Int. J. Dev. Biol. 42: 423-429 (1998)

trithorax and the regulation of homeotic gene expression in *Drosophila*: a historical perspective

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ABSTRACT Animals homozygous for a spontaneously arising allele of the *trithorax (trx)* gene exhibit highly variegated homoeotic transformations of their thoracic and abdominal segments. This paper retraces the sometimes tortuous history of the analysis of *trx* function, from the fortuitous recovery of the first *trx* allele, to the present understanding that *trx* encodes a highly conserved chromatin binding protein that is required to maintain the expression of the *Antennapedia* and *Bithorax complex* genes.

KEY WORDS: *cell-herekity, trithorax, homeotic, Drosophila*

Introduction

Exactly twenty years ago I had the good fortune to isolate a spontaneous allele of the *Drosophila* gene that I subsequently named *trithorax (trx).* This event not only provided me with the basis for my doctoral thesis but more significantly gave me an opportunity, early in my career, to meet and discuss my research with Antonio García-Bellido. Coincidentally, the experiment that led to the serendipitous recovery of trx^1 –though unrelated to the analysis of the regulation of the Bithorax (BX-C) complex in which *trx* is implicated– had itself been inspired by García-Bellido's seminal work on compartmentalization and selector genes (García-Bellido *et al.,* 1973,1976; García-Bellido 1975).

As a student of genetics I had received an all too brief exposure to the revolutionary new ideas that were emanating from the Univesidad Autonoma in Madrid in the mid-1970s –but the brilliant exposition of the Compartment Hypothesis and the role of the Selector gene *engrailed* given one winter's evening by Peter Lawrence (Lawrence and Morata, 1976) had been enough to convince me that the future of developmental biology lay in understanding the genetic basis of lineage restrictions. Armed with this conviction, I arrived in the laboratory of J.R.S. Whittle at the University of Sussex, England determined to identify other genes that, like *engrailed*, might be involved in regulating the development of specific compartments. My first strategy was to scour the Red Book (Lindsley and Grell, 1968) for mutants with phenotypes that could be construed as being compartment specific. Amongst the most promising candidates, the first two that I selected for study were *shifted (shf)* and *elbow (el)*. The narrowing of the spacing between the LIII and LIV veins in *shf* wings suggested a possible effect on the growth or patterning of the anterior compartment, whilst the curvature and reduction of size of *el* mutant wings seemed to me to indicate a defect specific to the posterior compartment of the wing. With little other rationalization than this, I decided to investigate the effects of combining both mutations in the same animal. But what was to arise from the crosses performed to generate the double mutants was certainly nothing that I had anticipated. Amongst the F2 progeny of my *shf* and *el* flies were a number of animals with enlarged and misshapen halteres that, on closer inspection, clearly exhibited signs of being partially transformed to wing tissue (see Fig. 1). Satisfied that my experiment had worked, albeit with an unexpected outcome, I preserved these homoeotically transformed flies in 70% ethanol before showing them to my adviser! His reaction when he saw the pickled flies was predictable, since, unlike me, he immediately realized that this phenotype was almost certainly unrelated to either the *el* or *shf* mutations. Fortunately, the cultures were still producing a few pupae and from these I was able to obtain enough flies from which to attempt to recover what we now suspected to be a new spontaneously arising mutation.

A variegated homoeotic mutation

Much to our relief, the following generation yielded the same assortment of flies displaying variable homoeotic transformations of their halteres (see Fig. 1). I now attempted to establish a pure line of

Abbreviations used in this paper: BX-C, bithorax complex; ANT-C, Antennapedia complex; bx, bithorax; trx, trithorax; shf, shifted; el, elbow; iab, infra-abdominal; Antp, Antennapedia; Scr, Sex combs reduced; Pc, Polycomb; esc, extra sex combs; Dfd, Deformed; en, engrailed; Ubx, Ultrabithorax; pbx, postbithorax; bxd, bithoraxoid; TRE, trithorax response element; Mll, Mixed lineage leukaemia; bp, base pairs; KDa, kilo-Dalton; DNA, deoxy ribonucleic acid.

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0214-6282/98/$10.00

Printed in Spain

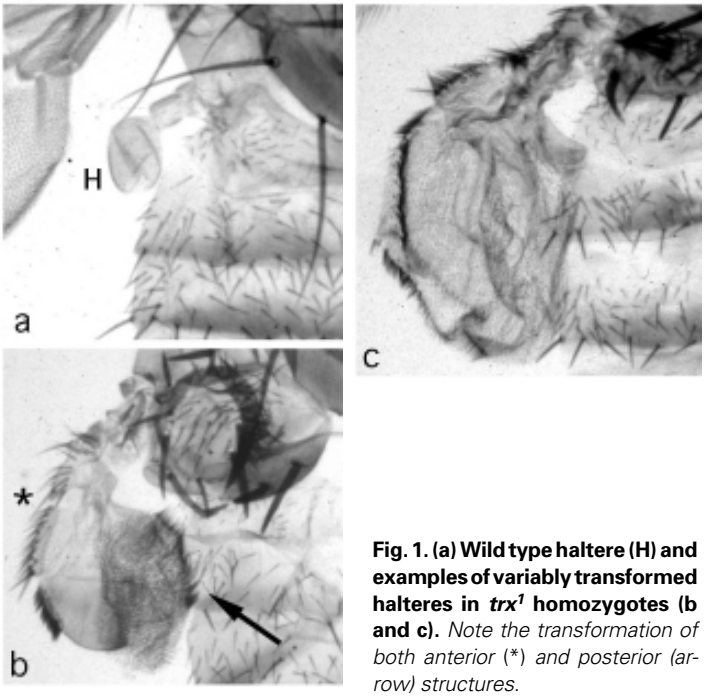

Fig. 1. (a) Wild type haltere (H) and examples of variably transformed halteres in *trx*^{1} homozygotes (b and c). *Note the transformation of both anterior (*) and posterior (arrow) structures.*

the putative new mutation by selecting phenotypically mutant animals and inter crossing them. The results were again surprising: in the first place, not all of the progeny exhibited the homoeotic phenotype; but amongst those that did, the transformations were frequently much more severe than had been observed in the previous generation. In extreme cases almost the entire haltere was replaced by a near complete wing composed of both anterior and posterior compartment structures, a phenotype reminiscent of that of flies homozygous for both the *bithorax; postbithorax (bx;pbx)* homoeotic mutations (Lewis, 1963,1964). Moreover, it was clear that the homoeosis was not restricted to the haltere: large apical bristles, characteristic of the distal tibia of the mesothoracic leg were present on the metathoracic leg. This finding was not unprecedented, since E.B. Lewis had already shown that *bx* and *pbx* mutations transform the metathoracic legs as well as the halteres towards a mesothoracic character (Lewis, 1963,1964). What was without precedent, however, was the finding that the same large apical bristles were also present on the prothoracic legs of the affected flies –and in line with this representing a transformation of the prothorax towards mesothorax, the sex comb of the prothoracic leg in males was found to be either reduced in size or completely replaced by thin bristles typical of the mesothoracic basitarsus.

While the effects on the metathorax were consistent with the new mutation being a hypomorphic BX-C mutation –the *bx*^{1} allele for instance exhibits a similarly variable phenotype though its effects are restricted to the anterior compartment of the metathorax– the effects of the new mutation on prothoracic development suggested otherwise. Although *Ubx* function would subsequently be shown to be required in the posterior prothorax (Morata and Kerridge, 1981), at this time no involvement of the BX-C in prothoracic development was recognized. In any case, the effects of this new mutation were largely restricted to the anterior compartment of the prothoracic leg, a compartment for which no selector gene function had yet been identified. Moreover, a minority of affected individuals also showed signs of a transformation of the dorsal prothorax to mesothorax; in the most extreme cases, this resulted in the development of flies with six wings (see Fig. 2), a phenotype from which the mutation derived its name -*trithorax (trx)*. The phenotype of the *trx*^{1} mutation –as we now designated it (Ingham and Whittle, 1980)– was thus intriguing for several reasons: not only did it imply the existence of a gene distinct from the BX-C that controls metathoracic development; it also provided support for the notion advanced by García-Bellido (García-Bellido, 1975), amongst others, that each body segment is a derivative of a ground state of differentiation represented by the mesothorax.

While complementation analysis with various BX-C mutations supported the view that *trx*^{1} is not a BX-C allele, the definitive proof came from recombination analysis. Meiotic mapping placed *trx* proximal to the BX-C at 54.3 on the right arm of chromosome 3, a position confirmed by its inclusion in various deficiencies of the cytogenetic region 88B that include the *red* locus (which itself maps to 54) (Ingham and Whittle, 1980). Interestingly, this map position corresponds closely to that determined for the mutation *tetraptera*, whose phenotype, as described by Astauroff (Astauroff, 1930), bears a remarkable resemblance to that of *trx*. Unfortunately, this mutation had long since been lost so allelism could not be established, but it seems more than likely that *tetraptera*, discovered nearly half a century earlier, was in fact the original *trx* allele.

trithorax is a maternal effect homoeotic mutation

The localization of *trx* to 88B was highly significant since García-Bellido (García-Bellido, 1977) had previously reported that individuals heterozygous for certain deficiencies of this region exhibit, at low penetrance, weak *bithorax*-like transformations of the haltere. Moreover, such transformations were apparently only observed when the *Df(3)red* chromosome was introduced from the maternal parent, leading to the suggestion that the deficiency uncovers a maternally expressed gene required for the correct activation of BX-C genes. This proposal fitted well with the characteristics of the *trx* mutation described above; in particular, it suggested that the observed increase in phenotypic strength of the progeny of their relatively weakly transformed parents reflected the fact that the latter were most likely derived from mothers which were heterozygous for the mutant *trx* allele and therefore carried one wild type copy of the gene. To confirm this interpretation, reciprocal crosses were performed between females and males known to be either heterozygous or homozygous for the *trx* allele; as expected, *trx* homozygotes derived from homozygous females showed a much higher penetrance and expressivity of the phenotype than those derived from heterozygous mothers (Ingham and Whittle, 1980). These experiments thus clearly established that expression of *trx* is required both during oogenesis and embryogenesis for the normal development of the adult segments.

To investigate at what stage of embryonic development *trx* activity is required we took advantage of the fact that the *trx*^{1} allele is temperature sensitive. Thus when homozygotes are reared at 18°C the penetrance and expressivity of the phenotype was much reduced compared to siblings raised at 25°C. By manipulating the culture temperature at different times during embryogenesis it was found that there is a critical requirement for the gene during the first four hours of embryogenesis (Ingham and Whittle, 1980). Not only was this timing consistent with the maternal effect of the mutation; more significantly, it coincided with the developmental stage at

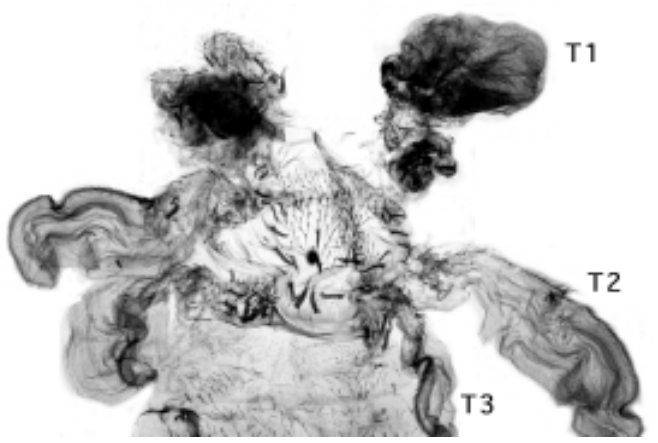

Fig. 2. A six winged fly! *Each of the three thoracic segments (T1-3) bears a wing in some shape or form - those in the prothoracic (T1) segment have not everted properly and remain partly surrounded by peripodial membrane, hence their rather ugly appearance.*

which cells are known to become committed to a single segment, as deduced by cell lineage analysis. Taken together, these characteristics suggested that *trx* might itself be somehow involved in the initiation of segmental fates.

trithorax and ether-induced *bithorax* phenocopies

The temperature sensitive period of trx^1 also suggested a link between the normal function of the gene and a curious phenomenon described many years earlier by (Gloor, 1947) and (Waddington, 1956) These authors had found that variable homoeotic transformations of the haltere to wing –so-called *bithorax* phenocopies– could be induced by exposure of embryos to ether vapor; and more recently, several authors had established the critical period for exposure to ether to be around the blastoderm stage, i.e., 3-3.5 h post-fertilization (Capdevila and García-Bellido, 1974; Bownes and Seiler, 1977). That this corresponds closely to the temperature sensitive period of trx^1 was especially intriguing, since the transformations induced by ether treatment are virtually indistinguishable from those typical of trx^1 homozygotes. So could ether be affecting a mechanism regulated by *trx* activity? Studies by Capdevila and García-Bellido (1978) had already demonstrated that heterozygosity for *Df(3)red* renders embryos more sensitive to ether treatment –so it was not surprising to find that ether induced transformations are also significantly increased in frequency in embryos heterozygous for trx^1. Crucially, the transformations induced by ether treatment in these animals affect the prothoracic as well as the metathoracic segments (Ingham and Whittle 1980) –thus the effects of ether exposure should more properly be referred to as *trithorax* not *bithorax* phenocopies.

Consideration of the phenomenon of ether-induced homoeotic transformation had led Capdevila and García-Bellido to propose a model for the specification of segmental identity (Capdevila and García-Bellido, 1978), based on the notion of the differential activation of the BX-C developed by E.B. Lewis. Following his detailed genetic analysis of the complex, Lewis (1978) had postulated that the BX-C is comprised of a series of genes arranged in a proximal-distal order that reflects the spatial requirement for the function of each gene; thus, the *bx* and *pbx* genes, located at the centromere proximal end of the complex specify metathoracic character while distal to these, a series of *infra-abdominal (iab)* genes specify the development of successively more posterior abdominal segments. Increasing activation of the complex in a proximal to distal direction along the antero-posterior axis of the body would lead to successively more genes of the complex being expressed and hence to more posterior fates being specified. To explain how such differential activation of the BX-C along the antero-posterior body axis could be accomplished, Capdevila and García-Bellido (1978) invoked the existence of a maternally deposited inducer molecule, itself distributed in a concentration gradient along the antero-posterior axis of the egg. This inducer would act only transiently, initiating levels of BX-C expression that would be stably maintained throughout subsequent divisions by cell heredity. The effects of ether could thus be explained in terms of this model by postulating that ether somehow disrupts the activity or distribution of the inducer in the blastoderm embryo; according to this view, ether induced transformations should be clonally propagated –and evidence that this is the case came from cell lineage analysis of phenocopied animals (Capdevila and García-Bellido, 1974).

Because of the increased sensitivity of *Df(3)red* heterozygotes, the idea that this deletion might uncover the locus encoding the putative inducer seemed an attractive one. Moreover, Capdevila and García-Bellido (1978) had found that deficiency heterozygotes exhibit transformations of their abdominal as well as metathoracic segments; these posterior to anterior transformations were exactly of the type expected to result from reduced activity of the different *infra-abdominal* genes, again strengthening the idea that the *Df(3R)red* deletes a global inducer of the BX-C. While relatively weak and infrequent in *Df(3)red* heterozygotes, such abdominal transformations occur with high penetrance and expressivity in trx^1 homozygotes (Ingham and Whittle, 1980; see Fig. 3). Thus trx^1 showed all the characteristics expected of a point mutation in the putative inducer locus: it has a maternal effect, a temperature sensitive period around the blastoderm stage of embryogenesis and transforms both the metathoracic and the abdominal seg-

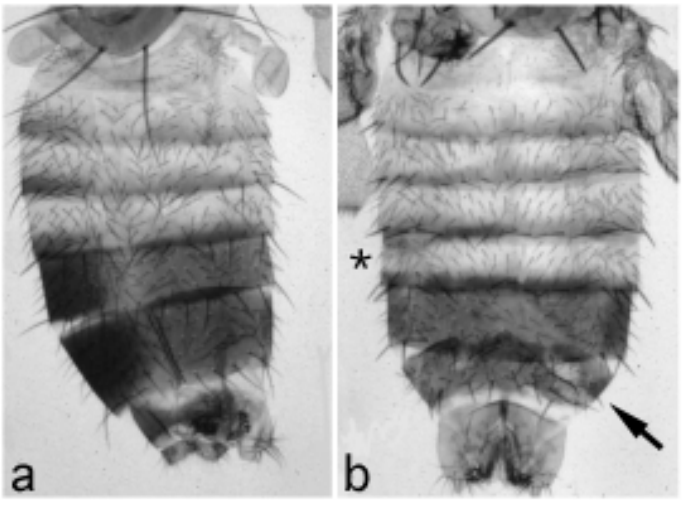

Fig. 3. Wild-type (a) and trx^1 homozygote (b) male abdominal segments. *Note the loss of dark pigmentation typical of the 5th abdominal segment (*) and the additional darkly pigmented tergite posterior to the sixth segment (arrow) in the mutant; both of these features are indicative of a transformation of the segments to more anterior identities.*

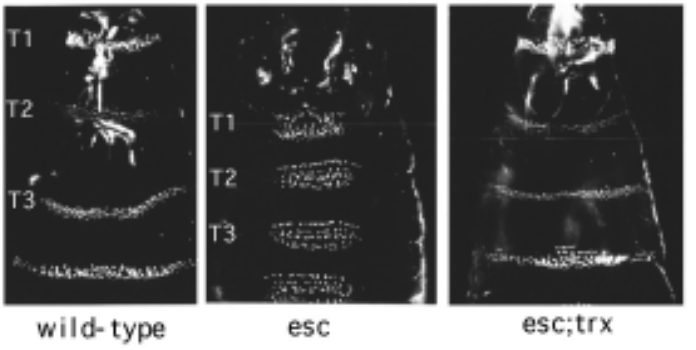

Fig. 4. Thoracic segments of wild-type, *esc* and *esc; trx* mutant larvae. *In the esc mutant, all the segments are transformed to eigth abdominal character, a transformation that is completely suppressed by the removal of trx activity.*

ments to more anterior segmental fates. But how could the prothoracic transformations seen in trx^1 homozygotes be reconciled with this model?

trithorax mimics mutations at the Antennapedia complex

An answer to this question was soon to be provided by the discovery of the Antennapedia complex (ANT-C) by Thomas Kaufman and colleagues. Although *Antennapedia (Antp)* mutations had been known for many years (having first been described in the 1940s) the function of the gene identified by these mutations had remained enigmatic –certainly it had not been considered to be analogous to the genes of the BX-C. Perhaps part of the reason for this was that all of the *Antp* mutations were dominant gain of function alleles; the transformation of antenna to leg caused by these mutations, while dramatic could in isolation give no clear indication of the normal function of the gene. Moreover, although dominant mutations in other members of the ANT-C had been described, these had been erroneously mapped to the left arm of chromosome three, thus obscuring their clustering with *Antp* in a single complex. Through careful genetic and cytological analysis, Kaufman established that *Antp* is one of a complex of homoeotic genes, including the previously described *proboscipedia* and a new locus named *Sex combs reduced (Scr),* required for the development of the thoracic and gnathal segments (Lewis *et al.,* 1980a,b; Denell *et al.,* 1981; Wakimoto and Kaufman, 1981). The discovery of *Scr* was particularly significant since, as its name implied, reduction in function of the gene leads to a partial transformation of the prothoracic leg towards its mesothoracic equivalent, precisely the phenotype seen in trx^1 homozygotes. The *trx* phenotype thus suggested a link between the regulation of the BX-C and the newly defined ANT-C, the inactivation of *trx* apparently resulting in a loss of function not only of BX-C genes but also of at least one ANT-C gene. In terms of the Capdevila and García-Bellido model however, this presented something of a problem, since it was not clear how a simple anterior to posterior gradient of *trx* could accomplish the activation of BX-C genes posterior to the mesothorax while at the same time activating ANT-C genes in segments anterior to the mesothorax.

Moreover, the inducer model suffered from another more serious conceptual flaw. The specification of segmental fate must somehow be closely co-ordinated with the subdivision of the embryo into segmental units and it was difficult to see how the graded activity of an inducer could activate the ANT-C and the BX-C with the precision needed if segmental units and segmental identity are established by independent mechanisms. To achieve such precision, the activation of the homoeotic complexes needed to be integrated with the activation of genes that define the segmental units themselves. As it happened, such "segmentation" genes had just been discovered in the saturation mutagenesis screen of Nüsslein-Volhard and Wieschaus (1980) and their subsequent analysis established how the co-ordination of segmental sub-division and specification of segmental identity is achieved. We now know that a regulatory hierarchy between the gap and pair-rule genes establishes a series of positional cues in the blastoderm

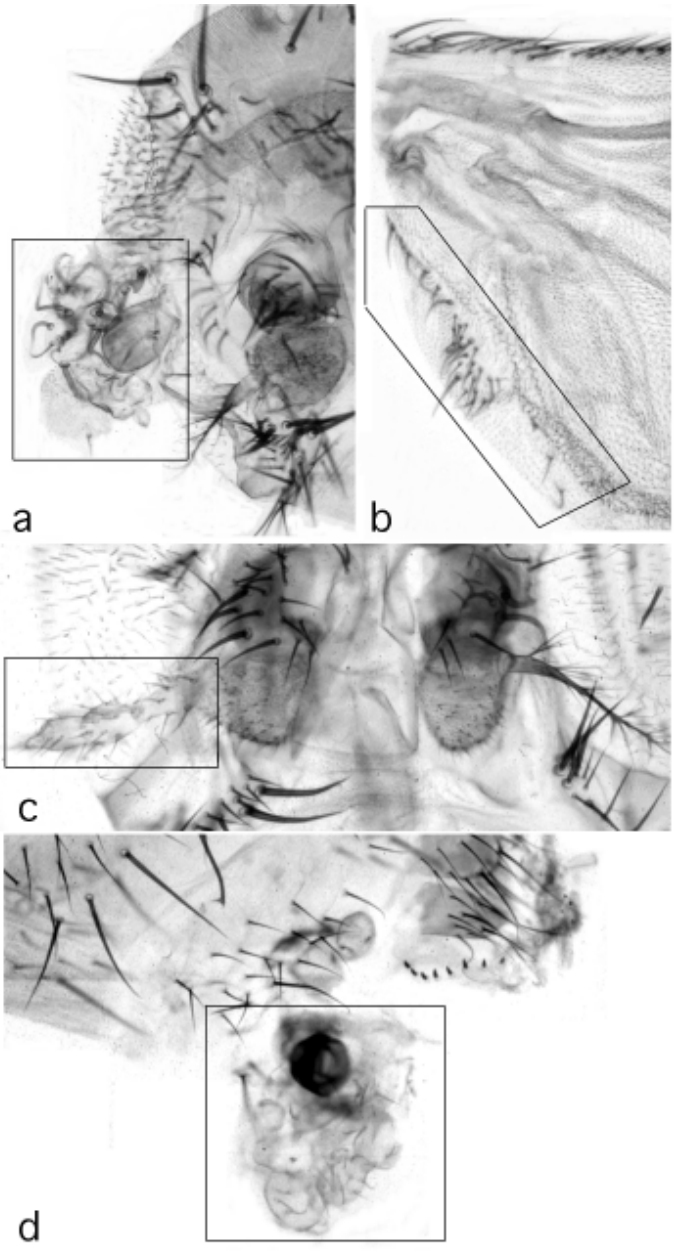

Fig. 5. Transformation of clones of cells lacking *trx* activity in the eye (a), posterior wing (b) antenna (c) and female genitalia (d). *The clonal tissue is indicated by the boxes.*

embryo (Ingham, 1988); these cues serve to activate expression both of the BX-C and ANT-C genes (Ingham and Martinez-Arias, 1986) and of genes such as *wingless (wg)* and *engrailed (en)* (Ingham *et al.,* 1988), whose activities define and maintain segmental borders.

Apart from these theoretical considerations, it was in any case evident from the analysis of larvae homozygous for amorphic alleles of *trx* that its activity cannot be essential for initiating the differential expression of the two gene complexes. In contrast to the major transformations of the larval cuticle associated with complete loss of the BX-C or ANT-C, larvae homozygous for either amorphic point mutations of *trx* (Ingham, 1983) or for *Df(3)red* (Capdevila and García-Bellido, 1978) exhibit relatively minor modifications of their segmental pattern. Even if all maternal *trx* activity is also eliminated from such larvae, the pattern remains remarkably unaffected, with a clear distinction between thoracic and different abdominal segments easily discernible (Ingham, 1983). By contrast, mutations in putative repressors of the BX-C and ANT-C, such as *Polycomb (Pc)* (Lewis, 1978) or *extra sex combs (esc)* (Struhl, 1981) do have major effects on the larval pattern, even head and thoracic segments exhibiting a transformation towards the eighth abdominal segment (see Fig. 4) , a phenotype predicted to result from the complete de-repression of both gene complexes. From this point of view, both *Pc* and *esc* seemed much better candidates for genes involved in initiating the differential expression of the homoeotic gene complexes. But one important finding ruled out such a role for both genes; despite the relatively mild effects of *trx* mutations on normal larval development, the same mutations act as potent suppressers of both the *Pc* and *esc* phenotypes (Capdevila and García-Bellido, 1981; Ingham, 1983). Thus in either *Pc trx* or *esc; trx* double mutants the transformation of head and thoracic segments towards the eighth abdominal is completely suppressed (see Fig. 4); so it is clear that the differential activation of ANT-C and BX-C genes is independent of all three genes.

This conclusion has since been confirmed by the direct visualization of BX-C and ANT-C gene expression in embryos mutant for *trx*, *esc* and *PC*. In all cases, the initial patterns of expression in gastrulating embryo are indistinguishable from wild-type; however, as embryogenesis proceeds changes in the expression of both complexes become apparent. While in the case of *Pc* and *esc* mutant embryos, genes of the BX-C become ectopically expressed in anterior segments (Struhl and Akam, 1985; Simon *et al.,* 1992), the effects of *trx* are more subtle. In general, all of the ANT-C and BX-C genes are expressed in their normal domains but in most cases the levels of expression appear reduced (Breen and Harte, 1993; Sedkov *et al.,* 1994). The effect is most pronounced in the case of the BX-C genes, each of which shows a significant reduction in its transcript level from about stage 10. Effects on expression of *Scr*, *Antp* and *Dfd*, by contrast are much weaker and are first detectable only at around stage 16 of embryogenesis. Thus *trx* function seems to be necessary to maintain the appropriate levels of ANT-C and BX-C gene expression within their normal domains, a function that persists well beyond the completion of embryogenesis.

To establish exactly where and for how long *trx* function is required, clones of cells homozygous for null alleles of the locus were induced in the imaginal discs at successively later stages of development (Ingham, 1985). The initially surprising finding was that *trx* is required in every disc until very late in development to maintain the appropriate identity of each segment. In the absence of *trx* activity, cells are autonomously transformed to a mesothoracic identity (Ingham, 1985) –thus clones in dorsal structures, such as the eye or haltere differentiate notal or wing tissue whereas clones in ventral structures, such as the proboscis, antenna or genitalia are transformed into mesothoracic leg (see Fig. 5). While incompatible with a role for *trx* as the embryonic inducer of ANT-C and BX-C expression, these transformations are perfectly consistent with a role for *trx* in the cell heredity mechanism postulated to maintain the appropriate state of homoeotic complex activation during successive cell divisions. But the clonal analysis revealed that *trx* function is not restricted to the regulation of just the ANT-C and BX-C: even in the wing *trx* clones exhibited a mutant phenotype. In the posterior compartment, clones including the wing margin differentiated socketed bristles, indicative of a transformation to anterior compartment identity (Ingham, 1985), a transformation previously only associated with mutations at the *engrailed* locus (Morata and Lawrence, 1975). This was intriguing since although not a member of either the BX-C or ANT-C, *en* had been classified with the latter as a selector gene, that is a gene that controls the identity of a specific developmental compartment. Like the ANT-C and BX-C genes, *en* expression was postulated to be initiated at the blastoderm stage and subsequently maintained in each posterior compartment by cell heredity (Lawrence and Morata, 1976). Thus the finding that *trx* clones have an *en*-like phenotype strongly suggested that the maintenance of expression of all selector genes is controlled by the same *trx*-dependent mechanism. Subsequent molecular studies have confirmed that *en* expression does indeed depend upon *trx* activity at least in the embryo (Breen *et al.,* 1995), though this has yet to be confirmed in the imaginal discs. In addition *trx* clones in the anterior compartment of the wing also exhibit a mutant phenotype: in this case, clones including the distal wing margin bear bristles characteristic of more proximal regions (Ingham, 1981; Ingham *et al.,* 1985). Unlike the posterior to anterior transformation, this phenotype does not correspond to that of any previously described mutation; however, it seems likely that this represents the loss of activity of some other selector gene that specifies distal versus proximal development, a function that in the ventral appendages at least is executed by the homoeobox gene *Distalless*.

The molecular characterization of *trithorax*

Molecular cloning and sequence analysis of *trx* has revealed that it encodes two large proteins of 365 KDg and 405 KDg (Mazo *et al.,* 1990; Sedkov *et al.,* 1994). The most notable feature of these proteins –apart from their size– is the presence of novel zinc finger-like motifs, suggesting a possible interaction between the TRX proteins and DNA. Such an interaction would, of course, be consistent with the hypothesized role of *trx* in regulating the expression of ANT-C, BX-C and other genes. Indirect evidence that TRX protein binds to the regulatory regions of ANT-C and BX-C genes has come from various studies. In the case of the ANT-C, mutations in *trx* were found to enhance repression of transgenes by a putative cis-regulatory region of the *Scr* gene (Gindhart and Kaufman, 1995), whereas in the case of the BX-C, mutations in cis-regulatory regions of the *Ubx* gene were found to be enhanced by mutations in *trx* (Castelli-Gair and García-Bellido, 1990). More recently, a relatively small (440bp) fragment from the *pbx/bxd* regulatory region of *Ubx* has been shown to be sufficient to

mediate *trx* dependent activation of a reporter gene both *in vivo* and in transfected tissue culture cells (Chang *et al.,* 1995). Activation of transcription via this so called TRE (*trithorax* response element) requires the zinc finger binding domain of the TRX protein (Chang *et al.,* 1995). However, there is no evidence for a direct interaction between TRX and this DNA element. Interestingly, the same 440bp fragment also mediates repression of transcription by the PC protein (Chang *et al.,* 1995). This protein is thought not to bind directly to DNA but rather to associate with chromatin via other proteins, some of which may have sequence specific DNA binding activity. Indirect immunofluorescence studies have revealed that both PC and TRX proteins bind to specific sites in the polytene chromosomes of larval salivary glands some of which are shared by the two proteins (Kuzin *et al.,* 1994; Chinwalla *et al.,* 1995); as expected, these sites include the ANT-C and BX-C the best characterized targets of both *Pc* and *trx* activity. These studies suggest that the proper regulation of the ANT-C, BX-C and other target genes may be achieved by a competition between PC and TRX for binding to chromatin sites around the target loci.

Analysis of *trx* transcription has revealed that the gene gives rise to multiple splice variants which unexpectedly are expressed in spatially and temporally distinct patterns in the embryo. Four different forms are expressed in the embryo prior to 7 h post fertilization, the predominant form of which is expressed both maternally and in the early zygote, peaking at around 2-4 h post fertilization (Sedkov *et al.,* 1994), a period that corresponds approximately to the cellular blastoderm stage. Interestingly, expression is first detected at this stage in the posterior half of the embryo, resolving into four alternating stripes that correspond to the parasegments in which the *Ubx* gene is initially activated (Sedkov *et al.,* 1994). This early phase of expression correlates with the early temperature sensitive period of the trx^{1} allele and may indicate a particular requirement for *trx* function at this stage of development.

Finally, the molecular cloning of *trx* has led to the discovery that the gene has been conserved both structurally and functionally in the course of evolution. The vertebrate homolog of *trx*, the *Mixed-lineage leukaemia (Mll)* gene was, as its name suggests, initially identified as an oncogene (Djabali *et al.,* 1992). Analysis of mice mutant for the murine *Mll* homolog however, has more recently shown that this gene, like its *Drosophila* counterpart, plays a crucial role in homoeotic gene regulation. As in flies, the murine *trx* gene is haplo-insufficient so that heterozygous animals display fusions of the sternal vertebrae as well as transformations of the cervical, thoracic and lumbar vertebrae in anterior and posterior directions (Yu *et al.,* 1995). This is mirrored by shifts in the anterior limits of *Hoxa7* and *Hoxc9*, while in homozygous *Mll* mutants, expression of both genes completely disappears (Yu *et al.,* 1995). The continuing analysis of *trx* in *Drosophila* thus provides a powerful approach not only to understanding the development of the fly but also towards understanding the specification of the vertebrate body plan and of tumor progression. The field has certainly come a long way in the last twenty years!

References

ASTAUROFF, (1930). Analyse der erblichen Störungsfälle der bilateralen Symmetrie im Zusammenhang mit der selbständigen Variabilität ähnlicher Strukturen. *Z. indukt. Abstamm. Vererb. 55:* 183-262.

BOWNES, M. and SEILER, M. (1977). Developmental effects of exposing *Drosophila* embryos to ether. *J. Exp. Zool. 199:* 9-24.

BREEN, T. and HARTE, P. (1993). trithorax regulates multiple homeotic genes in the Bithorax and Antennapedia complexes and exerts different issue-specific, parasegment-specific an promoter-specific effects on each. *Development. 117:* 119-134.

BREEN, T., CHINWALLA, V. and HARTE, P. (1995). trithorax is required to maintain engrailed expression in a subset of engrailed-expressing cells. *Mech. Dev. 52:* 89-98.

CAPDEVILA, M. and GARCÍA-BELLIDO, A. (1974). Development and genetic analysis of bithorax phenocopies in Drosophila. *Nature. 250:* 500-502.

CAPDEVILA, M. and GARCÍA-BELLIDO, A. (1978). Phenocopies of b*ithorax* mutants. Genetic and developmental analysis. *Roux Arch. Dev. Biol. 185:* 105-126.

CAPDEVILA, M. and GARCÍA-BELLIDO, A. (1981). Genes involvd in the activation of the Bithorax complex of D*rosophila. Roux Arch. Dev. Biol. 190:* 339-350.

CASTELLI-GAIR, J. and GARCÍA-BELLIDO, A. (1990). Interactions of the P*olycomb* and *trithorax* with cis regulatory regions of Ultrabithorax during the development of *Drosophila melanogaster. EMBO J. 9:* 4267-4275.

CHANG, Y., KING, B., O'CONNOR, M., MAZO A. and HUANG, D. (1995). Functional reconstruction of trans regulation of the *Ultrabithorax* - promoter by the products of two antagonistic genes, *trithorax* and *Polycomb. Mol. Cell. Biol. 15:* 6601-6612.

CHINWALLA, V., JANE, E. and HARTE, P. (1995). The *Drosophila* trithorax protein binds to specific chromosomal sites and is co-localized with polycomb at many sites. *EMBO J. 14:* 2056-2065.

DENELL, R., HUMMELS, K., WAKIMOTO, B. and KAUFMAN, T. (1981). Developmental Studies of Lethality Associated with the Antennapedia Gene Complex in *Drosophila melanogaster. Dev. Biol. 81:* 43-50.

DJABALI, M., SELLERI, L., PARRY, P., BOWER, M., YOUNG, B. and EVANS, G. (1992). A *trithorax-like* gene is interrupted by chromosome 11q23 translocations in acute leukemias. *Nature Genet. 2:* 113.

GARCÍA-BELLIDO, A. (1975). Genetic control of wing disc development in *Drosophila*. In Cell Patterning (Ed. S. Brenner) Ciba Foundation Symposium 29 Associated Scientific Publishers Amsterdam, pp. 161-177.

GARCÍA-BELLIDO, A. (1977). Homoeotic and atavic mutations in insects. *Am. Zool. 17:* 613-629.

GARCÍA-BELLIDO, A., RIPOLL P. and MORATA, G. (1973). Developmental compartmentalisation of the wing disc of Drosophila. *Nature New Biol. 245:* 251-253.

GARCÍA-BELLIDO, A., RIPOLL P. and MORATA, G. (1976). Developmental compartmentalization in the dorsal mesothoracic disc of Drosophila. *Dev. Biol. 48:* 132-147.

GINDHART, J. and KAUFMAN, T. (1995). Identification of Polycomb and trithorax group responsive elements in the regulatory region of the *Drosophila* homeotic gene *Sex combs reduced. Genetics. 139:* 797-814.

GLOOR, H. (1947). Phänokopie-Versuche mit Äther an *Drosophila. Rev. Suisse Zool. 54:* 637-712.

INGHAM, P.W. (1981). trithorax: A new homoeotic mutation of *Drosophila melanogaster.* II. *Roux Arch. Dev. Biol. 190:* 365-369.

INGHAM, P.W. (1983). Differential expression of bithorax complex genes in the absence of the ex*tra sex combs* and *trithora* genes. *Nature. 306:* 591-593.

INGHAM, P.W. (1985). A clonal analysis of the *trithorax* gene in *Drosophila. J. Embryol. Exp. Morphol . 89:* 349-365.

INGHAM, P.W. (1988). The molecular genetics of embryonic pattern formation in *Drosophila. Nature. 335:* 25-34.

INGHAM, P.W. and MARTINEZ-ARIAS, A. (1986). The correct activation of Antennapedia and bithorax complex genes requires the *fushi-tarazu* gene. *Nature. 324:* 592.

INGHAM, P.W. and WHITTLE, R. (1980). trithorax: a new homoeotic mutation of Drosophila *melanogaster* causing transformations of abdominal and thoracic imaginal segments. *Mol. Gen. Genet. 179:* 607-614.

INGHAM, P.W., BAKER N. E. and MARTINEZ-ARIAS, A. (1988). Regulation of segment polarity genes in the Drosop*hila* blastoderm by *fushi-tarazu* and *even-skipped. Nature. 331:* 73-75.

INGHAM, P.W., MARTINEZ-ARIAS, A. LAWRENCE, P.A. and HOWARD, K. (1985). Expression of *engrailed* in the parasegment of *Drosophila. Nature. 317:* 634-636.

KUZIN, B., TILLIB, S., SEDKOV, Y., MIZROKHI, L. and MAZO, A. (1994). The drosophila trithorax gene encodes a chromosomal protein and directly regulates the region-specific homeotic gene fork head. *Genes Dev. 8:* 2478-2490.

LAWRENCE, P.A. and MORATA, G. (1976). The compartment hypothesis. In *Insect Development* (Ed. P.A. Lawrence) Blackwell Scientific Publications, Oxford. pp. 132-149.

LEWIS, E.B. (1963). Genes and developemtal pathways. *Am. Zool. 3:* 33-56.

LEWIS, E.B. (1964). Genetic control and regulation of developmental pathways. In *Role of Chromosomes in Development* (Ed. M. Locke) Academic Press, New York, pp. 232-251.

LEWIS, E.B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature. 276:* 565-570.

LEWIS, R., KAUFMAN, T., DENELL, R. and TALLERICO, P. (1980a). Genetic analysis of the Antennapedia Gene Complex (ANT-C) and adjacent chromosomal regions of *Drosophila melanogaste*r. I Polytene chromosome segemnts 84B-D. *Genetics. 95:* 367-381.

LEWIS, R., WAKIMOTO, B., DENELL, R. and KAUFMAN, T. (1980b). Genetic analysis of the Antennapedia Gene Complex (ANT-C) and adjacent chromosomal regions of *Drosophila melanogaste*r. II Polytene chromosome segemnts 84A-84B1,2. *Genetics. 95:* 383-397.

LINDSLEY, D. and GRELL, E. (1968). *Genetic variations of Drosophila melanogaster*. Carnegie Inst. Washington Publ. No. 627.

MAZO, A., HUNG, D-H., MOZER, B. and DAWID, I. (1990). The t*rithorax* gene, a trans-acting regulator of the bithorax complex in *Drosophila*, encodes a protein with zinc-binding domains. *Proc. Natl. Acad. Sci. USA. 87:* 2112-2116.

MORATA, G. and KERRIDGE, S. (1981). Sequential functions of the Bithorax complex in *Drosophila*. *Nature 290:* 778-781.

MORATA, G. and LAWRENCE, P.A. (1975). Control of compartment development by the en*grailed* gene in *Drosophila*. *Nature 255:* 624-627.

NÜSSLEIN-VOLHARD, C. and WIESCHAUS, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature 287:* 795-801.

SEDKOV, Y., TILLIB, S., MIZROKHI, L. and MAZO, A. (1994). The Bithorax complex is regulated by *trithorax* earlier during *Drosophila* embryogenesis than is the Antennapedia complex, correlating with a *bithorax*-like expression pattern of distinct early *trithorax* transcripts. *Development. 120:* 1907-1917.

SIMON, J., CHIANG, A. and BENDER, W. (1992). Ten different Polycomb group genes are required for spatial control of the abdA and AbdB homeotic products. *Development. 114:* 493-505.

STRUHL, G. (1981). A gene product required for correct initiation of segmental determination in *Drosophila*. *Nature 293:* 36-41.

STRUHL, G. and AKAM, M. (1985). Altered distributions of Ultrabithorax transcripts in extra sex combs mutant embryos of Drosophila. *EMBO J. 4:* 3259-3264.

WADDINGTON, C. (1956). Genetic assimilation of the *bithorax* phenotype. *Evolution 10:* 1-13.

WAKIMOTO, B. and KAUFMAN, T. (1981). Analysis of Larval Segmentation in Lethal Genotypes Associated with the Antennapedia Gene Complex in D*rosophila melanogaster*. *Dev. Biol. 81:* 51-64.

Yu, B., HESS, J., HORNING, S., BROWN, G. and KORSMEYER., S. (1995). Altered *Hox* expression and segmental identity in *Mll* mutant mice. *Nature 378:* 505-508.