The location of bithorax complex transcripts in the *Drosophila* embryo
by *in situ* hybridization

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Products of the bithorax complex (BX-C) control the developmental fate of segments in the *Drosophila* embryo (Lewis, 1978).

I have localized transcripts from the *Ubx* (*Ultrabithorax*) region of the BX-C using *in situ* hybridization with tritium-labelled probes (Akam, 1983) prepared from cloned genomic fragments of the complex (Bender *et al.* 1983), encoding the major 5' exon of the *Ubx* transcripts.

In the cellular blastoderm, *Ubx* transcripts accumulate principally in a narrow band of cells approximately midway between anterior and posterior poles of the egg, a location which, from fate mapping, corresponds to the primordia for the third thoracic and/or first abdominal segments.

After gastrulation, *Ubx* transcripts are detectable in a larger region of the germ band, extending from the third thoracic to the eighth abdominal segments. Both mesoderm and ectoderm are labelled in the first seven abdominal segments, but in the third thoracic and eighth abdominal segments only certain cells of the ectoderm are labelled.

Homo geneic mutations, which disrupt the normal expression of the bithorax complex, alter the distribution of these transcripts.


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**Analysis of defects in temperature sensitive embryonic lethal mutants of the nematode *Caenorhabditis elegans***

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We have isolated a set of temperature sensitive lethal mutants in *C. elegans*, defining 30 genes essential for embryogenesis (*emb*) and characterized them genetically and cell culturally (Cassada *et al.* 1981; Isnenghi *et al.* 1983; Denich *et al.* 1984). I have now begun a more detailed analysis of a few selected mutants from this set.

The mutant *emb-22* is being investigated as a possible ts actin mutant, based on its rather pleiotropic phenotype and genetic location. Defects include absence of polar bodies, pseudopodia, abnormal cytoplasmic streaming, diffuse spindle, and slow cell division (1/3 normal) (Denich *et al.*). Two- and three-factor crosses (Cassada *et al.*) place it within 10 genes or so of the 3 cloned actin genes on chromosome V, mapped by DNA restriction polymorphisms (Hirsh *et al.*; Waterston *et al.*). I am complementing with five deficiencies spanning the region (obtained from others) to localize the gene further. I have also been able to use slow post-embryonic growth as a recessive phenotype for complementation of *emb-22* to known actin mutants (dominant uncoordinated movement) (Waterston *et al.*), which also show recessive slow growth, but have no embryonic phenotype. (Nor is *emb-22* uncoordinated.)

The mutant *emb-18* has some of the phenotypic properties of *emb-22* and maps near it, to the left. Neither *emb-22* nor *emb-18* has any significant suppressor activity on mutations in *unc-15* (paramyosin), as does *sup-3*, a muscle-specific gene mapping just left of actin (Riddle and Brenner, 1978).

The mutant *emb-29*, one of the few strictly zygotic (non maternal-effect) mutants in our set, arrests with some 300 cells. These cells are all in G2, not expressing a mitosis-specific protein kinase (Hecht *et al.*). I am mapping the gene (left arm of V) with respect to interstrain DNA polymorphisms, as a first step to gene isolation (with R. Hecht). Progress will be described.

The mutant *emb-21* is the only pattern-coordinate-altering mutant, its early division pattern resembling a double-posterior pattern. Investigations on the basis of the defect will be reported.

Finally, I have isolated a wild-type *C. elegans* in Freiburg with several differences from the Bristol strain, including a mutator activity, to be described.

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Gene interactions controlling sexual phenotype in the nematode C. elegans

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Sex determination in Caenorhabditis elegans is being studied in order to analyse a set of genes that control the choice between three possible pathways of development: male, hermaphrodite, or female. In the wild type, the primary mechanism of sex determination is chromosomal: XO animals develop into males and XX animals develop into hermaphrodites; females do not occur naturally. Null mutations in a single autosomal gene, tra-1, cause both XO and XX animals to develop into fertile males, while constitutive tra-1 mutations cause both XO and XX animals to develop into fertile females. Six other autosomal genes that affect sex determination have been identified, as well as three genes that affect X chromosome dosage compensation. Analysis of these genes provides evidence for the existence of a cascade of gene interactions that mediate between the primary sex determining signal, the ratio of X chromosomes to autosomes, and the critical gene tra-1, so that this gene is fully repressed in XO animals (permitting male development) and active but transiently repressed in XX animals (permitting hermaphrodite, as opposed to female, development). The cascade consists of five steps: the X/A ratio; the single gene her-1; the two genes tra-2 and tra-3; the three genes fem-1, fem-2, and fem-3; and the single gene tra-1.

The implications of this model will be discussed, and the mechanism of sex determination in C. elegans will be compared with that of Drosophila.

Molecular and genetic analysis of segmentation in Drosophila

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The process of segmentation in Drosophila requires the activity of a number of genes, many of which were recently identified by Nusslein Volhard and Wieschaus (1980). These genes can be broadly grouped into three classes according to their mutant syndromes. We are studying the genes of the pair-rule class, and in particular the hairy (h) gene. The effect of absence of the h+ gene during embryogenesis is reflected in the patterning of the larval cuticle. The posterior region of each odd numbered segment together with the anterior region of each even numbered segment is deleted; the deleted region includes the segment border, so that h- larvae possess only half the normal number of segments. Combinations of h- mutations with various homeotic mutations suggest that each of these segments has qualities of two wild type segments. Analysis of embryogenesis in h- animals by conventional histology and electron microscopy has revealed that these defects result from a failure of the normal subdivision of the extended germ band, accompanied by localised cell death in the ectoderm. This indicates an early requirement for the h+ gene; pole cell transplantation experiments suggest this to be met entirely by zygotic gene expression. We have cloned DNA corresponding to the h gene and are using this to study the expression of the gene by Northern blot analysis and by in situ hybridisation.

In addition to its requirement for correct segmentation during embryogenesis, h+ is required much later in development for the appropriate patterning of sensory organs of the adult peripheral nervous system. This functional complexity has been investigated by molecular and genetic analysis. Complementation studies suggest the existence of two functional units within the h gene, one exclusive to the adult function, one required for both functions. Molecular mapping of over 30 mutations of the gene has revealed a clustering of mutations according to phenotypic effect, suggesting that the two functional units are physically separated in a gene spanning at least 20 kb of DNA.

Developmental genetics of invertebrates

Cell lineage of some internal organs of *Drosophila*

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We now know a good deal about the cell lineage and developmental genetics of the *Drosophila* epidermis. Much less is understood about the basis of pattern formation in the CNS, muscles, gut and other soft parts. What is the dependence of the internal organs on the bithorax complex, on *engrailed* and on other homoeotic genes? Are any of the internal organs divided into segmental or subsegmental compartments? Which of the germ layers are segmented? These questions can be approached with mosaics made by using genetically marked clones and nuclear transplantation. Some preliminary results will be discussed.

Evidence for a DNA ligase change related to early development in sea urchin

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A definite change in the molecular form of DNA ligase appears when the sea urchin egg enters cleavage (*Psammechinus miliaris*). Sucrose gradient analysis show that a slower migrating form (7S) of enzyme exists in unfertilized egg and in sperm. A faster migrating form of DNA ligase (7.5S) is present in developing egg at the 8 cell stage as well as in artificially activated unfertilized egg. The timing of this early biochemical event has been determined, and the situation closely parallels what has been described in Amphibia.

The change in molecular form of DNA ligase has been shown to be sensitive to antimetabolites inhibiting protein synthesis (cycloheximide), gene transcription (amanitin), and DNA replication (aphidicolin, Ara C). Consequently the appearance of the fast migrating form of DNA ligase is assumed to result from the expression of the corresponding gene, switched on following egg activation. This assumption is in line with the situation established in Axolotl.
Developmental genetics of invertebrates

The genetic structure of the bithorax complex of Drosophila

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We have isolated a number of lethal and viable mutations within the confines of Df(3R)P9, a 4-6 band deletion including the entire bithorax complex (BX-C). By deficiency mapping and complementation analysis we have defined 5 complementation groups. The two genes located at both ends, left lethal and right lethal appear to be necessary for cell proliferation but do not affect segment development. The other three complementation groups form the BX-C and each of them defines a particular functional domain in the fly. The most proximal one, Ultrabithorax is responsible for the normal segment pattern of the posterior mesothorax, metathorax and anterior first abdominal segment. The intermediate one, abdominal-A controls the development from the posterior first to the fifth abdominal segment. The most distal gene Abdominal-B is responsible for the development of sixth, seventh and eighth abdominal segments. Each of these major genes may contain a number of distinct functions that specify the development of each compartment within the domain.

Correlations between defects in embryogenesis and defects in transcription caused by a temperature-sensitive RNA polymerase II mutation in Drosophila

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Embryos, which are deficient for a locus that encodes the large subunit of RNA polymerase II, the Rpll215 locus, develop to the late embryo stage before dying, relying entirely on maternally loaded polymerase. These dead embryos are morphologically wild type. Embryos possessing a temperature-sensitive mutation in this locus, Rpll215ts, are viable to permissive temperature (22 °C); however, development is arrested at either the late embryo stage or first larval instar stage if fertilized eggs are placed at restrictive temperature (29 °C). These dead individuals are also morphologically normal at the time of death. This contrasts with embryos that develop from eggs produced by Rpll215ts females at restrictive temperature. The resulting embryos display holes in the pseudocephalon, presumably caused by a failure of head involution, and are missing ventral hypoderm. The defects can only be partially corrected by allowing embryogenesis to proceed under permissive conditions. We have attempted to correlate the aforementioned defects with the state of the RNA polymerase II at the time development is arresting. This was done by heat shocking late embryos at 37 °C for 40 min following the same treatments described above. Northern analyses revealed that HSP70 mRNA is accumulated to the same level in Rpll215ts and wild-type embryos raised at 22 °C. However, following oogenesis or embryogenesis at restrictive temperature, late embryos fail to accumulate HSP70 mRNA to the same levels as similarly treated controls. Thus, treatments of Rpll215ts flies that affect development also affect the ability of embryos to accumulate HSP70 mRNA. This is consistent with the notion that the developmental defects result from defects in transcription. This work was supported by a grant no. GM26693 from the NIH. DAH was supported by a postdoctoral training grant no. GM7227.
The role of the X chromosome in sex determination

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In Drosophila, the ratio of X chromosomes to sets of autosomes (X:A) is the primary genetic signal for two closely linked processes, sex determination and dosage compensation. An X:A ratio of 1.0 triggers female differentiation, a ratio of 0.5 leads to male development. Early work with triploids carrying variable amounts of X chromosome material led to the conclusion that the female determining effect of the X chromosome is of quantitative nature, i.e. that a large number of equally important female determining genes are spread all over the X chromosome. Attempts to localize such genes failed.

The aim of this work was to test whether the female determining effect of the X chromosome is purely quantitative or whether there are qualitative differences between different regions of the X. In the latter case specific genes needed for female differentiation might even be defined. We chose to investigate the sexual phenotype of flies with two sets of autosomes and an intermediate X:A ratio by providing them with more than one, but less than two X chromosomes. Such aneuploids for larger parts of the X chromosome are lethal. Thus, we produced mosaic flies carrying the desired genotype in clones.

Our results show that essential female determining genes are located distally on the X chromosome. One such gene is Sxl which was previously described to be involved in both sex determination and dosage compensation (Cline, 1978, Genetics 90, 683–698). We defined a new region (3E8 to 4F11) that is essential for female differentiation. In addition, we demonstrated a quantitative effect of the X chromosome inasmuch as intermediate X:A ratios activate the gene Sxl to a level that is intermediate between the female level (Sxl is active in females) and the male level of activity (Sxl is not active in males).
Developmental genetics of invertebrates

Scanning electron microscopy of Drosophila: formation of the caudal segments in wild type embryos and the phenotype of larvae deficient for the genes of the bithorax complex

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a) Wild type embryos show transient dorsal segment borders during late stages of germ band shortening separating putative segments A9, A10 and A8. b) The location of segments A9 and A10 coincides with specific posterior structures visible at the end of embryogenesis. c) Dorsally BX-C- larvae [Df(3R)P9] have the anterior parts of the MS, MT, and abdominal segments A1 to A8 transformed into MS and the posterior parts into PRO. Ventrally the posterior part of the transformed segments cannot be unambiguously classified for lack of markers. d) BX-C- larvae lack some of the posterior sense organs located dorsally between the base of the posterior spiracles and anterior to the tuft, and have an altered spinule pattern there. This area also bears dorsal pits laterally characteristic of thoracic segments. These results confirm an earlier study of development of the caudal segments indicating that during embryogenesis there are ten abdominal segments, even though the segmental boundaries between extreme caudal segments are transient. BX-C- larvae show that dorsally, the MS, MT and the first eight abdominal segments are transformed into a MS/PRO mosaic pattern, indicating that the BX-C contains gene functions promoting posterior MS as opposed to posterior PRO. This difference between transformed anterior and posterior compartments has been reported elsewhere (see review by Lawrence and Morata, 1983). The dorsal transformation of some structures posterior to A8 in BX-C- larvae, leaving unaltered the most posterior structures, indicates that there are at least 10 abdominal segments, the first nine of which require gene functions located with the BX-C.


Genetic and developmental analysis of the bithorax-complex and its environs

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The bithorax-complex (89E1-4) comprises a number of gene functions required for the determination of thoracic and abdominal segments. We have used EMS and γ-rays to induce lethal and visible mutations within and outside the complex, utilising two deficiencies, Df (3R) bxdm (89 B5,6; 89E2,3) and Df (3R) P115 (89B; 89E5,7). Within the 89B5,6 to 89E5,7 interval, 26 lethal complementation groups and one visible group (ss*) were identified.

Five lethal groups (here numbered I–V) fall within Df(3R)P9 which presumably uncovers the entire complex. Group I mutants have no apparent change in larval morphology and probably define the left boundary of the complex. Group II is the haplo-insufficient locus Ultrabithorax (Ubx) which has a domain of function from posterior mesothorax to anterior abdominal-1. Three viable Ubx alleles were recovered having weaker but similar effects to those of the lethal alleles. Group III is allelic to iab-2, and is defined by the right breakpoint of Df (3R) P10, and transforms abdominal segments 2–8 towards abdominal-1, leaving the other segments unaffected. Group IV is a haplo-insufficient locus; heterozygous males have an additional tergite posterior to tergite 6 and an extra sternite with bristles. Male and female hemizygous escapers of one allele have an extra abdominal 8 segment. In females, the genitalia are transformed into an abdominal segment but the analia are unaffected. In males the genitalia and analia are normal. Abdominal segments 5–8 resemble abdominal 4 in both larvae and adults. Group V has no apparent abnormality and may represent the right-hand boundary of the complex.

Within 89E3,5; E5,7 there were four lethal groups. Only one group affects larval phenotype; the number of ventral denticles and dorsal spinules in each segment are reduced and deranged, meso- and metathoracic denticles are coarser and darkly pigmented, and ventral pits, Keilin's organs and abdominal 8, 9 and 10 sense organs are absent. These effects may not be homoeotic transformations.

We shall discuss the organisation of the bithorax-complex in the light of this evidence that it contains only three lethal complementation groups.
**Developmental genetics of invertebrates**

**Chromatin diminution in Ascaris: a molecular approach**

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During early cleavage divisions of Ascaris lumbricoides a process called chromatin diminution takes place: about a quarter of the total amount of germ line DNA is expelled from the presumptive somatic cells. Hybridization experiments revealed that the germ line and somatic DNA contain the same percentage of ribosomal genes. Therefore, chromatin diminution does not serve the purpose of discarding large amounts of rRNA genes from the germ line cells. On the other hand, over 99%, but not all of the satellite DNA sequences being present in the germ line genome, are eliminated from the presumptive somatic cells by chromatin diminution. Molecular cloning and sequence analysis of different restriction enzyme fragments isolated from the germ line satellite DNA indicate that this eliminated satellite is composed of a whole set of related variant sequences, which differ by several point mutations. Members of the same variant class are tandemly linked and therefore physically separated from other variant classes. The comparison of all the determined sequences allowed to establish a 121 bp long and AT rich consensus sequence which itself is composed of an 11 bp long subrepeat. There is no indication for transcriptional activity of the satellite DNA sequences at any stage or tissue analysed. We have evidence that the eliminated DNA contains also other DNA sequences apart from the class of highly repetitive satellite DNA.

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**Larval phenotypes of the mutations affecting the function of the abdominal domains of the bithorax-complex in Drosophila melanogaster**

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Genetic analysis of lethal mutations affecting the bithorax complex functions has allowed their grouping by complementation in 3 domains, one thoracic and two abdominals: Ubx, affecting the thoracic segments, abdA, affecting the forward abdominal segments and AbdB, affecting the rear abdominal segments (Sánchez-Herrero et al. unpublished).

In this communication we present the description of the larval phenotypes of the last two groups of mutants which is in agreement with this subdivision of the Bithorax complex.

Individual larvae homozygous or hemizygous for abdA present a strong transformation of all the ventral abdominal denticle belts towards a combination of T3p-A1a segment. A similar transformation is visible in the dorsal cuticle. The anterior part of both ventral and dorsal sides is clearly transformed into A1a. On the other hand, the posterior part is transformed into T3p, as indicated by the absence of a typical row of chitinized spines in the posterior part of the dorsal side of the abdominal segments and the presence with some variability of 'mono-hairs', the posterior component of the Keilin's organ, in the ventral side of the abdominal segments. Towards the end of the animal the transformation to T3p-A1a, although still recognizable, becomes less clear in a gradient-like fashion, beginning at the 5th or 6th abdominal segment. This is particularly conspicuous in the dorsal side, since the dorsal abdominal 8th segment is practically indistinguishable from the wild type.

Individual larvae homozygous or hemizygous for AbdB present a transformation of the last abdominal segments toward more anterior ones. Interestingly, the abdominal 8th segment appears transformed both in its anterior as in its posterior part, since the posterior spiracles appear strongly reduced or absent. The anal pads and tuft appear normal. It is difficult to decide which is the more anterior segment in which the function of AbdB appears to be already required but it certainly includes up to the anterior abdominal 6th. The results are in agreement with the hypothesis that the larval abdominal cuticle is differentiated under the influence of the functions coded by genes included in these two abdominal domains.
Developmental genetics of invertebrates

Interactions among 3 mutations in the 2d chromosome affecting the pair-rule segmental pattern formation of Drosophila melanogaster

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In a systematic search for embryonic lethal mutants of Drosophila melanogaster, Nüsslein-Volhard & Wieschaus (1) have identified multiple loci (> 15) that, when mutated, alter the segmental pattern of the embryo. Our interest in early morphogenetic events in Drosophila embryogenesis has prompted us to study the genetic interactions among some of these loci. In this communication a description of the phenotypes of the three double mutants odd-skipped, even-skipped and paired is presented. As reported (1), these mutants of the 2d chromosome produce pattern deletion in alternating segments.

The cuticular pattern of late embryos of the double mutant odd-skipped, even-skipped suggests additivity of the two phenotypes since all ventral denticle belts are absent. Interestingly, one set of Keilin’s organs and ventral pits is still present.

The phenotype of the double mutant odd-skipped, paired indicates a functional interaction between them, since the ventral denticle belts are basically similar to those of a weaker allele of paired(prd^2). Moreover, additivity of action in relation to the Keilin’s organs and ventral pits is suggested by their complete absence.

In contrast to even-skipped and paired, which form half the normal number of bulges in the surface of the 9 h embryos (1), odd-skipped homozygous embryos form the normal number of segments and only later does fusion of the odd segments occur.

Finally, the phenotype of the double mutant odd-skipped, paired is more extreme than the simple addition of the two of them, showing a very clear effect during gastrulation leading to the elimination of nearly all cuticular features.

Results suggest early and co-operative functions for even-skipped and paired while odd-skipped seems to be more of a maintenance gene. Moreover, some independence in the formation of the ventral cuticular markers (dentine belt vs. Keilin’s organs and ventral pits) is also suggested.


We acknowledge the kind gift of the mutant from Dr Ch. Nüsslein-Volhard.