REFERENCES


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Note added in proof

The regulation of the subcellular localization of EXD has also been reported by Mann, R. S. and Abu-Shaar, M. (1996). Nuclear import of the homeodomain protein Extradenticle in response to Wg and Dpp signalling. Nature 383, 630-633.