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abdominal A specifies one cell type in *Drosophila* by regulating one principal target gene

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SUMMARY

The Hox/homeotic genes encode transcription factors that generate segmental diversity during *Drosophila* development. At the level of the whole animal, they are believed to carry out this role by regulating a large number of downstream genes. Here we address the unresolved issue of how many Hox target genes are sufficient to define the identity of a single cell. We focus on the larval oenocyte, which is restricted to the abdomen and induced in response to a non-cell autonomous, transient and highly selective input from *abdominal A* (*abdA*). We use Hox mutant rescue assays to demonstrate that this function of *abdA* can be reconstituted by providing Rhomboid (Rho), a processing factor for the EGF receptor ligand, secreted Spitz. Thus, in order to make an oenocyte, *abdA* regulates just one principal target, *rho*, that acts at the top of a complex hierarchy of cell-differentiation genes. These studies strongly suggest that, in at least some contexts, Hox genes directly control only a few functional targets within each nucleus. This raises the possibility that much of the overall Hox downstream complexity results from cascades of indirect regulation and cell-to-cell heterogeneity.

Key words: Hox/homeotic, EGFR, *Drosophila*

INTRODUCTION

The Hox/homeotic genes encode homeodomain transcription factors that generate morphological diversity along the major body axis during animal development (Lewis, 1978; Wakimoto and Kaufman, 1981; McGinnis and Krumlauf, 1992; Mann and Morata, 2000). Work in *Drosophila* suggests that they fulfil this role by regulating a large overall number of downstream loci (reviewed by Akam, 1998; Graba et al., 1997). Studies focusing on the individuation of serially homologous appendages indicate that very different subsets of the downstream gene pool are likely to be deployed in each developmental context. For example, during the formation of a dorsal appendage, the haltere, *Ultrabithorax* (*Ubx*) suppresses wing development by regulating *Serrate*, *spalt* (*sal*), *wingless* (*wg*), *vestigial*, *blistered*, *achaete* and probably many other downstream genes (Weatherbee et al., 1998). In the case of leg development, the major function of *Antennapedia* (*Antp*) in distal and medial regions appears to be to repress the antennal selector gene *homothorax* (*hth*) but in more proximal territory other, as yet unknown, *Antp* targets are involved (Casares and Mann, 2001). In the abdomen, leg development is completely blocked as the resident Hox genes suppress components of the ventral appendage programme itself, such as *Distal-less* (Vachon et al., 1992).

Implicit in the above examples is the idea that Hox genes function to modify an underlying metameric pattern or ground state (Lewis, 1978; Struhl, 1983). We use this term in a developmental context to refer to the body plan formed without any Hox inputs. At present, the mechanisms that link the inputs from Hox genes and ground state genes to final morphological readouts are unknown. This is largely because it has not yet been possible to characterise a complete battery of Hox targets sufficient for any one patterning process. In order to identify such a target set in a relatively simple and well-defined system, we have initiated a single-cell resolution study of the larval oenocyte, a specialised secretory cell that is restricted to the larval abdominal segments (Bodenstein, 1950; Gould et al., 2001). Two recent reports have shown that oenocytes are derived from the dorsal embryonic ectoderm by a local induction involving epidermal growth factor receptor (EGFR) activation within the presumptive oenocyte itself (Elstob et al., 2001; Rusten et al., 2001). The relevant ligand, secreted Spitz (sSpi), is made by a nearby cell of the peripheral nervous system: a chordotonal organ precursor called C1. Importantly, the zinc-finger transcription factor encoded by the *sal* gene is required to prepattern the responding ectoderm so that induction results in an oenocyte-specific EGFR output.

Here we investigate why oenocyte formation is restricted to abdominal segments. We use genetic analysis to show that the formation of this cell type requires an input from *abdA* that can not be substituted for by the closely related *Ubx* gene. Using the GAL4/UAS system, we show that *abdA* plays no direct role during oenocyte differentiation but acts transiently in C1 during the induction phase. We then employ various Hox mutant rescue assays to demonstrate that this non-cell autonomous role of *abdA* is mediated by only one principal target gene *rhomboid* (*rho*), required in C1 for processing the

sSpi signal. This function of AbdA prolongs sSpi production until the Hox-independent oenocyte prepattern has been fully assembled in the responding ectoderm. Thus, in this context, a single principal Hox target is sufficient only because all cell-type specificity information is present in the ground state and *abdA* merely provides the permissive inducing signal to uncover it.

MATERIALS AND METHODS

Fly stocks

en-GAL4, *sal-GAL4*, *UAS-rho*, *UAS-sspi*, *svp-lacZ*, *rho-lacZ*, *ato*^{1} and Df(3R)p13 were as described (Elstob et al., 2001). Other stocks were: *Antp*^{RW10}, *abdA*^{M1}, *Scr*^{4} *Antp*^{25}, *Ubx*^{bxd100}, *ato-GAL4 10* (Hassan et al., 2000), *UAS-abdA.M* (Michelson, 1994), *UAS-Ubx1a.C* (Castelli-Gair et al., 1994), *UAS-EGFR*^{ACT} (Queenan et al., 1997), also called *UAS-EGFR*$^{top4.2}$. *exd*^{B108} mutants, derived from germline clones, were made as described (Rauskolb et al., 1993). Crosses were made at 25°C except for those shown in Fig. 2F-H, Fig. 3F,J,K, and Fig. 4A-D,F, which were made at 29°C.

Immunolabelling

Immunolabelling and confocal microscopy were as described (Elstob et al., 2001). Fig. 2 shows single confocal sections, other figures show projections of several confocal sections. Primary antibodies were as described (Elstob et al., 2001) with the following additions: anti-Ato (Jarman et al., 1995) at 1:2,000, mouse anti-AbdA (Kellerman et al., 1990) at 1:1,000 or rat anti-AbdA (Macias et al., 1990) at 1:500, and mouse anti-Ubx (White and Wilcox, 1984) at 1:20. RNA in situ hybridisation used an *Alas* probe, as described previously (Ruiz de Mena et al., 1999).

RESULTS

A selective, transient and non-cell autonomous requirement for *abdA*

Oenocytes are present in clusters of approximately six cells in each of the abdominal segments A1-A7 (Fig. 1A). In the thorax, there is no EGFR induction around C1 and no specific serial homologue of the oenocyte. In order to score unambiguously the presence of oenocytes in a range of different genetic backgrounds, we identified a panel of seven immediate-early, early and late markers (Fig. 5; E. Gutierrez and A. G., unpublished). To determine why oenocyte formation is restricted to the abdomen, embryos lacking various Hox genes or *extradenticle* (*exd*), which encodes a Hox co-factor (Mann and Chan, 1996), were examined. These experiments indicate that oenocyte formation requires *exd* and *abdA* but not two other Hox genes that are also expressed in the abdomen: *Antp* and *Ubx* (Fig. 1B-E). To assess whether oenocytes form in the absence of all Hox functions, we examined the T1 segment in embryos lacking *Sex combs reduced* (*Scr*) and *Antp* activities (Struhl, 1983; Macias and Morata, 1996). No oenocytes are produced in this context, and therefore these cells are not part of the ground state (Fig. 1F). However, the ground state does contain both the signalling and responding cell types involved in oenocyte induction: C1 and the Sal-positive dorsal ectoderm (data not shown).

Two types of GAL4/UAS assay (Brand and Perrimon, 1993) were used to test the potential of genes to form oenocytes. The ectopic assay reveals whether gene products can trigger oenocyte formation in the T1-T3 thoracic segments (Fig. 1, Fig. 3) while the rescue assay tests the potential of genes to

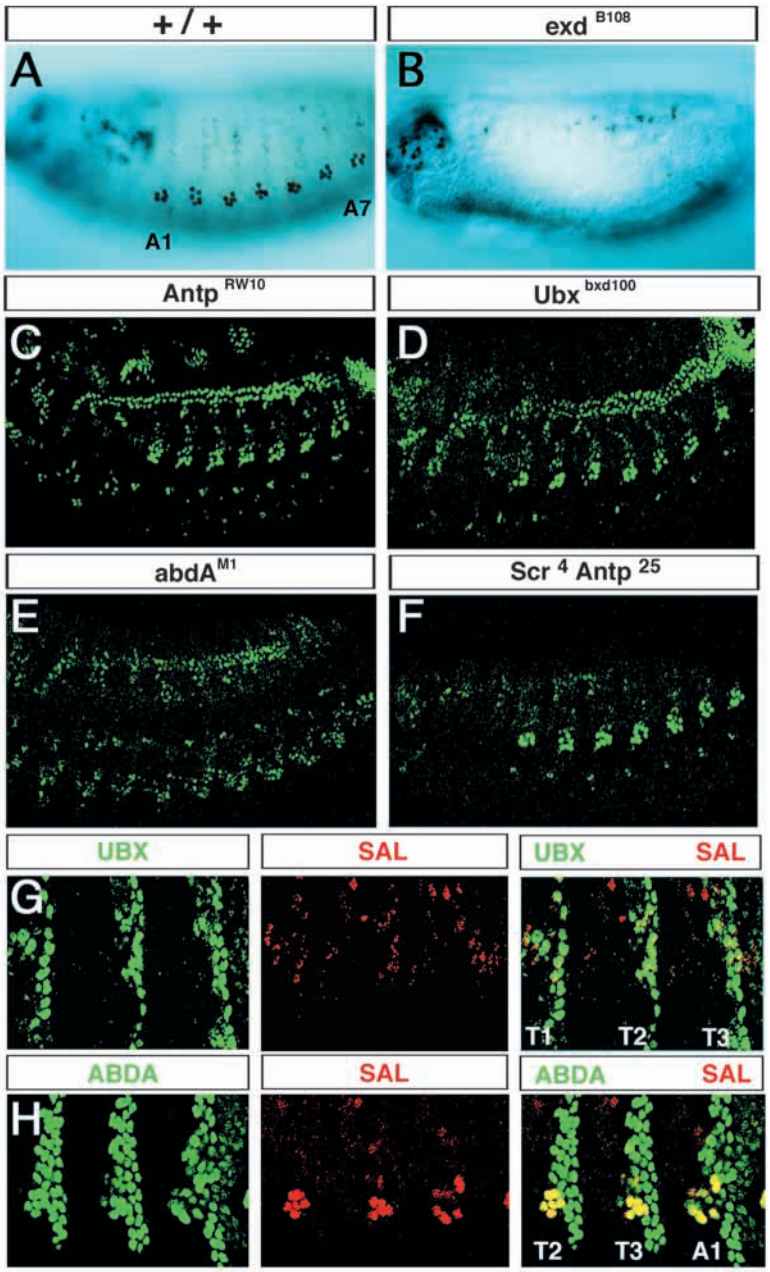

Fig. 1. *abdA* is necessary and sufficient to specify oenocytes. In this and subsequent figures, oenocytes are labelled with anti-Sal unless otherwise stated. (A,B) Anti-β-galactosidase immunostaining of late embryos carrying *svp-lacZ* showing oenocyte clusters present in A1-A7 (indicated) in a wild-type background (A) but missing in an *exd*^{B108} mutant (B). (C-F) Late embryos homozygous for *Antp*^{RW10} (C) or *Ubx*^{bxd100} (D) display normal oenocyte clusters whereas those homozygous for *abdA*^{M1} (E) do not. *Scr*^{4} *Antp*^{25} (F) double mutants show a wild type oenocyte pattern. (G,H) Using *en-GAL4* to drive *UAS-Ubx* (G) or *UAS-abdA* (H) indicates that AbdA but not Ubx can specify oenocytes in T1-T3 (indicated).

overcome the oenocyte deficit in *abdA* mutants (Fig. 4). First, we used *en-GAL4* to express AbdA or Ubx in ectodermal stripes that include the oenocyte precursors (Elstob et al., 2001). In this ectopic assay, only AbdA could produce oenocytes in T1-T3 (Fig. 1G,H). Together with the preceding results, this indicates that *abdA* provides a highly selective patterning input and is both necessary and sufficient for the oenocyte fate.

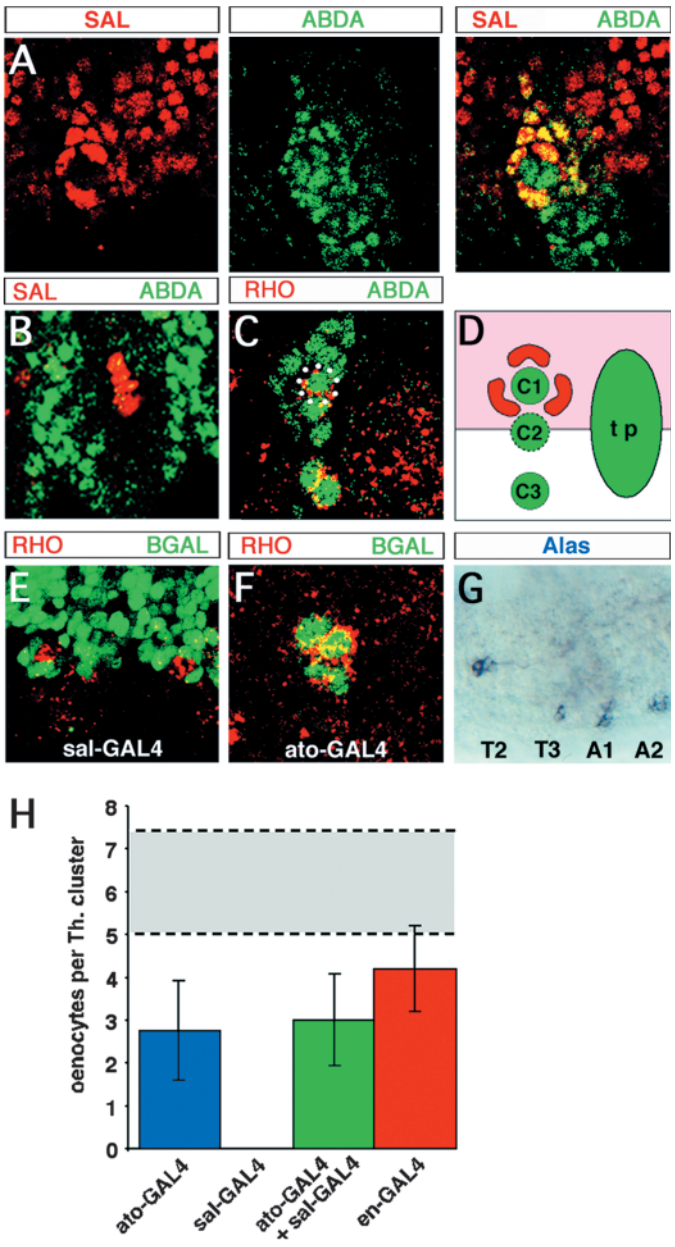

Fig. 2. AbdA misexpression in the thoracic C1 lineage induces oenocytes. (A,B) Oenocyte precursors strongly express AbdA at stage 11 (A) but not stage 13 (B). (C) C1 (circled here and subsequently), labelled with anti-Rho, transiently expresses AbdA at stage 11. (D) Cartoon representing oenocyte precursors (red), chordotonal organ precursors C1-C3 (green), the tracheal pit (tp, green) and the dorsal Sal domain (pink) (Elstob et al., 2001) (E,F) Marking the C1-lineage with anti-Rho, reveals that *sal-GAL4* and *ato-GAL4* drive complementary expression of *UAS-nlslacZ* in the dorsal ectoderm including the oenocyte precursors (E) or in the C1 lineage (F) respectively. (G) *ato-GAL4* driving *UAS-abdA* produces *Alas*-positive oenocytes in the thorax. (H) Numbers of thoracic oenocytes produced by misexpressing AbdA with the drivers indicated. This and subsequent graphs show the mean±1 s.d. for experimental (error bars) and wild-type abdominal counts (grey zone).

At the time of oenocyte induction during stage 11, we find a transient burst of AbdA expression in both C1 and oenocyte precursors (Fig. 2A-D). To ascertain where *abdA* function is required, we used two drivers that, unlike *en-GAL4*, have complementary expression in the oenocyte precursors (*sal-GAL4*, Fig. 2E), or in the C1 lineage (*ato-GAL4*, Fig. 2F). Driving AbdA with *ato-GAL4* is sufficient to induce a late oenocyte marker in thoracic segments (Fig. 2G,H) and also to rescue oenocyte formation in *abdA* mutants (see Fig. 4A,H). In each assay, using *sal-GAL4* to drive AbdA in the dorsal ectoderm fails to produce oenocytes and using both drivers together does not augment the numbers of oenocytes formed with *ato-GAL4* alone (Fig. 2H, see Fig. 4H). These experiments demonstrate clearly that *abdA* is required in the C1 lineage but not in the presumptive oenocyte itself, despite being transiently expressed there. It therefore follows that although *abdA* switches on an extensive hierarchy of early-to-late differentiation genes within the oenocyte, all this regulation must be indirect.

abdA maintains the transcription of *rhomboid* in C1

We looked for potential *abdA* targets from amongst the genes known to play a role in the specification or function of C1. This particular sensory organ precursor produces a type of stretch receptor, the chordotonal organ, that is defined by the proneural gene *atonal* (*ato*) (Jarman et al., 1993). *ato* is also required for oenocyte formation (Elstob et al., 2001; Rusten et al., 2001) but it is similarly expressed in thoracic and abdominal C1, is not regulated by *abdA* and is downregulated prior to oenocyte induction (Fig. 3D and data not shown). We then examined *rho*, a gene downstream of *ato* and rate-limiting for the production of sSpi by cleavage from an inactive membrane-bound precursor (mSpi) in the Golgi apparatus (Lee et al., 2001). Like *ato*, *rho* is also required for oenocyte formation (Elstob et al., 2001). Rho protein is first expressed in C1 at stage 10, after it has delaminated from the dorsal ectoderm. As with Ato at this stage, early Rho is present at similar levels in thoracic and abdominal C1 precursors and is not under *abdA* control (Fig. 3A). During stage 11, however, thoracic Rho becomes extinguished while abdominal Rho persists at a similar level in the C1 lineage (Fig. 3B,C). Unlike the early expression, this late phase correlates with the time of oenocyte induction and is missing in *abdA* mutants (Fig. 3E). Furthermore, driving AbdA in the C1 lineage during stage 11, either in the thorax of a wild-type embryo, or in the abdomen of an *abdA* mutant, is sufficient to prolong Rho expression (Fig. 3F, Fig. 4B). Together, these results indicate that the maintenance but not the establishment of Rho expression is under *abdA* control. Analysis of a *rho-lacZ* line, expressed at stage 11 but not stage 10, suggests that this late regulation is at the transcriptional level and is mediated by a different enhancer than that controlling the early phase of expression (Fig. 3G-I).

Maintaining Rho is sufficient to rescue oenocyte formation in *abdA* mutants

Next, we asked whether the rather simple Rho timing difference between the thorax and the abdomen is responsible for deciding

whether a segment is going to form oenocytes. *en-GAL4* or *ato-GAL4* were used to extend the time-window of Rho expression in the thorax. Remarkably, using either driver results in the formation of bona fide oenocytes, albeit that they are frequently unclustered and dorsally misplaced (Fig. 3J,M). The sufficiency of *rho* in the absence of AbdA can be clearly demonstrated as *ato-GAL4* driven expression of Rho rescues oenocyte formation in *abdA* mutants (Fig. 4H). Hence, prolonging the expression of Rho in the C1 lineage is all that is needed to reconstitute the oenocyte identity function of *abdA*. Next, we used *ato-GAL4* to drive AbdA or Rho expression in *Scr Antp* double homozygotes. As ectopic oenocytes are formed in both cases in the mutant T1 segment, representing the ground state, we can rule out any redundant requirement in the responding ectoderm arising from functional equivalence of Hox proteins (Fig. 3K and data not shown).

The above experiments do not reveal whether C1 also produces some other oenocyte signal that is normally present in both thorax and abdomen. Addressing this issue, *en-GAL4* was used to express Rho in a genetic background lacking *ato*, and therefore missing a functional C1 cell (Elstob et al., 2001; Jarman et al., 1995). In this mutant context, oenocytes can still be induced, indicating that the only role that C1 plays during oenocyte specification is to express Rho and thus provide a source of sSpi signal (Fig. 4F).

Maintenance of Rho expression by *abdA* is predicted to extend the period of Spi secretion, so that the abdominal C1 lineage signals for longer than its thoracic homologue. To test directly whether prolonging active ligand production could induce oenocytes, *ato-GAL4* was used to drive a constitutively active form of sSpi (Schweitzer et al., 1995), in the C1 lineage after stage 10. This resulted in ectopic oenocytes in the thorax and, more importantly, rescued oenocyte formation in *abdA* mutants (Fig. 3M, Fig. 4C,H). Providing sSpi prematurely, from stage 9 onwards using *en-GAL4*, also produces thoracic and rescued abdominal oenocytes but the onset of induction remains restricted to the normal time window during stage 11 (Fig. 3L,M, Fig. 4D,H). Together, these results demonstrate that the oenocyte specification function of *abdA* can be rescued by adding back either Rho or sSpi in C1 during the period of ectodermal competence. Given that the oenocyte role of *abdA* is synonymous with prolonging Rho and thus sSpi synthesis in C1, then activating the EGFR in the dorsal ectoderm at the appropriate time would be expected to have the same effect. Consistent with this prediction, expressing constitutively active EGFR ($EGFR^{ACT}$) under the control of *sal-GAL4* is sufficient to trigger oenocyte formation in *abdA* mutants, completely rescuing their number, position and clustering (Fig. 4G,H).

DISCUSSION

A transient and selective role for *abdA* in oenocyte induction

Oenocyte formation is under the positive control of AbdA and its co-factor Exd. The temporally restricted pulse of AbdA expression in C1 reflects a transient function in prolonging the oenocyte-inducing signal during stage 11. This type of hit-and-run Hox function appears to be widespread and has previously been observed for other ectodermal derivatives (Castelli-Gair et al., 1994). The misexpression experiments clearly indicate that the oenocyte-promoting role of AbdA is highly selective and can not be substituted for by Ubx. This is explained in molecular terms, as only AbdA is capable of

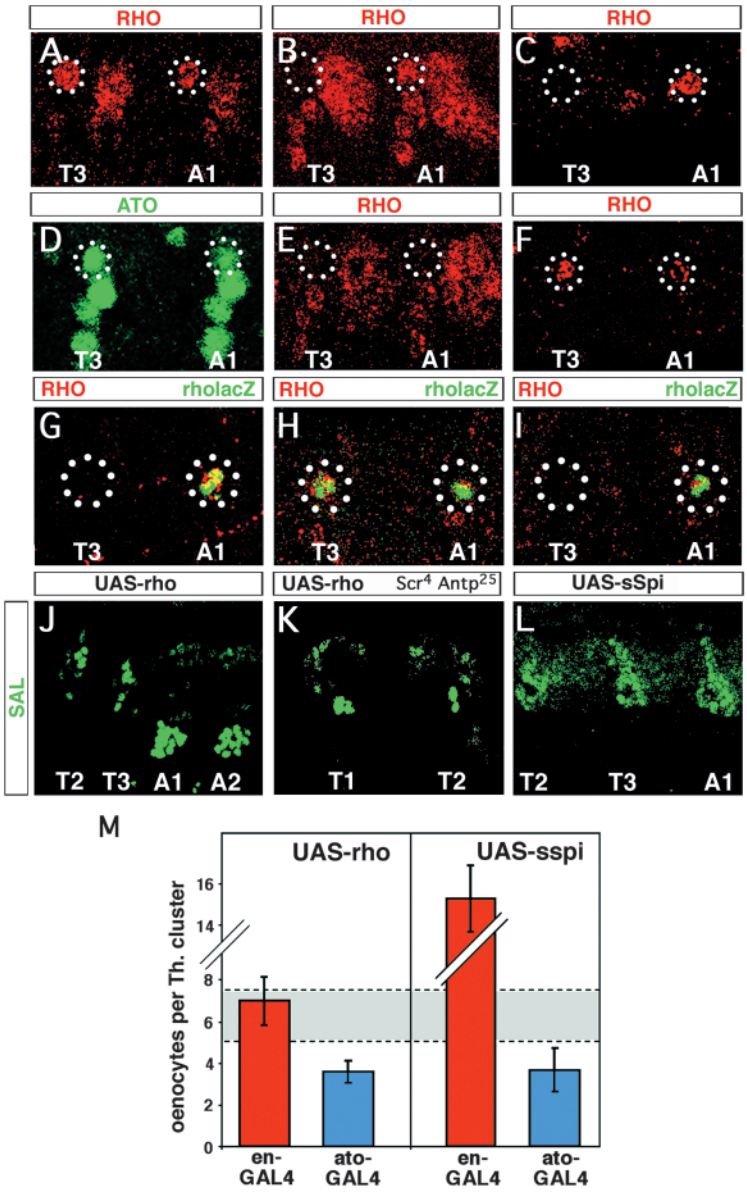

Fig. 3. *rho* expression in C1 is regulated by *abdA* and is sufficient to induce thoracic oenocytes. (A-C) Rho is initiated in all C1 homologues at stage 10 (A) but is maintained at early (B) and late (C) stage 11 only in abdominal C1. (D) Ato is expressed in both thoracic and abdominal C1 at stage 10. (E) Rho fails to be maintained in $abdA^{M1}$ mutants. (F) Rho is prolonged in the thorax by driving AbdA with *ato-GAL4*. (G-I) *rho-lacZ* recapitulates Rho maintenance in abdominal C1 (G) and is ectopically expressed in stage 11 thorax by driving AbdA (H) but not Ubx (I) with *ato-GAL4*. (J,K) *ato-GAL4* driving Rho in either a wild type (J) or Scr^4 $Antp^{25}$ (K) background produces oenocytes in T1-T3. (L) The timing of induction is not altered by prematurely providing sSpi using *en-GAL4*. (M) Numbers of thoracic oenocytes produced by misexpressing Rho or sSpi with the drivers indicated.

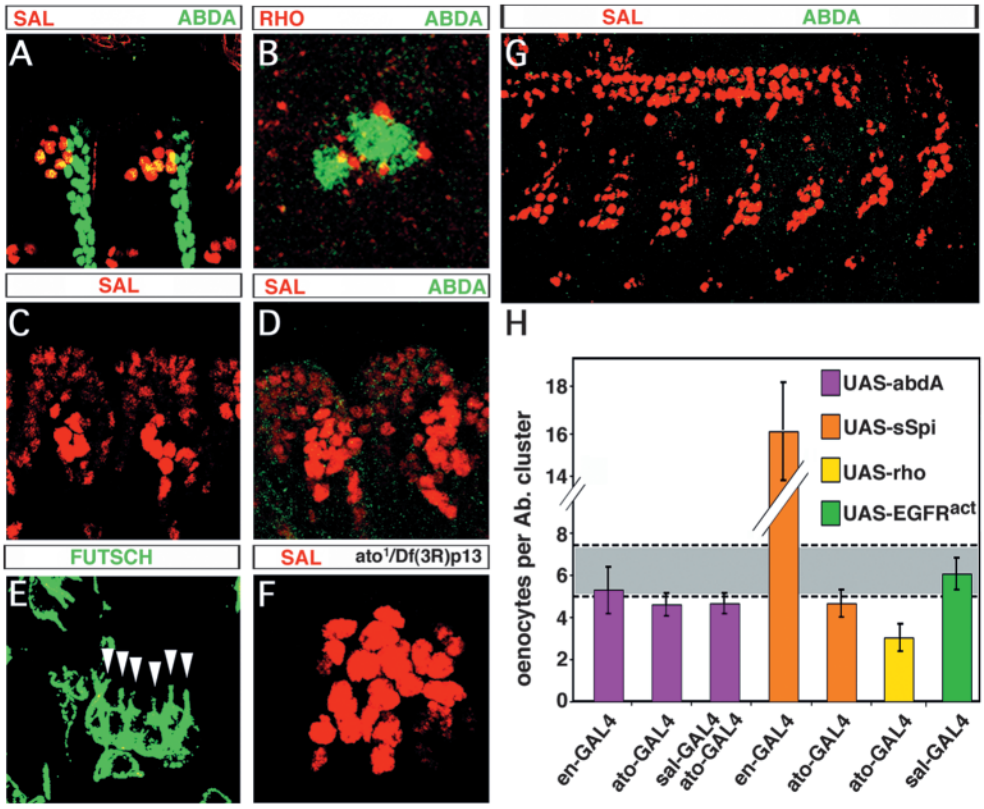

Fig. 4. Activating the EGFR pathway rescues the oenocyte deficit in *abdA* mutants. (A,B) AbdA driven by *en-GAL4* (A) or *ato-GAL4* (B) rescues oenocyte formation or Rho maintenance respectively in $abdA^{M1}$ homozygotes. (C-E) sSpi driven by *ato-GAL4* (C) or *en-GAL4* (D) in an $abdA^{M1}$ background rescues oenocyte formation. In addition, anti-Futsch/22C10 labelling reveals that a dorsal or lateral array of 5-7 chordotonal organs (arrowheads) is produced with *en-GAL4* (E), instead of the dorsal triplet found in *abdA* mutants (Heuer and Kaufman, 1992). (F) The lack of oenocytes in ato^{1}/Df(3R)p13 transheterozygotes (Elstob et al., 2001), is rescued by driving Rho with *en-GAL4*. (G) Providing $EGFR^{ACT}$ with *sal-GAL4* in an $abdA^{M1}$ background produces a normal oenocyte pattern. (H) Numbers of oenocytes per abdominal cluster produced with the GAL4-driver/UAS combinations indicated.

maintaining the transcription of *rho* in the C1 lineage. Such selectivity contrasts with the equivalent biological activities of Ubx and AbdA proteins in promoting haltere formation (Casares et al., 1996). In this regard, we note that *exd* is required to make an oenocyte but not a haltere (Gonzalez-Crespo and Morata, 1995) and therefore may allow these two Hox proteins to discriminate between different targets, as has been suggested previously (Mann and Chan, 1996).

The oenocyte function of *abdA* is mediated by one principal target, *rho*

The results reported here allow us to add sSpi to the growing list of intercellular signalling molecules that are known to be targets of the Hox genes (Immergluck et al., 1990; Reuter et al., 1990; Szuts et al., 1997; Szuts et al., 1998; Wiellette and McGinnis, 1999). For example, during abdominal denticle patterning, it has been shown that *Ubx* and *abdA* positively regulate Serrate signalling, in turn expanding *rho* expression and creating an additional row of denticulate cells (Szuts et al., 1997; Wiellette and McGinnis, 1999). In another context, the visceral mesoderm, these same two Hox genes directly or indirectly regulate the production of at least three signals required to induce appropriate levels of Labial in the adjacent endoderm: Decapentaplegic, Wg and Vein (Yu et al., 1996; Szuts et al., 1998). Interestingly, different levels of Wg can induce two alternative cell fates in the gut, copper cells or large flat cells, thus indicating that this signalling input plays an instructive rather than a permissive role (Hoppler and Bienz, 1995).

Although many different signalling molecules and also a wide range of other types of gene product are all known to be Hox targets, a set of these sufficient for any one patterning process had not yet been clearly defined. For the first time, we have presented a stringent proof of sufficiency by rescuing a cellular Hox phenotype with the gene products of the relevant downstream targets. In the context of the oenocyte, this has revealed that only one target gene, *rho*, is sufficient to execute all aspects of *abdA* function. Our experiments do not distinguish whether the transcriptional maintenance of *rho* by *abdA* in C1 is direct or indirect. Either way, the complex downstream genetic cascade triggered by Rho-dependent activation of sSpi is sufficient to substitute for an input from *abdA*. Importantly, even late differentiation markers such as *alas* are switched on in the oenocyte by providing *abdA* function specifically in the C1 lineage. Hence, *abdA*, via its one principal target *rho*, plays a non-cell autonomous role in promoting the differentiation of the complex oenocyte fate.

As the numerical deficit of chordotonal organs that is found in *abdA* mutants (Heuer and Kaufman, 1992) can also be rescued with sSpi (Fig. 4E), it appears that *rho* may be the principal target of *abdA* in this system too. Although no other single-cell functions of Hox genes have yet been clearly defined by rescue, we think it likely that in most, if not all, developmental contexts, Hox genes directly control only a few critical targets within each nucleus at any one time. In this scenario, the overall downstream complexity that has been observed previously would largely arise from cell-to-cell heterogeneity and cascades of indirect regulation. Both of these factors will need to be given careful consideration whenever genome-wide approaches, such as microarrays, are employed for the identification of biologically relevant Hox targets.

A prepattern for a segment-specific cell type is Hox independent

In the absence of any Hox input, oenocytes are completely

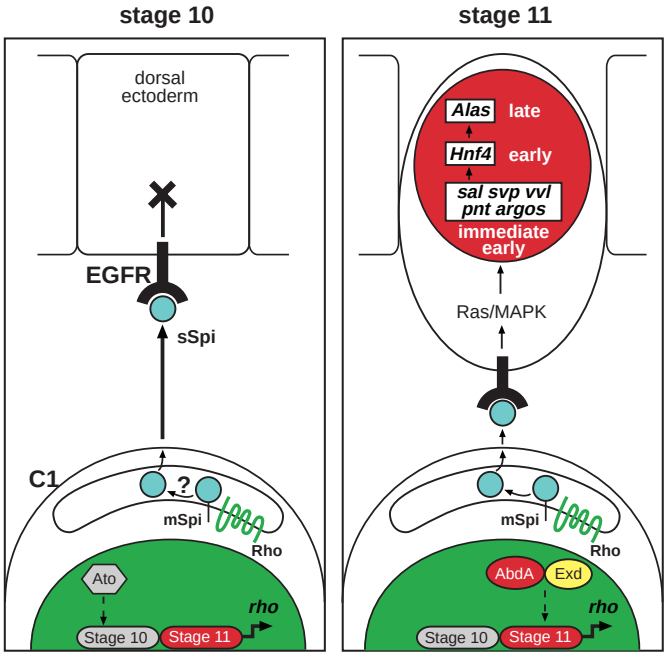

Fig. 5. Permissive model for oenocyte specification by *abdA*. At stage 10, Ato activates *rho* transcription in C1. Rho may or may not (?) process mSpi to sSpi in the Golgi apparatus at this stage, either way the response in the dorsal ectoderm is blocked (cross). By stage 11, the dorsal ectoderm acquires the full oenocyte prepattern and becomes competent for induction. At this time, Ato is no longer present but AbdA and its co-factor Exd maintain *rho* transcription, thus keeping sSpi available to activate the EGFR and in turn the hierarchy of oenocyte differentiation genes including: *pointed* (*pnt*), *argos*, *spalt* (*sal*), *seven up* (*svp*), *ventral veins lacking* (*vvl*), *Hepatocyte nuclear factor 4* (*Hnf4*) and *delta-aminolevulinate synthase* (*Alas*).

missing and therefore are not an overt part of the ground state. At first sight, it might seem that for cell types that have no morphological representation in the ground state, such as oenocytes, Hox genes must necessarily play a classic instructive role in defining the appropriate pathway of differentiation. However, as we will now argue, this is not the only way that Hox genes can direct the formation of segment-specific cell types.

Previously, we described two lines of evidence that the sSpi signal from C1 is permissive in the sense that it does not itself contain any oenocyte specificity information (Elstob et al., 2001). First, providing ectopic sSpi signal outside of a restricted dorsal zone around C1 fails to induce oenocytes. And second, the degree of sSpi signalling influences the number of induced cells rather than their identity. In contrast, it has been demonstrated that all of the cell-type specificity information is encoded in the dorsal ectoderm as an oenocyte prepattern (Elstob et al., 2001). One crucial component of this prepattern is encoded by *sal*. The Sal zinc-finger transcription factor acts to prime the EGFR response in favour of the oenocyte fate. In its absence, there is a fate switch and sSpi signalling now induces secondary chordotonal organs (Elstob et al., 2001; Rusten et al., 2001). Thus, it has been shown that oenocyte specificity is provided by the *sal*-dependent prepattern and not by the sSpi-inducing signal.

We have analysed the segmental restriction of oenocyte induction and provide evidence supporting a model where there is no Hox input into the prepattern but the timing of the sSpi-inducing signal is controlled by *abdA* (Fig. 5). Together with our previous finding that sSpi signalling is permissive, we now conclude that *abdA* does not directly specify the oenocyte identity, rather it determines which segments will form oenocytes. This involves modifying the signalling properties of C1, a serially reiterated cell type that is part of the ground state. In turn, this provides a permissive trigger that uncovers a cryptic oenocyte identity also present in the ground state. Hence *ato* and *sal*, two of the genes that contribute to the ground state, are essential for specifying the C1 cell type and the complete oenocyte prepattern respectively. Another important feature of our model is that the dorsal ectoderm is not competent for oenocyte induction until stage 11. This makes the prediction that if competence were to be acquired earlier, when C1 expresses Rho in both the thorax and abdomen, then oenocytes would be produced in all trunk segments independently of Hox genes.

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REFERENCES

Akam, M. (1998). *Hox* genes: from master genes to micromanagers. *Curr. Biol.* **8**, R676-R678.
Bodenstein, D. (1950). The postembryonic development of Drosophila. In *Biology of Drosophila* (ed. M. Demerec). New York: John Wiley & Sons.
Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.
Casares, F., Calleja, M. and Sanchez-Herrero, E. (1996). Functional similarity in appendage specification by the Ultrabithorax and abdominal-A Drosophila HOX genes. *EMBO J.* **15**, 3934-3942.
Casares, F. and Mann, R. S. (2001). The ground state of the ventral appendage in *Drosophila*. *Science* **293**, 1477-1480.
Castelli-Gair, J., Greig, S., Micklem, G. and Akam, M. (1994). Dissecting the temporal requirements for homeotic gene function. *Development* **120**, 1983-1995.
Elstob, P. R., Brodu, V. and Gould, A. P. (2001). *spalt*-dependent switching between two cell fates that are induced by the *Drosophila* EGF receptor. *Development* **128**, 723-732.
Gonzalez-Crespo, S. and Morata, G. (1995). Control of *Drosophila* adult pattern by *extradenticle*. *Development* **121**, 2117-2125.
Gould, A. P., Elstob, P. R. and Brodu, V. (2001). Insect oenocytes: a model system for studying cell-fate specification by Hox genes. *J. Anat.* **199**, 25-33.
Graba, Y., Aragnol, D. and Pradel, J. (1997). *Drosophila Hox* complex downstream targets and the function of homeotic genes. *BioEssays* **19**, 379-388.
Hassan, B. A., Bermingham, N. A., He, Y., Sun, Y., Jan, Y. N., Zoghbi, H. Y. and Bellen, H. J. (2000). *atonal* regulates neurite arborization but does not act as a proneural gene in the *Drosophila* brain. *Neuron* **25**, 549-561.
Heuer, J. G. and Kaufman, T. C. (1992). Homeotic genes have specific functional roles in the establishment of the *Drosophila* embryonic peripheral nervous system. *Development* **115**, 35-47.
Hoppler, S. and Bienz, M. (1995). Two different thresholds of *wingless* signalling with distinct developmental consequences in the *Drosophila* midgut. *EMBO J.* **14**, 5016-5026.

Immergluck, K., Lawrence, P. A. and Bienz, M. (1990). Induction across germ layers in Drosophila mediated by a genetic cascade. *Cell* **62**, 261-268.
Jarman, A. P., Grau, Y., Jan, L. Y. and Jan, Y. N. (1993). *atonal* is a proneural gene that directs chordotonal organ formation in the *Drosophila* peripheral nervous system. *Cell* **73**, 1307-1321.
Jarman, A. P., Sun, Y., Jan, L. Y. and Jan, Y. N. (1995). Role of the proneural gene, *atonal*, in formation of *Drosophila* chordotonal organs and photoreceptors. *Development* **121**, 2019-2030.
Kellerman, K. A., Mattson, D. M. and Duncan, I. (1990). Mutations affecting the stability of the *fushi tarazu* protein of *Drosophila*. *Genes Dev.* **4**, 1936-1950.
Lee, J. R., Urban, S., Garvey, C. F. and Freeman, M. (2001). Regulated intracellular ligand transport and proteolysis control EGF signal activation in *Drosophila*. *Cell* **107**, 161-171.
Lewis, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565-570.
Macias, A., Casanova, J. and Morata, G. (1990). Expression and regulation of the *abd-A* gene of *Drosophila*. *Development* **110**, 1197-1207.
Macias, A. and Morata, G. (1996). Functional hierarchy and phenotypic suppression among *Drosophila* homeotic genes: the *labial* and *empty spiracles* genes. *EMBO J.* **15**, 334-343.
Mann, R. and Chan, S.-K. (1996). Extra specificity from extradenticle: the partnership between HOX and PBX/EXD homeodomain proteins. *Trends Genet.* **12**, 258-262.
Mann, R. S. and Morata, G. (2000). The developmental and molecular biology of genes that subdivide the body of *Drosophila*. *Annu. Rev. Cell Dev. Biol.* **16**, 243-271.
McGinnis, W. and Krumlauf, R. (1992). Homeobox genes and axial patterning. *Cell* **68**, 283-302.
Michelson, A. M. (1994). Muscle pattern diversification in *Drosophila* is determined by the autonomous function of homeotic genes in the embryonic mesoderm. *Development* **120**, 755-768.
Queenan, A. M., Ghabrial, A. and Schupbach, T. (1997). Ectopic activation of *torpedo/Egfr*, a *Drosophila* receptor tyrosine kinase, dorsalizes both the eggshell and the embryo. *Development* **124**, 3871-3880.
Rauskolb, C., Peifer, M. and Wieschaus, E. (1993). *extradenticle*, a regulator of homeotic gene activity, is a homolog of the homeobox-containing human proto-oncogene *pbx1*. *Cell* **74**, 1101-1112.
Reuter, R., Panganiban, G. E. F., Hoffmann, F. M. and Scott, M. P. (1990). Homeotic genes regulate the spatial expression of putative growth factors in the visceral mesoderm of *Drosophila* embryos. *Development* **110**, 1031-1040.
Ruiz de Mena, I., Fernandez-Moreno, M. A., Bornstein, B., Kaguni, L. S. and Garesse, R. (1999). Structure and regulated expression of the delta-aminolevulinate synthase gene from *Drosophila melanogaster*. *J. Biol. Chem.* **274**, 37321-37328.
Rusten, T. E., Cantera, R., Urban, J., Technau, G., Kafatos, F. C. and Barrio, R. (2001). Spalt restricts EGFR mediated induction of chordotonal precursors in the embryonic PNS of *Drosophila*. *Development* **128**, 711-722.
Schweitzer, R., Shaharabany, M., Seger, R. and Shilo, B. Z. (1995). Secreted Spitz triggers the DER signaling pathway and is a limiting component in embryonic ventral ectoderm determination. *Genes Dev.* **9**, 1518-1529.
Struhl, G. (1983). Role of the *esc+* gene product in ensuring the selective expression of segment-specific homeotic genes in *Drosophila*. *J. Embryol. Exp. Morphol.* **76**, 297-331.
Szuts, D., Freeman, M. and Bienz, M. (1997). Antagonism between EGFR and Wingless signalling in the larval cuticle of *Drosophila*. *Development* **124**, 3209-3219.
Szuts, D., Eresh, S. and Bienz, M. (1998). Functional intertwining of Dpp and EGFR signaling during *Drosophila* endoderm induction. *Genes Dev.* **12**, 2022-2035.
Vachon, G., Cohen, B., Pfeifle, C., McGuffin, M. E., Botas, J. and Cohen, S. M. (1992). Homeotic genes of the Bithorax complex repress limb development in the abdomen of the Drosophila embryo through the target gene *Distal-less*. *Cell* **71**, 437-450.
Wakimoto, B. T. and Kaufman, T. C. (1981). Analysis of larval segmentation in lethal genotypes associated with the Antennapedia gene complex in *Drosophila melanogaster*. *Dev. Biol.* **81**, 51-64.
Weatherbee, S. D., Halder, G., Kim, J., Hudson, A. and Carroll, S. (1998). Ultrabithorax regulates genes at several levels of the wing-patterning hierarchy to shape the development of the *Drosophila* haltere. *Genes Dev.* **12**, 1474-1482.
White, R. A. and Wilcox, M. (1984). Protein products of the bithorax complex in *Drosophila*. *Cell* **39**, 163-171.
Wiellette, E. L. and McGinnis, W. (1999). *Hox* genes differentially regulate *Serrate* to generate segment-specific structures. *Development* **126**, 1985-1995.
Yu, X., Hoppler, S., Eresh, S. and Bienz, M. (1996). *decapentaplegic*, a target gene of the *wingless* signalling pathway in the *Drosophila* midgut. *Development* **122**, 849-858.