Trans-regulatory functions in the Abdominal-B gene of the bithorax complex

JORDI CASANOVA¹ and ROBERT A. H. WHITE²

¹Centro de Biología Molecular CSIC-UAM, Facultad de Ciencias, Universidad Autónoma de Madrid, Canto Blanco, Madrid 28.049, Spain
²Department of Anatomy, University of Cambridge, Downing Street, Cambridge, CB2 3DY, UK

Summary

We have investigated the functional organization of the Abdominal-B gene in the bithorax complex using the expression of the Ultrabithorax gene as an assay for Abdominal-B trans-regulatory functions. Using Polycomb mutants to relax the normal spatial control of Ultrabithorax expression, we have examined the effects of Abdominal-B mutations on the expression of Ultrabithorax protein products in parasegment 14. The results support the hypothesis that the Abdominal-B gene contains two trans-regulatory functions: the m element active in parasegments 10–13 and the r element acting exclusively in parasegment 14.

Key words: bithorax complex, Drosophila, homeotic genes, Polycomb, Abdominal-B.

Introduction

The functional organization of the bithorax complex has been approached by both genetic and molecular analysis (Lewis, 1963, 1978; Bender et al. 1983; Beachy, Helfand & Hogness, 1985; Karch et al. 1985; Sánchez-Herrero, Vernos, Marco & Morata, 1985). Together these indicate that the complex includes three complementation groups Ultrabithorax (Ubx), abdominal-A (abd-A) and Abdominal-B (Abd-B) (Sánchez-Herrero et al. 1985; Tiong, Bone & Whittle, 1985) corresponding to three homologous transcription units, each containing a homeobox sequence (Regulski et al. 1985). The products of the Ubx transcription unit are expressed from parasegment 5 (Martinez-Arias & Lawrence, 1985) to parasegment 13 (Akam, 1983; Akam & Martinez-Arias, 1985; White & Wilcox, 1984, 1985a; Beachy et al. 1985); abl-A products are expressed from about parasegment 7 posteriorly (Harding, Wedeen, McGinnis & Levine, 1985; Regulski et al. 1985) and Abd-B transcripts are found from about parasegment 10 posteriorly (Harding et al. 1985; Regulski et al. 1985). The products of these genes act in concert to specify the individual characteristics of parasegments 5–14 (Lewis, 1978; Sánchez-Herrero et al. 1985).

The Ubx gene is the best characterized unit both molecularly and genetically. There are two classes of loss of function mutations: Ubx mutations (Lewis, 1963) either abolish or reduce the expression of functional products from the Ubx transcription unit (Bender et al. 1983; Beachy et al. 1985) while the subfunction mutations abx, bx, pbx and bxd (Lewis, 1963, 1978, 1981; Casanova, Sánchez-Herrero & Morata, 1985) appear to affect cis-acting control regions and result in altered distribution of Ubx products (Beachy et al. 1985; White & Wilcox, 1985b).

The Abd-B gene has been subjected to extensive mutational analysis (Sánchez-Herrero et al. 1985; Tiong et al. 1985; Karch et al. 1985; Whittle, Tiong & Sunkel, 1986) and recently it has been suggested that its functional organization may differ considerably from that of Ubx (Casanova et al. 1986). Following the terminology of Casanova et al. (1986) there are three classes of Abd-B mutations. Class I mutations transform parasegments 10–13 of the embryo towards parasegment 9. In class II mutations only parasegment 14 is affected and it is transformed into a more anterior structure. Class III mutations show transformations from parasegments 10–14. Parasegments 10–13 are transformed towards parasegment 9 and parasegment 14 exhibits sclerotic plates symptomatic of a failure to repress head-forming genes in this unit. Mutations of class I complement those of class II whereas both I and II fail to complement class III mutations. So far this could be fitted into the Ubx paradigm; however, Casanova et al. (1986) present
evidence that the class II mutations affect a function that is only required in parasegment 14 and that acts in trans to repress other homeotic genes. Abd-B is already known to encode a function that down-regulates Ubx in parasegment 13 (Struhl & White, 1985). This suggests that there are two trans-acting functions within the Abd-B unit. The function affected by class I mutations is called the m element and that affected by class II mutations is called the r element. This interpretation of the functional organization of Abd-B is based entirely on the analysis of larval and adult cuticle phenotypes. In this paper, we support this interpretation using Ubx protein expression in the embryo to provide a molecular assay of trans-regulatory functions in Abd-B.

Materials and methods

Stocks

The stocks used have been described previously (Casanova et al. 1986). Abd-B mutations were analysed in cis combinations with Pc (Lewis, 1980). As representatives of the three classes of Abd-B mutations we used: Class I Abd-B; Class II X25-1; Class III Abd-B. We also studied Pcabd-A (Abd-B Class I) and Pcabd-A Abd-B (Abd-B Class III). The Abd-A allele is a null or almost null allele (Sánchez-Herrero et al. 1985). Embryos were collected from balanced heterozygous stocks. We report the phenotypes of the homozygous mutant embryos which constituted approximately one quarter of the progeny.

Immunohistochemistry

The monoclonal antibody FP.3.38 was used to detect the Ubx protein products (White & Wilcox, 1984). Ubx expression in the ventral nervous system of 13–15 h embryos was studied by immunofluorescence on dissected embryo wholemounts, as described previously (White & Wilcox, 1984). The total nuclear pattern was revealed by labelling with Hoechst 33258. Labelling of undissected wholemounts was based on Mitchison & Sedat, 1983, and Carroll & Scott, 1985. Fixation was for 40 min at 20°C in 3:1 heptane:4% paraformaldehyde, 0-1 M-Pipes (pH 6-9), 2 mM-EGTA, 1 mM-MgSO4. For immunoperoxidase we used the Vectastain ABC kit (Vector laboratories).

Results

In the wild type, Abd-B is not involved in the down-regulation of Ubx in parasegment 14 since, even in the deletion of the entire Abd-B gene (e.g. Df C4), Ubx is not expressed in this unit (Struhl & White, 1985). Thus, we have to remove the normal positional constraints on Ubx expression in order to use Ubx as an indicator of trans-regulatory function in parasegment 14. In homozygous Polycomb (Pc) mutant embryos, Ubx expression in the 12–15 h ventral nervous system clearly extends outside its normal confines (Beachy et al. 1985; White & Wilcox, 1985b; Fig. 1). This enables us to test the activity of the r element of Abd-B by comparing the Ubx distribution in class I and class III Abd-B mutations in a Pc background. According to the two element model, in class I mutations only the m element is defective (m r+) and in class III mutations the function of both the m and the r elements is impaired (m r+). Fig. 1F shows the Ubx pattern in the ventral nervous system of Pc m r embryos. Ubx labelling extends to the posterior limit of the ventral nervous system. The Pc m r+ embryos (Fig. 1D) look very similar except that there is a dramatic reduction in Ubx expression in parasegment 14. Thus, the r element appears, even in Pc− embryos, to act specifically on parasegment 14 and to be capable of down-regulating Ubx.

Although Ubx is released from its normal positional constraints in Pc− Abd-B+ embryos, the expression pattern is not uniform (Fig. 1; Beachy et al. 1985; White & Wilcox, 1985b) and there is a particularly low level in parasegment 14. Presumably this is due to the activity of the r element in this unit. A repression of Ubx activity in parasegment 14 is also seen in esc− embryos and in esc− Abd-B− embryos, but not in esc− Abd-B+ (m r+) mutants (see fig. 3, Struhl & White, 1985).

Examining earlier embryos allowed us to investigate the effects of Abd-B mutants on trans-regulatory functions in the epidermis and also provided an interesting insight into the development of the Pc− phenotype. The pattern of Ubx expression is initially wild type in Pc− mutant embryos (Wedeen, Harding & Levine, 1986) but by the time the germ band has contracted there is clear ectopic expression of Ubx products (Fig. 2). This ectopic expression develops in a reproducible pattern. In the epidermis, Ubx expression extends anteriorly and many nuclei in parasegment 4 become labelled. Anterior to this, there is only scattered Ubx labelling. In contrast to this heterogeneity in the epidermis, the ventral nervous system exhibits a more homogeneous pattern. What determines this pattern of ectopic Ubx expression? One possibility is that it is determined, at least in part, by the pattern of cell division. Two lines of evidence support this view. First, the difference between epidermis and ventral nervous system correlates with mitotic activity in these tissues (Hartenstein & Campos-Ortega, 1985) and second, isolated ectopic Ubx expression often appears as two nuclei close together (data not shown). A close connection between cell division and ectopic homeotic gene expression would fit with the view that Pc mutations result in an instability in the transmission of determined states (Struhl, 1981; Denell & Frederick, 1983).
Trans-regulatory functions in Abdominal-B

Fig. 1. Ubx protein expression in the ventral nervous system of Pc^ and Pc^ Abd-B embryos. (A,B) Pc^ homozygous embryo; (A) labelling with Hoechst 33258 to reveal total pattern of nuclei; (B) Ubx protein expression pattern revealed by FP3.38 monoclonal antibody followed by Texas-Red-conjugated sheep anti-mouse IgG. Ubx proteins are expressed in a heterogeneous pattern over the whole extent of the ventral nervous system. Posteriorly, the labelling declines with parasegment 13 exhibiting weak labelling and in parasegment 14 the labelling is very weak. (C,D) Pc^ Abd-B^M5 homozygous embryo (Abd-B Class I, m^-r^+); (C) Hoechst labelling; (D) Ubx expression extends rather uniformly posteriorly up to the end of parasegment 13. There is very little labelling in parasegment 14. (E,F) Pc^ Abd-B^M1 homozygous embryo (Abd-B Class III, m^-r^-); (E) Hoechst labelling; (F) Ubx labelling extends rather uniformly to the posterior end of the ventral nervous system.

The parasegmental units in the posterior ventral nervous system are indicated. In A and B these are derived from the commissure pattern as revealed by labelling with anti-horseradish peroxidase (Jan & Jan, 1982; Thomas, Bastiani, Bate & Goodman, 1984; White & Wilcox, 1985a). In C–F the landmarks are derived from the repeat units in the Ubx expression pattern (White & Wilcox, 1985a).

Ubx expression in the epidermis of Pc^-Abd-B class III (m^-r^-) and Pc^-Abd-B class I (m^-r^+) embryos correlated well with the results obtained in the ventral nervous system. In Pc^-m^-r^- embryos, there is considerable Ubx expression in parasegment 14 and in Pc^-m^-r^+ there is little Ubx expression in this unit (data not shown). We also examined Pc^-Abd-B class II (m^+-r^-) mutant embryos. The Ubx expression pattern supports the hypothesis that class II mutants affect a trans-regulatory function active only in parasegment 14. Before the end of germ band expression there is ectopic expression of Ubx in parasegment 14 at a considerably higher level than is seen in Pc^-Abd-B^+ embryos (Fig. 3) and at the same time parasegment 13 shows its characteristic wild type pattern. As this Ubx pattern in parasegment 13 is dependent on down-regulation by Abd-B (Struhl & White, 1985 for ventral nervous system; J.C. & R.A.H.W. – unpublished observations for early epidermal pattern) the Abd-B trans-regulatory function in this unit is active in the m^-r^- mutant whereas the parasegment 14 specific function is deficient.
Fig. 2. Ubx protein expression in wild type and Pc3 embryo wholemounts. (A) Wild type embryo (stage 13: Campos-Ortega & Hartenstein, 1985). The Ubx protein pattern is revealed by labelling with FP.3.38, followed by immunoperoxidase. Ubx expression extends from parasegment 5–13. (B) Pc3 homozygous embryo (late stage 12). In comparison to the wild type, there is more uniform expression in parasegment 5 and ectopic expression in parasegment 4. There is little Ubx expression anterior to this. (C) Pc3 homozygous embryo (stage 13). Ventral view. Centrally, in the ventral nervous system, the Ubx expression extends far anteriorly (the most anterior regions of the ventral nervous system bend dorsally and lie out of focus). More laterally, as in B, the labelling in the epidermis extends to parasegment 4 with many labelled nuclei in this unit and anterior to this there are few labelled nuclei.

We have also examined Ubx protein expression in Pc3 Abd-B M8 embryos (data not shown). Our results support the conclusion of Casanova et al. (1986) that the r function does not work indirectly through the abd-A gene. In Pc3 Abd-B M8 homozygous embryos, the entire ventral nervous system exhibits a uniform high level of Ubx expression because the Ubx gene is released from the down-regulation due to abd-A and Abd-B (Struhl & White, 1985). In contrast, in Pc3 Abd-B M8 homozygous embryos, the high level of Ubx expression extends posteriorly only to parasegment 13 and there is little Ubx expression in parasegment 14. Thus, in the absence of abd-A function, the r element of Abd-B is capable of down-regulating Ubx expression in parasegment 14.

Discussion

The results we have presented support the analysis of Abd-B function based on cuticle phenotype and argue in favour of the existence of two trans-regulatory functions in Abd-B: the m element acting in parasegments 10–13 and the r element acting in parasegment 14 (Casanova et al. 1986). This model is consistent with the phenotypes of a large number of mutant combinations and, in particular, it can explain the different cuticular phenotypes in parasegment 14 of class I, II and III Abd-B mutations. In class I (m− r−), there is no effect on parasegment 14 as the r function is unimpaired; in class II (m− r+) an ectopic denticle...
band is produced in parasegment 14 due to the ectopic expression of the m function in this unit in the absence of the normal down-regulation of the m function by the r element; finally, in class III (m\(^{r_{-}}\)) the lack of any Abd-B trans-regulatory functions allows the expression of head-forming genes in parasegment 14 resulting in the appearance of sclerotic plates.

An alternative model of the functional organization of Abd-B, based on the Ubx paradigm, in which class I and II mutations merely affect the positional control of an Abd-B transcriptional unit is more difficult to reconcile with the observed phenotypes.

What is the molecular reality that lies behind the model of two trans-regulatory functions in Abd-B? Three additional lines of evidence bear on this issue. (1) In situ hybridization to embryos with a short probe containing the Abd-B homeobox shows accumulation of transcripts in parasegments 10–14 (Harding et al. 1985; Regulski et al. 1985). This suggests that both the m and r functions may be mediated by transcripts containing the Abd-B homeobox. This agrees with the observed functional similarity between the m and r elements as both show the ability to down-regulate the head-forming genes and other homeotic genes. (2) Class III mutants inactivate both functions yet are not generally cytologically detectable (Karch et al. 1985). That both elements can be affected by small DNA lesions also suggests that the m and r functions may have sequences in common; despite these similarities, the two functions are distinct as replacing r\(^{+}\) activity with m\(^{+}\) activity in parasegment 14 (in class II Abd-B mutants) results in a homeotic transformation (Casanova et al. 1985). (3) The positional specificity of the m element is relaxed in Pc mutants whereas the trans-regulatory function of the r element appears to be restricted to parasegment 14 even in the absence of Pc\(^{+}\) function. This insensitivity to loss of Pc\(^{+}\) function, though not unique among homeotic genes as the expression pattern of Deformed appears little altered in Pc\(^{-}\) embryos (Wedgeen et al. 1986), suggests that the regulation of m element and r element may be under quite distinct controls. The emerging picture of two promoters controlling the expression of transcripts that share common sequence elements has a precedent in the structure of the Antennapedia gene (Schniewul, Kuroiwa, Baumgartner & Gehring, 1986).

Much of this work was done at the MRC Laboratory of Molecular Biology, Cambridge, and we would like to thank Peter Lawrence and Michael Wilcox for their help. We would also like to thank Gines Morata and Ernesto Sánchez-Herrero for encouragement and discussions and Gary Struhl for advice on immunoperoxidase labelling. J.C. is grateful to EMBO for a short-term fellowship.

References


(Accepted 27 May 1987)