The Clonal Basis of Development

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Preface

The idea that embryos consist of a complex array of morphogenetic fields formed the conceptual basis of developmental biology for many years. Increasingly, however, field theories of development are under attack. They have not provided satisfactory answers to questions of why the field boundaries are so sharp, or why there is no gradient of cell type within the field. And attempts to identify morphogenetic substances that might be the chemical basis of the field have not met with general success.

An alternative view that has emerged is that the early embryo is a cell clone within which subclones differentiate to give rise to specific structures or parts of structures. This view of the developing organism has progressed farthest in *Drosophila* where the ability to mark cell clones by mutations has provided a powerful stimulus to progress, and has extended to mammalian embryological studies with the introduction of chimeric technology. Quite independently botanists had been making chimeric plants since the pioneering work of Winkler in the early 1900s, but because they had been articulating their results in a different terminology the similarities between the plant and animal results had until recently been overlooked. The 36th Symposium of the Society focused on clonal aspects of development, to see where we have come from, where we are now, and where we are going with this approach to developmental analysis. The five sessions examined clonal analysis in *Drosophila*, in mammals, in plants, and in lateral organs of plants and animals, and concluded with an examination of genetic mechanisms of clone initiation.

The Symposium was held on the campus of North Carolina State University, and its success was due in large part to the excellent work of the local committee headed by John Scandalios, and to the efforts of Claudia Foret who was our liaison with the local committee. Financial support provided by the Developmental Program of National Science Foundation made it possible to bring an outstanding group of scientists to speak at the symposium. The Society deeply appreciates the continuing financial support provided to us by the National Science

Foundation, and recognizes the importance of this to progress in the whole field of developmental biology.

With this volume we change editors for the symposium series. John Papaconstantinou, edited volumes 33-35. The Society expresses its thanks to him for his excellent services during this time.

Ian M. Sussex

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I. Invertebrates

The Initiation and Maintenance of Gene Activity in a Developmental Pathway of *Drosophila*

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I. INTRODUCTION

Determination during embryonic development can be considered as a discrete event preceding and leading to terminal differentiation. Whereas differentiation could be molecularly defined by a spectrum of specific cell products, determination is an operational concept with unknown genetic or molecular bases. However, the existence of tissue-specific hormone receptors or of tissue cell lines stable upon transplantation and culture suggests that the final inventory of gene products was somehow already defined at the genetic level. Thus, the problem of cell differentiation is implicit in the problem of determination. This in turn could depend on the mechanism of activating certain genes or groups of genes and maintaining them in an active state in subsequent cell generations.

It was possible to elucidate the mechanism involved in gene activation in microorganisms because the elements involved could be manipulated. The minimum number of elements is two: the specific gene to be activated (structural gene) and an extrinsic factor (inductor)

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specific for the activation. Since this specificity is probably based on molecular recognition, inductor molecules would presumably first interact with a gene product in order to affect the DNA. Therefore, in the simplest scheme of regulation two genes are involved: a regulator and a structural gene. Very little is known of the mechanisms of gene activation during embryonic development for several reasons: the lack of well defined genetic variants, the lack of knowledge about the specific inductor molecules and the difficulties involved in the manipulation of both in cells.

We will discuss here some data which suggest that regulatory mechanisms function during the determination of the metathoracic developmental pathway in *Drosophila*. This study is based, on the one hand, on the possibility of manipulating different genetic variants that affect this pathway. Since such genetic variants can be defined at the cellular level, their cellular phenotypes are a reliable indication of gene activity. On the other hand, it is based on the possibility of experimentally varying the local distribution of extrinsic factors apparently functioning during the initiation of the pathway.

II. THE GENETIC ELEMENTS

A. *Genetic Interactions*

The development of a normal metathoracic segment requires the function of the wild type alleles of the bithorax gene complex (see Lindsley and Grell, 1968 for details about the genetic variants mentioned). Genetic analysis has shown that this gene complex codes for different complementing functions (Lewis, 1964, 1967), (Fig. 1). It is located in the bands 89E1-4 in salivary chromosomes. Certain mutant alleles in the bithorax system cause transformations between mesotho-

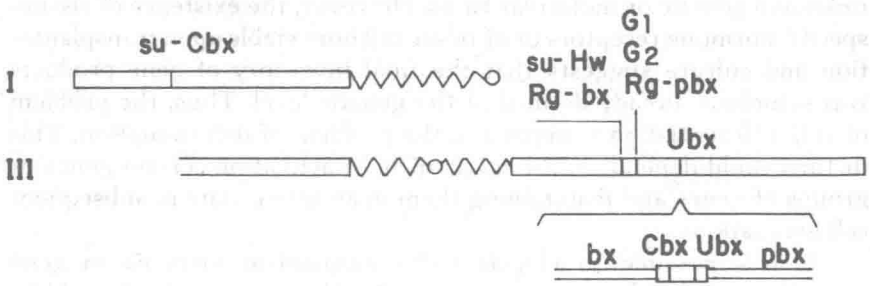

Fig. 1. Distribution in the *Drosophila* genome of the loci involved in the metathoracic pathway. Description of genotypes and mutant interactions in text.

racic and metathoracic segments (Table I). In the adult fly, such transformations are visible in all cuticular derivatives of the mesothorax and metathorax. Mutant alleles fall into two main groups:

TABLE I

Genetic, Phenotypic, Clonal, and Phenocopy Properties of Different Alleles and Loci Related to the Metathoracic Pathway

				Phenotype in Flies			Phenotype in Clones		Phenocopy	
Locus	Allele	HZ	HM	Penetrance	Expressivity	Specificity	Clonality	Fidelity	Via ♀	Via ♂
bithorax	bx^{34e}	+	bx	T	P	C	P	short	1.0	
	bx^{3}	+	bx	T	T-P	C	T-P	short	0.9	1.0
	pbx	+	pbx	T	T-P	C	T-P	short	1.1	
	Ubx	Ubx	L(bx,pbx)	T	T	C		short	0.9	
	Ubx^{130}	Ubx	L(bx,pbx)	T	T	C		short	1.7	
	Cbx	Cbx	Cbx	T	P	C	P		0.9	
	Hm	Cbx	L	T	P	C	T-P		0.6 (xx)	
Rg-pbx	Rg-pbx	pbx^{v}	L(x)	P	P	V	T	long	2.4	2.9
	$Rev(G_1)$	+	L(x)	+	+	–		long	0.9	
	$Rev(G_2)$	+	L(x)	+	+	–		long	0.8	
Rg-bx	Rg-bx	bx^{v}	L(x)	P	P	V	T(?)	long	3.0	
	Df(3)red	bx^{v} & pbx	L(x)	P	P	V		C.L	3.2	1.8
su-Cbx	su-Cbx	+	su-Cbx	T	T	C	T	short	1.0	1.3
	Df(1)KA14	+	L	T	T	C		C.L	0.8	
su-Hw	su^{2}-Hw	+	su-bx^{3}	T	P	C			1.0	

HZ: heterozygous; HM: homozygous. Phenotypes; +: wildtype; L: lethal; in parentheses the phenotype of the lethal embryo; (x): apparently normal segmentation. Penetrance and expressivity (T: complete, P: partial) specificity (C: constant; V: variegated or variable). Clonality: penetrance in cells of the same clone. Fidelity: perdurance of the maternal phenotype in mitotic recombination clones. C.L: cell lethal. Phenocopy: data presented as the ratio of phenocopy frequencies of mutant zygotes to wildtype sib flies. Via ♀ or via ♂ : maternal or paternal origin of the mutant in the zygote. (x x) phenocopies only in the notum; the wing to capitellum transformation is not changed by the ether treatment.

recessive and dominant. The recessive alleles show, in homozygous condition, transformation either of the anterior *(bithorax (bx)* alleles) or of the posterior (*postbithorax, (pbx)* alleles) developmental compartments of the metathoracic segment into the corresponding ones of the mesothoracic segment. All the *bx* and *pbx* mutants which have been studied are recessive over a single dose of their wildtype alleles. Mutant alleles in homozygous flies show total penetrance but variable expressivity. Their maximal expressivity is restricted to either the anterior or posterior compartment. In weak, "leaky", mutant alleles the partial expressivity has, however, a constant specificity: they transform specific regions within the compartment affected. It is characteristic of these leaky mutants to vary their expressivity under different conditions of temperature and genetic background (Villee, 1945; Kaufman *et al.*, 1973).

Two types of dominant mutants are known in this system: The first group includes the *Ultrabithorax (Ubx)* alleles. All of these correspond to the lack of function of both bithorax and postbithorax wildtype alleles located in cis-configuration. The same phenotype can be caused by point mutations, mapping between *bx* and *pbx* loci, and also by chromosome rearrangements with breakpoints in 89E1-2 and by chromosome deficiencies for those bands. The phenotype of *Ubx*, or of *Df*(89E1-2) in heterozygous flies with one wildtype dose is a slightly swollen haltere. This phenotype disappears in heterozygous flies with the wildtype system duplicated. Thus, the *Ubx* dominant phenotype corresponds to haplo-insufficiency of the system. *Ubx* is lethal in homozygous flies. This phenotype can be studied in embryos or in mitotic recombination clones (see below) in the adult cuticle. Under these conditions they show the double syndrome of extreme *bx* and *pbx* mutants (Lewis, 1964; Morata and García-Bellido, 1976). Embryos homozygous for DF(89E1-4) show segmental transformations in the epidermis and the nervous system (Lewis, pers. comm.). It is possible that the genetic information of the bithorax system is required for the proper segmental development of all the germ layer derivatives.

The second group of dominant mutants includes the *Contrabithorax (Cbx)* alleles. These are dominant over one or several doses of wildtype bithorax systems. They include point mutations *(Cbx)*, mapping close to *Ubx* and between the loci of *bx* and *pbx*, and a rearrangement *(Haltere mimic, Hm)* with a breakpoint in 89E1-2 (Lewis 1964, and pers. comm). The phenotype of these mutants show the transformation of mesothoracic structures into metathoracic ones, which is opposite to that caused by *Ubx*. The penetrance of these mutations is complete, but the expressivity is partial, increasing with the number of doses of mutant

alleles. The specificity varies between different alleles, but is constant for a given allele.

The effects of the different mutant bithorax alleles suggests that the wildtype function of the bithorax system is to create a metathoracic pathway as an alternative to the mesothoracic one. Genetic analysis of the system suggests that it contains structural loci (*bx* and *pbx*) and cis-regulatory loci (*Ubx* and *Cbx*). The genetic behaviour of the *Ubx* alleles indicates that they correspond to operator deficient mutations (0^o) whereas *Cbx* alleles can be interpreted as operator constitutive (0^c) mutations (Lews, 1964, 1967). The phenotypic transformations caused by *Cbx* alleles suggests that bithorax system wildtype products are released in the mesothorax. This leads to the suppression of the mesothoracic pathway and the appearance of its metathoracic alternative. That this is due to the derepression of the bithorax system is confirmed by the cis-suppression effect of bx^3 on *Cbx* in bx^3 *Cbx*/+ + flies (Lewis, 1964). That *Cbx* is also derepressed in other segments, is strongly suggested by the effect of *Cbx* in double mutant combinations with other homeotic mutations which transform other segments to mesothorax. For example, head structures transformed into dorsal mesothoracic structures in *Opthalmoptera* (*Opt*) flies (Goldschmidt and Lederman-Klein, 1958) is further converted into metathoracic structures in *Opt; Hm* flies (Capdevila, unpublished).

The existence of mutations, mapping outside the bithorax system, which show a bithorax phenotype on their own or that interact with the expression of the bithorax mutant alleles, suggests that the metathoracic developmental pathway requires the normal function of other genes besides those of the bithorax system. We will summarize some genetic data relating to these mutants. (Table I, Fig. 1).

The mutant called *Regulator* of *postbithorax* (*Rg-pbx*, Lewis, 1968) is a recessive lethal which shows a dominant visible phenotype over its wildtype allele. The penetrance in heterozygotes is not complete but high (ca. 90%) as is its expressivity. In addition its specificity is variable; heterozygous flies show, in an asymmetric and erratic fashion, mesothoracic transformation patches. These appear anywhere within the posterior compartment of the metathoracic segment. The mutant condition is associated with one of the breakpoints of the *In(3R)* 85B; 88B. A duplication carrying the left hand region of this inversion on the Y chromosome shows the same dominant phenotype as the original inversion. Penetrance and expressivity are similar in zygotes receiving the mutation from either parental gamete. Penetrance and expressivity vary with the number of wildtype doses of the bithorax system present in the genome; both decrease with increasing number

of these wildtype genes (Lewis, 1967). This behavior led E. B. Lewis to suggest that the mutation may lead to a superrepressor condition in a regulator locus with trans effects on the *pbx* gene. It is therefore interesting to note that the mutant phenotype is suppressed in *Rg-bx* +/+ *Cbx* flies (Capdevila, unpublished). Two independent revertants (G_1 and G_2) of the *Rg-pbx* original inversion have been isolated by E. B. Lewis. Both are homozygous lethal and lethal in heterozygous combination with *Rg-pbx*. It is reasonable to assume that the reversion of the phenotype is associated with the lack of function of the *Rg-pbx* gene. Thus, if the *Rg-pbx* mutation corresponds to a mutation of the trans-regulator gene, its amorphic condition would lead to an extreme phenotype in homozygous flies. However, both the *Rg-pbx* and the two revertant mutations in homozygous mutant embryos do not show segmental transformations. Although if only specific for the *pbx*+ function, they might not be detectable in cuticular structures.

The point mutant named *Regulator* of *bithorax* (*Rg-bx*, Lewis, pers. comm.) is included in a deficiency, *Df(3)red* (88B), which lacks two or three bands including the loci of *red* and *su-Hw* (see below). Both *Df(3)red* and *Rg-bx* are recessive lethals. Both show, in heterozygous condition over their wildtype homologs, a low penetrance and expressivity. The phenotype is expressed in patches of mesothoracic structures, located in variegated or random fashion (variable specificity), in both anterior and posterior compartments of the metathorax. Thus, the phenotype of *Rg-bx* seems to correspond to the haploinsufficiency of this locus. Although *Df(3)red* and *In(3)Rg-pbx* have breakpoints in the same chromosome region, they are not allelic. Both *Df(3)red* and *Rg-bx* mutants are viable in flies doubly heterozygous for either *Rg-pbx* or the G_1 or G_2 revertants.

The penetrance of the transformation varies depending on whether the mutation was carried to the zygote by the male or by the female gamete. The penetrance also varies depending on the genetic constitution of the zygote with respect to the *bithorax* system and the *Rg-pbx* locus (Capdevila, 1977). This penetrance is 4% in *Df(3)red*/+ flies of mutant mothers but zero in mutant zygotes of mutant fathers and in non-mutant zygotes of mutant mothers. When the zygotes are also double heterozygotes for *In(3)Ubx*^{130} (or *Df(3)89E1-4*) the penetrance increases to about 30% if *Df(3)red* was carried by the female gamete and to about 4% if carried by the sperm. The doubly heterozygous *Df(3)red* +/+ *Rg-pbx* zygotes of *Df(3)red*/+ mothers show the typical high penetrance of posterior transformation caused by *Rg-pbx* but the