GENETIC PROGRAMMING FOR DEVELOPMENT IN *DROSOPHILA*

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INTRODUCTION

From the start we wish to make one point perfectly clear. Genetic programming for development in multicellular organisms is not understood. Any attempt to write on this subject must necessarily be speculative if not fanciful. To some degree this article will be both speculative and fanciful. Nevertheless, if one is to understand and elucidate the characteristics of programming for development in higher organisms and if one accepts the premise that development is dependent on gene regulation, then it is logical to first investigate and delineate the organization of the eukaryotic genome. *Drosophila melanogaster,* because of its advanced genetic characterization, is more suitable for such an investigation than any other multicellular organism. In addition, the polytene chromosomes of *Drosophila,* and other members of the Diptera, offer a particularly useful tool for studying the function of chromatin. Relying mainly on genetic studies in *Drosophila,* observations on polytene chromosomes, and studies on the molecular biology of chromatin, we will first attempt to delineate the nature of the organization of the *Drosophila* genome in terms of the geography of gene distribution and in terms of function. We will use this information in an appraisal of limited aspects of *Drosophila* development and to derive a provisional model of regulation during development. Although the information in this article is mainly restricted to *Drosophila,* the picture which emerges may well be applicable to developmental processes in eukaryotes in general.

THE NUMBER OF GENES IN *DROSOPHILA*

One of the critical factors affecting the types of mechanisms required for regulating a genome is the number of genes present. Before discussing the means of estimating gene number in *Drosophila* it is necessary to come to a working definition of the term "gene" as applied to eukaryotic organisms. The classic definition of a gene is a sequence of base pairs coding for a polypeptide or RNA molecules with definable functions (e.g., rRNA, tRNA). However, the genetic detection of such entities may not always be possible. For example, it is unlikely that point mutations in genes coding for histones could be recovered because of the redundancy of these genes.[1] Nor, would it be possible to separate by complementation tests a

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TABLE 1

Estimates of the Number of Genes in the Haploid Genome of *Drosophila melanogaster*

Method of Estimation	Number of Genes
1. DNA content	100,000
2. Spontaneous mutation rate	
A. lethal mutations	500
B. minor deleterious mutations	5–10,000
3. Complementation groups/chromomere	5,000
4. Intragenic map distance	112,000

single structural gene from an adjacent regulatory gene which is *cis*-dominant. None of the induced mutations would complement and all would appear to reside in a single genetic element. Furthermore, there might be regions of DNA which are transcribed, but in which the base sequence or the precise length is not critical. These regions would not be detected as functional except by the induction of appropriate deletions. However, all of these regions under the appropriate test might be identified as genes in the classic sense. There may also be areas of the genome in which the function of the DNA is purely structural (e.g., centric heterochromatin) and hence not involved in an assignable "genetic" function. We realize that this is a debatable point; however, we chose not to consider centric heterochromatin in this article because no specific genes have been assigned to it and we must of necessity be limited to regions of the DNA with assignable functions. We shall refer to genes in which we are relatively certain that some functional product is produced as "cistrons" (because they should be delineated by the classical *cis-trans* test)[2] or "structural genes." We will use the more general term "gene" when we are uncertain if an identifiable functional product is produced. With these definitions in mind we can turn our discussion to a consideration of the methods of estimating gene number in *Drosophila*.

1. Total amount of DNA per haploid genome (Table 1).

An estimate of the number of genes can be derived by dividing the number of base pairs in the haploid genome by the average length of a gene. The accuracy of this estimate depends on the availability of accurate estimates of the amount of DNA, determination of the average size of a gene, and determination of the proportion of the DNA which is devoted to genes. Estimates of the DNA quantity per haploid genome in *Drosophila* agree within a factor of about three. They range from the lowest estimate of 0.07 pg haploid nucleus[3] to 0.2 pg/haploid nucleus[4] with intermediate estimates of 0.14 pg[5] and 0.18 pg.[6] We prefer the value of 0.14 pg/haploid nucleus because it is based on renaturation kinetics.[5] The higher value of 0.18 pg is based on microdensitometric measurements using a cytophotometric standard. The amount of DNA in the standard has been debated. If the lower of two values for the standard is used, the amount of DNA in the haploid genome of *Drosophila melanogaster* from microdensitometric measurements would be about 0.15 pg/haploid nucleus,[6] essentially identical with the value obtained from renaturation kinetics. The proportion of the genome not devoted to function (i.e., without genes) in *Drosophila* (perhaps centric heterochromatin) may amount to about 25% of the total DNA.[7] Assuming an average gene is 1,000 base pairs long and that the genome contains 0.14 pg of DNA/haploid nucleus (about 1.3×10^8 nucleotide pairs), only 75% of which codes for structural genes (leaving 0.975×10^8 nucleotide pairs), there is enough DNA for about 100,000 genes. A critical question about the validity of this estimate is whether there are other regions of the genome, in addition to centric heterochromatin, which do not contain genes. If the other regions are substantial in number, then the above estimate is excessive.

2. Mutation rate per genome divided by mutation rate per gene (Table 1).

A. Mutations to lethal conditions. The units resulting from the division of mutation rate per genome by mutation rate per gene are genes per genome. Available estimates of mutation rates in *Drosophila* have recently been reviewed.[8] The most comprehensive data are provided by Wallace[9,10] who estimates that the spontaneous

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mutation rata per genome is about 1.5% recessive lethals per generation in males. Wallace's estimates are slightly higher than those obtained for the X and the second chromosome by other workers.[11-14] Mutation rates for specific non-lethal genes have generally been found to be about 3×10^{-5} per gene per generation in male germ cells.[15,16] Using these two estimates, then, it is possible to calculate that the *Drosophila* genome contains only about 500 genes (0.015 mutations per genome/0.00003 mutations per gene = 500 genes per genome). The validity of this estimate of the number of genes in *Drosophila* is based on two assumptions. First, the majority of mutations are phenotypically lethal and detectable by the classical methods of *Drosophila* genetics. Second, the estimates of mutation rate per gene are accurate within an order of magnitude. The first assumption will be discussed below. As for the second there is no way of knowing whether the estimates of mutation rate per gene produced so far are characteristic of the entire genome since only a very few loci have been investigated. Furthermore, many, but not all, of the loci used apparently involve comparatively large amounts of DNA (see Table 3) and thus may not be suitable for estimating average mutation rates. Although one might argue that the genes used for estimating mutation rates per gene represent a random selection, all of the loci used[15,16] were originally identified through spontaneous mutations recovered before 1925. They may, as reflected by their early recovery, be characterized by higher spontaneous mutation rates than the average gene. Suffice it to say, if the spontaneous mutation rate were an order of magnitude lower, the above estimate of 500 genes in the genome of *Drosophila melanogaster* would be increased tenfold.

B. Mutation rates of genes resulting in only minor decreases in viability. One possible way to circumvent the problem limiting studies on mutation rate per genome to lethals and near lethals is to study rates per genome for genes with only minor viability effects. Estimated mutation rates by one such method are 10 to 20 times greater than mutation rates per genome to lethals.[17,18] Based on these data it is possible to argue that the number of genes in *Drosophila melanogaster* is about 5 to 10,000 (10 to 20 times 500). There is no reason to believe minor deleterious mutations and lethals involve different populations of genes. They may involve alterations in the same genes with less drastic effects. There is good evidence that at some loci some alleles have minor effects while others are lethal. At the same time there is no *a priori* reason to expect that all loci which give rise to minor deleterious mutations must also be capable of mutating to lethality. Still, one is not certain that the induction of mutational changes with minor deleterious effects will identify the majority of loci present. Many mutant stocks, e.g., ones involved in eye color mutants, or involving null mutants for particular enzymes (e.g., alcohol dehydrogenase), show no demonstrable reduction of viability to the casual observer. One wonders if there is a significant number of genes which if mutated have no effect on any detectable phenotypic parameter. While there is no guarantee that the approach utilizing minor deleterious mutations will involve all loci, it is clear that it identifies substantially more loci than tests dependent only on lethals. The problem of detection of mutations will be discussed again in the next section.

3. Relationship between complementation groups and bands (chromomeres) (Table 1).

If we accept, tentatively, that a complementation group in a higher organism involves a structural gene, then the geographic distribution and spacing of complementation groups should provide an estimate of the total number of genes. There is a growing body of evidence (recently reviewed)[19] indicating a 1:1 relationship between polytene chromosome bands (chromomeres) and genes. Of particular importance is a recent paper by Judd, Shen, and Kaufman (1972) studying visible, lethal, and semilethal mutations. These workers have demonstrated that for a region of the X chromosome mainly between *zeste* and *white* there are 16 complementation groups and 15 or 16 bands. By cytological studies using a series of deletions Judd and his co-workers were able to assign many of the complementation groups to specific bands or interbands. Thus, for this study there appears to be a 1:1 relationship between bands (chromomeres) and genes. Similar, but less detailed, work by Lifschytz and Falk, in another region of the X chromosome, shows an approximate 1:1 relationship between complementation groups and bands.[20,21] Also, the study by Hochman[22] on the fourth chromosome shows an approximate equality between bands and complementation groups since there are about 40

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complementation groups and approximately 50 bands.[23-25] There are about 5,000 bands in *Drosophila melanogaster*[23-25] and therefore about 5,000 structural genes by this estimate.

Lefevre and Green[26] however, have apparently demonstrated at least one band which does not have any assigned phenotypic effect. The white locus resides at band 3C2 on the X chromosome. The simultaneous deletion of 3C2 and 3C3 results in no additional detectable phenotypic change and therefore suggests that 3C3 involves a nonessential function which has yet to be identified. This reemphasizes the fact that there are genetic functions which cannot be detected by classical approaches in *Drosophila*. Cistrons without readily identifiable phenotypic effects (we shall call them "neutral" cistrons) may reside at loci (bands, chromomeres) associated with cistrons which have demonstrable phenotypic effects. Hence, there may be several cistrons per band, most of which are "neutral." If, for example, the mutants identified by Mukai and his co-workers[17,18] causing minor deleterious effects involve a separate population of genes from the lethal ones identified by the classical approaches of Judd et al. (1972), Lifschytz and Falk,[20,21] and Hochman,[22] then one could estimate that there are 50- to 100,000 structural genes in *Drosophila melanogaster* (5,000 lethal genes (one per band) x 10 to 20 (difference in spontaneous mutation rates) = 50- to 100,000). If the above calculation were true, it would imply that the average mutation rate is an order of magnitude lower than estimated.[15,16] [It is interesting to note, however, that the mutation rate per gene per generation calculated from Mukai's data[18] assuming 5,000 structural genes is 6×10^{-5} (0.3 mutations per genome per generation/5,000) which is in good agreement with the estimate of 3×10^{-5} per gene per generation previously noted.][15,16] Studies on loci coding for specific enzymes suggest that many of these loci are expendable. Of approximately 19 loci which have been demonstrated to contain the structural genes for different enzymes by the use of electrophoretic variants probably only one is known to be associated with a lethal effect under standard culture conditions (Figure 2), although the structural gene for xanthine dehydrogenase is associated with an eye color alteration. Some of these mutants, under alternative culture conditions, are lethal. For example, alcohol dehydrogenase null mutants are more sensitive to alcohol than wild type flies. Presumably such mutants would be detectable by approaches which identify minor deleterious mutants. However, it is still difficult to eliminate the possibility that neutral cistrons are present in substantial number in each chromomere.

Despite these types of objections, which may or may not be valid, the remarkable characteristic of the work of Judd and his co-workers (1972) is that there is no case of one chromomere containing two lethal complementation groups. The possibility that this would occur by accident is extremely low. Furthermore, in a subsequent paper from Judd's laboratory (Shannon et al., 1972) it has been demonstrated that the mutants located within a given complementation group, and hence a given chromomere, have similar patterns of lethality and morphogenetic characteristics. The similar developmental characteristics of the different alleles in a given band lead one to conclude that they all result from a defect in a common function. These observations strongly indicate that there is a specific function associated with each band, and throughout the entire set of chromosomes there is a 1:1 relationship between functions and bands (chromomeres, loci). That is not to suggest that all structural genes are associated with essential functions. Indeed, for the approximate region studied by Judd and his co-workers (1972) there is one band which contains a neutral cistron (3C3)[26] which suggests that about 1 in 10 loci may involve neutral cistrons. Furthermore, Hochman's data,[22] interpreted liberally, suggest that 1 in 5 loci contain neutral cistrons. Thus, about 500 to 1,000 loci might not be identified by classical genetic approaches. This is more than adequate to account for the number of loci with known products but no lethal effects.

4. Map distance per genome divided by map distance per gene (Table 1).

Yet another method of determining the number of genes is available. Namely, if the average intragenic recombination distance per gene were divided into the recombination distance of the total map length, then an estimate is obtained of the number of genes which can be packed into the available space. Such estimates are limited by the number of loci which have been subjected to fine structure mapping (about 16) and by other complications. The approach has a major advantage over estimates which depend on the detection of

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TABLE 2

Intragenic Map Distance in Single Cistrons

Locus	Intragenic Map Distance	Number of Genes in Genome*
brown	0.001 map units	287,000
deep orange	0.006 map units	47,800
garnet	0.005 map units	57,400
maroon-like	0.0014 map units	205,000
rosy	0.009 map units	31,900
vermilion	0.007 map units	41,000
		Average 111,700**

*Obtained by dividing 287 m.u. by intragenic recombination distance.
**Obtained by summing gene numbers for the six loci and taking the mean. An alternative approach dividing 287 m.u. by the mean of the intragenic map distances for the six loci gives a different estimate of the number of genes: 58,600. This value is also in reasonable agreement with the number of genes estimated from DNA content.

mutants (e.g., mutation rates per genome, or genes per chromomere) because it is potentially independent of the nature of the mutant gene, and dependent only on the map distance within the genetic unit. Intragenic recombination probably underestimates gene size because it is unlikely that mutant sites reside at the ultimate extremes of the genetic unit and thus the number of genes might be overestimated. Also, fine structure mapping is subject to huge statistical errors because of the small numbers of recombinants produced.[27] Further, there is no constant relationship between map distance and physical distance throughout the *Drosophila* genome.[28] Finally, the method is valid only if one is dealing with recombination within cistrons and not with a supragenic unit (e.g., structural gene plus *cis*-dominant regulatory elements). But it does appear possible, in some cases, to identify single cistrons (see below). The results summarized in Table 2 are drawn from data on genes which we consider to be single cistrons (*brown*,[29] *garnet*,[30] *rosy*,[31] *vermilion*,[32] *deep-orange*,[33] *maroon-like*[34]). The average estimate is 112,000 genes; i.e., the genome is large enough to hold 112,000 genes of this size. Of immediate interest is the similarity between this estimate and the estimate based on the total content of DNA in the genome of *D. melanogaster*. This correspondence, in itself, indicates that this approach is valid because if we are indeed dealing with single cistrons and fine structure mapping is reasonably representative of large-scale mapping, then we expect the two results to be similar.

In summary, different approaches give strikingly different estimates of gene number. It is clear that the genome, based on estimates utilizing the amount of DNA present or the recombinational length of the average cistron, is of sufficient size to contain about 100,000 cistrons. This places an approximate upper limit on the possible number of structural genes present. The other two approaches, based on mutation rates and the number of complementation groups per band, estimate that there are about 5- to 10,000 structural genes in the genome of *D. melanogaster*. These estimates place a lower limit on the number of genes present. The real number must lie somewhere in between. It is critically important, however, to determine which of the above estimates are closest to being correct. In the following section we present a further analysis of the proportion of DNA in *D. melanogaster* which is devoted to structural genes.

THE COMPOSITION OF CHROMOMERES IN *DROSOPHILA*

As discussed above, there is evidence from the work of Judd and his co-workers (1972) that each chromomere represents a functional unit. It is not clear, however, whether there is a single structural gene within each chromomere. Judd and his co-workers call attention to the fact that although they identify only one complementation group per chromomere that there is sufficient DNA, on the average, to code for 20 polypeptides (i.e., for 20 cistrons). Thus, on the surface, there appears to be

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a discrepancy between the amount of information packaged in a chromomere and the informational capacity of the chromomere. The question which immediately arises is whether the complementation group represents a single cistron or a supragenic element containing a cistron associated with one or more *cis*-dominant regulatory elements. If the latter were true, then it is clear that each chromomere does contain more than one gene (using the general definition from above). Indeed, Judd and his associates favor the interpretation that they have induced mutants in more than one genetic element.

In this section we will discuss the number of genetic elements which reside in a given chromomere. The problem of the number of genetic elements at a given locus (band, chromomere) has been mainly approached by investigators utilizing fine structure mapping and complementation studies. As indicated above, about 16 loci have been investigated. In general, the loci have been divided into two classes, simple loci and complex loci. The simple loci presumably involve single cistrons. The nature of the complex loci is open to discussion. It has been suggested that some complex loci contain multiple cistrons and others single cistrons. We feel that it is possible, by applying four tests to a given locus, to determine whether the locus (chromomere, band) involves single or multiple cistrons. Two of the tests which we will apply have been routinely used in the past by *Drosophila* workers. Two of them are comparatively new, at least in specific manipulations. The tests are as follows:

1. The distribution of mutant sites (M. Green, personal communication).

If the distribution of sites is continuous for a given region, then either the genes themselves are continuous or only a single gene is involved. A discontinuous distribution involving clustering of sites would suggest the existence of spacer DNA between cistrons or the presence of neutral genes. It is assumed that clustering of mutational sites does not result from differential sensitivity of the mutagen, although this possibility cannot be ruled out.

2. Characteristics of complementation.

Complementation between two mutant sites, if it exists, is either partial or complete. In the case of two cistrons at a locus one would expect to find complete complementation between two sets of mutant sites in an appropriate heterozygous double mutant because heterozygosity will exist for both genes. However, in situations where the amount of enzyme protein present is rate limiting, complementation between mutant sites in different cistrons within the same locus may be incomplete and result in a mutant phenotype. Complementation between alleles within the same cistron can also occur because a multimeric enzyme composed of peptides altered at different sites may be active. In general, in cases of complementation involving alleles within a cistron, via an active multimer, one expects that the complementation will be incomplete,[33] and result in a phenotype intermediate between wild type and the mutant one. It is possible, however, that the external phenotype of a fly will be wild type in appearance despite reduction in activity of a particular enzyme. Hence, the presence or absence of complementation does not provide a fool-proof method for determining whether mutant sites reside within one or more cistrons.

3. The ratio of classical recombinants to gene convertants.

One of the characteristics of fine structure mapping is the increased frequency of recovery of wild type progeny (gene convertants) which arise without reciprocal exchange of outside markers, but at frequencies substantially higher than can be accounted for by back mutation or double recombination. The study of gene conversion in *Drosophila* is difficult and only limited work has been done.[35] Gene conversion has been investigated in detail in yeast[36] and certain fundamental characteristics of this phenomenon have been established which appear to be applicable to other organisms. A model demonstrating the major results of gene conversion is presented in Figure 1 and follows the model presented by Sobell.[37] It is not our purpose here to discuss the validity of this model but to simply look at some of its consequences. The following characteristics are important for the present disussion: 1. Conversion is associated with the exchange of DNA and the formation of heteroduplex DNA. 2. When the heteroduplex contains one of the two mutant sites being studied the *possibility* exists that the site will be converted by a repair process to become identical in information content to its opposite homologous DNA. Thus, a mutant site could

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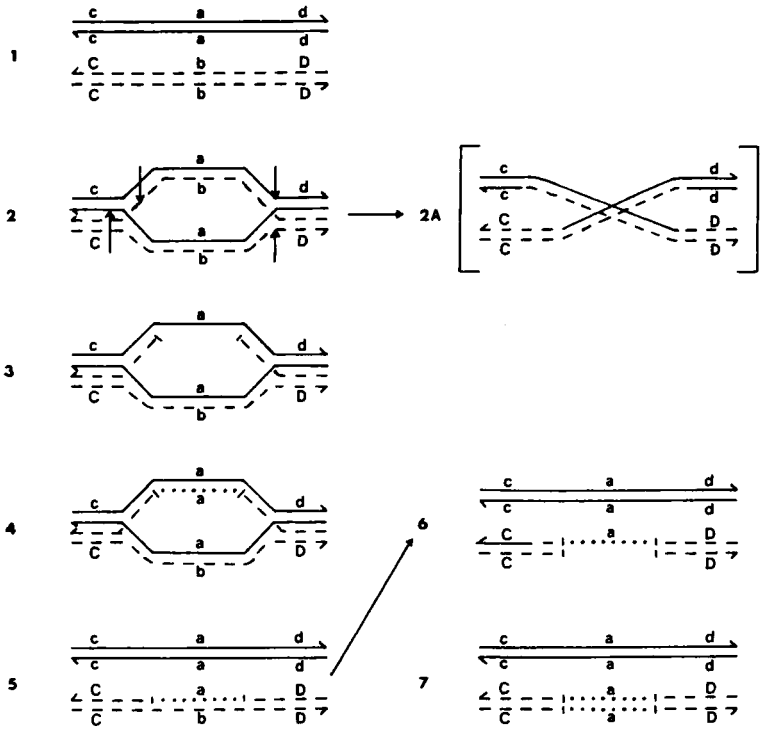

FIGURE 1. A model for gene conversion. 1) Two nonsister chromatids are shown (the other two chromatids in the tetrad are not pictured and are not involved in exchange. C,c and D,d represent outside markers; a and b represent different sites within a region which will be involved in the formation of a heteroduplex. 2) Heteroduplex regions are formed between antiparallel strands of the DNA. The arrows identify locations where nicks could occur resulting in the structure depicted in 2a. The configuration in 2a, when repaired, results in reciprocal exchange of outside markers. It is this type of event which is involved in classical recombination between the C,c sites and the D,d sites. An alternate sequence of events would result in gene conversion. 3) One strand is nicked by an appropriate "nickase" and then partially destroyed by an exonuclease. 4) The gap is repaired using the "a" strand as a template. The newly synthesized DNA is designated by (. . . . .). 5) The heteroduplexes separate and the original double helices reform. Note, however, that the (- - - -) helix contains regions of noncomplementing base pairs. 6) The (- - - - -) b strand is nicked and bases are removed by exonuclease activity. 7) The gap is repaired using the (. . . . .) "a" strand as the template. Note that the end result is the conversion of site "b" into site "a" without exchange of outside markers. Such conversion requires that the appropriate mutant site be included in the region forming the heteroduplex. This model is a static one and does not detail the possible mechanism for the initial formation of the heteroduplex. For a detailed exposition see Sobell.[37]

become wild type, or a wild type site mutant. The latter change would not be detected in *Drosophila* with conventional methods. 3. The frequency of conversion involving one of the studied markers increases as the map distance between the two markers decreases because the likelihood of the mutant sites being included in heteroduplex during exchange increases. 4. When sites are sufficiently close together the possibility exists that both sites will be included in the same heteroduplex and thus that both sites will be simultaneously converted ("co-converted"). If co-conversion occurs, the possibility of classical recombination is eliminated. 5. Co-conversion is particularly important in yeast when the sites are under 1,000 base pairs apart (i.e., in a single cistron, or in contiguous cistrons).[38] This information suggests that the size of the heteroduplex formed to initiate recombination

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TABLE 3

DNA Content of Different Loci in *Drosophila melanogaster*

Locus	Reference	Intralocus Map Distance	Number of Base Pairs	Number of Cistrons*
1. *brown*	29	0.001 m.u.	340	1
2. *deep orange*	33	0.006 m.u.	2,000	2
3. *garnet*	30	0.005 m.u.	1,700	2
4. *maroon-like*	34	0.0014 m.u.	480	1
5. *rosy*	31	0.009 m.u.	3,100	3
6. *vermilion*	32	0.007 m.u.	2,400	2–3
7. *bithorax*	43	0.03 m.u.	10,200	10
8. *dumpy*	27	0.11 m.u.	37,400	37
9. *forked*	45	0.012 m.u.	4,100	4
10. *lozenge*	44	0.14 m.u.	47,600	48
11. *miniature*	41	0.02 m.u.	6,800	7
12. *Notch*	42	0.14 m.u.	47,600	48
13. *rudimentary*	46	0.07 m.u.	23,800	24
14. *singed*	47	0.03 m.u.	10,200	10
15. *Star-asteroid*	2	0.012 m.u.	4,100	4
16. *white*	48	0.026 m.u.	8,800	9

The average number of base pairs is 1,670 for the first six loci, 20,600 for the last ten.

*Assuming 1,000 base pairs per cistron, and that all of the DNA is included in structural genes.

is on the order of magnitude of a 1,000 base pairs (i.e., about one cistron in length). The implication is that the frequency of conversion starts to approach the frequency of classical recombination when the mutant sites are about 1,000 base pairs apart, and should be higher when the sites are within a 1,000 base pairs of each other; i.e., one cistron. Thus, excess recovery of gene convertants relative to classical recombinants is a good indication that the mutant sites reside in the same cistron.

4. The molecular size of the intragenic recombination unit.

The genetic map of *Drosophila melanogaster* contains about 287 map units (m.u.)[39] and the haploid genome 1.3 x 10^8 nucleotide pairs.[5] About 25% of the DNA is located in the centromeric heterochromatin[7,40] and is essentially recombinationally inert as demonstrated by the map distance across centromeres.[39] Hence, only about 0.975 x 10^8 nucleotide pairs are involved in the portion of DNA which readily recombines. Using this figure, one nucleotide pair on the average equals 2.94 x 10^{-6} m.u. (287 m.u./0.975 x 10^8 base pairs) and an average cistron equals 2.94 x 10^{-3} m.u. An average band (chromomere, locus) equals 5.7 x 10^{-2} m.u. (287 m.u./5,000 bands), about 20 cistrons. It should be noted that this type of analysis, of course, is only possible in regions of the genome where recombination occurs. In Table 3 the map distances for loci subjected to fine structure mapping are presented. As can be seen the map sizes generally fall into two distinct groups in terms of the numbers of base pairs associated with a given locus. Generally, but not always, loci which have been characterized as being "complex" are an order of magnitude larger than those which have been considered to be simple. *Notch*, *bithorax*, *dumpy*, *lozenge*, *rudimentary*, *singed*, and *white* are all substantially larger than the "simple" loci *brown*, *vermilion*, and *rosy*. It should be kept in mind that the distinction between "complex" and "simple" loci has previously depended, in some instances, only on the presence of intralocus complementation.[34] Because of the existence of intragenic complementation such a distinction is not in itself valid for determining whether one or several cistrons are involved in the genetic function of a given locus. For example, both *deep-orange* and *maroon-like* alleles show partial complementation,[33,34] and

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hence are "complex", but based on map distance they both involve single cistrons as has previously been suggested.[33,34] If map distance is an accurate estimate of DNA content, it is clear that many "complex" loci are multicistronic. However, in order to accept this conclusion it is necessary to have independent estimates demonstrating that the number of base pairs involved in a cistron at a given locus can be accurately estimated from map distance. In this connection a previous study by Rudkin[49] estimates that 0.9% of the DNA of the polytene X chromosome (containing no heterochromatin)[89] is in the *zeste/white* interval. The map length of the *zeste/white* interval is 0.5 m.u. and the X chromosome contains about 70 m.u.[39] Thus, the region contains about 0.7% of the total map length and there is an approximate relationship between DNA content (0.9%) and map distance (0.7%). Lefevre[50] has demonstrated a positive correlation between band size and map distance. But, neither study demonstrates that such relationships hold at the fine structural level of mapping. In order to correlate fine structure map distance with DNA content it is necessary to have an estimate of the amount of DNA involved in the cistron itself. Such an estimate can be obtained if one knows the molecular weight of the polypeptide being coded for by a particular gene. Of the genes listed in Table 3, only four are associated with particular enzymic activities. In the case of two of the genes, *vermilion (v)* and *rosy (ry)* the molecular weights of the native proteins are known. Presumably *v* is the structural gene for tryptophan pyrrolase (see Figure 2), although no electrophoretic variants of the enzyme mapping at the *v* locus are known. The molecular weight of native tryptophan pyrrolase has been estimated at 150,000 D.[51] If it is a monomer, dimer, or tetramer, the polypeptides should contain, respectively, about 1,500, 750, or 375 amino acids. The maximum map distance at the *v* locus is 0.007 m.u. which corresponds to about 2,400 base pairs (0.007 m.u. divided by 2.94×10^{-6} m.u. per nucleotide pair) which would code for a polypeptide about 800 amino acids long. Thus, the estimated size of the polypeptide based on mapping data is very similar to the size estimated from molecular weight (and is particularly good if the enzyme is a dimer). Because of the absence of electrophoretic variants it has not been unequivocally established that *v* is the structural gene for tryptophan pyrrolase. However, electrophoretic variants of xanthine dehydrogenase (XDH) have been recovered and *ry* has been demonstrated to be the structural gene for the enzyme.[52] The molecular weight of the enzyme has been estimated to be 250,000 D.[53] Based on the presence of 3 XDH's with different electrophoretic mobilities in heterozygotes involving electrophoretic variants the protein must be at least a dimer. Although XDH may contain a cofactor specified by the *maroon-like* locus, the molecular weight of the subunits, assuming a dimer, should be about 125,000 D and contain about 1,250 amino acids. The maximum map distance of the *ry* cistron is 0.009 m.u. which corresponds to 3,100 base pairs and a polypeptide containing about 1,000 amino acids. In both of the above cases the size of the polypeptide estimated from map distance is in good agreement with the size estimated from the molecular weight of the native protein. One should keep in mind that both the estimated fine structure map distances and the molecular weight of the proteins are only approximations. However, the results from these preliminary tests, coupled with the results discussed above, indicate that map distance gives an approximate estimate of the number of base pairs involved in a structural gene and thus affords a technique for determining if multiple cistrons (i.e., 5 to 10 or more) are located in a given genetic locus. But it cannot easily distinguish between one cistron and two cistrons unless the cistrons are distinctly separated by spacer or neutral DNA.

The four tests outlined above have been applied to the 16 loci which have been studied by fine structure mapping. The summary of the results is presented in Table 4. The results of the four tests are highly consistent. Loci which are identified as involving multiple cistrons or single cistrons by one test are usually similarly identified by the other tests. It is worth noting that the test which is apparently most difficult to apply, as judged by consistency with the other tests, is the one involving complementation. Considering the complexity of regulation in eukaryotes this is not surprising. The conclusions about the nature of the loci are generally consistent with those drawn by the original investigators. For example, it has been previously suggested that *bithorax*[43] and *dumpy*[54] involve multiple cistrons. In contrast, *rosy*, *maroon-like*, and *deep-orange* have been proposed to involve only single cistrons. In the

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TABLE 4

Uni- or Multicistronic Categorization of *Loci*

Locus	Distribution of Alleles	Method of Characterization: Complementation	Method of Characterization: Recombinants vs. Convertants	Method of Characterization: Intralocus Map Distance
1. *brown*	U	U	U	U
2. *deep orange*	U	U	U	U
3. *garnet*	U	U	U	U
4. *maroon-like*	U	U	U	U
5. *rosy*	U	U	U	U
6. *vermilion*	U	U	U	U
7. *bithorax*	M	M	M	M
8. *dumpy*	M	M	M	M
9. *forked*	M	U	M	M/U
10. *lozenge*	M	M/U	M	M
11. *miniature*	M/U	M/U	M	M
12. *Notch*	M	M/U	M	M
13. *rudimentary*	M	M	M	M
14. *singed*	M	M/U	M	M
15. *Star-asteroid*	M	M	M	M/U
16. *white*	M	M/U	M	M

M indicates multicistronic, U indicates unicistronic, M/U ambiguous.

cases where single cistrons are responsible for the major phenotypic effect associated with a given locus it is likely that only a small part of the chromomere is involved directly with the mutant effect. For example, *v* is associated with bands 10A1,2, both of which contain average or greater amounts of DNA based on their staining intensity. Since the *v* gene involves an estimated 3,000 base pairs and the average band contains 20,000 base pairs, it follows that most of the DNA in this band is not delineated by mutational change resulting in the *v* phenotype. This is not to say that the remainder of the DNA in the chromomere has no function. Deficiencies involving the *v* locus, but not the *v* structural genes, might eliminate the function of the *v* locus. Apparently point mutations do not readily do so. If a *cis*-dominant regulatory element identifiable by point mutations is involved, it must be contiguous with the *v* cistron and not separated by any spacer DNA. Parallel arguments are applicable to other simple loci. Presumably the complementation groups defined by Judd and his co-workers (1972) also involve simple loci. Therefore, most of the DNA in the locus is not defined by the mutational tests conducted to date.

Many of the results of our analyses are consistent with conclusions drawn by workers actively involved in fine structure mapping. An important exception, which is particularly illuminating, involves *rudimentary* (*r*). Carlson (1971) has concluded that *r* must be a single cistron, while the results of our analyses indicate that multiple cistrons are involved. The basis of Carlson's conclusion rests heavily on the characteristics of complementation. Carlson has found a series of alleles which do not complement with any other alleles at the *r* locus. It is clear from mapping data that these alleles behave as point mutations. While most of the sites are located at one end of the locus, two are located toward the opposite end. Therefore, by analogy to prokaryotes, all of the sites cannot be polar mutants or involve *cis*-dominant regulatory genes. Carlson concludes that the existence of such sites is only consistent with a single cistron. Based on information from prokaryotes this is indeed a highly reasonable conclusion. But, since *r* apparently contains enough DNA for several cistrons, other possibilities should be considered. It is possible to imagine models of genetic organization involving multicistrons with which Carlson's data are consistent. For example, if transcription of a locus depends on unfolding of chromomeric DNA, it is possible that such unfolding requires action on both sides of the chromomere simultaneously. Thus, there might be two

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sites on either side of the locus which must be functional if the region is to be transcribed. Additionally, one might visualize that the polypeptides produced are involved in a large aggregate which is assembled in a step-wise fashion. One type of mutant polypeptide might preferentially bind to aggregates which contain only wild type polypeptides and exclude its homologous wild type polypeptide. Thus, mutations of this type might not complement with mutations located in separate cistrons because no active aggregate would be produced. In view of the fact that models can be constructed involving noncomplementing mutants located at different ends of a multicistronic array we prefer at this time to view *r* as a multicistronic locus.

The *r* locus is interesting for additional reasons. Recently, it has been demonstrated that *r* larvae require pyrimidines to develop and that the capacity to synthesize pyrimidines by *r* mutants has apparently been lost.[55,56] Since carbamyl aspartate[55] is capable of supplementing *r* homozygotes it appears that *r* mutants are defective in the capacity to synthesize carbamyl phosphate and/or carbamyl aspartate, and thus may be deficient in carbamyl phosphate synthetase and/or aspartyl transcarbamylase. In higher organisms these reactions are carried out by a single enzyme complex which has a molecular weight of about 650,000 D in *Neurospora,*[57] 800,000 D in yeast,[58] and 600,000 to 900,000 D in mammals.[59-61] Furthermore, in yeast and *Neurospora* the structural genes for both enzymes reside at a single multicistronic locus.[58,62] In mammalian systems the synthesis of the two enzymes has been found to be coordinated.[63] In all likelihood, aspartyl transcarbamylase and carbamyl phosphate synthetase will also be associated in a single enzyme complex in *Drosophila.* It is interesting that the maximal map distance in the *r* locus is 0.07 m.u. which corresponds to 23,800 nucleotide pairs and enough information to code for polypeptides with 8,000 amino acids and a molecular weight of about 800,000 D. Therefore, it is reasonable to speculate that the *r* locus contains the information necessary for the synthesis of all of the polypeptides involved in this enzyme aggregate. In this context, the proposal that some noncomplementing *r* mutants affect assembly of an aggregate is not an unreasonable one although, of course, speculative.

The average band contains about 20,000 nucleotide pairs, but the range is from 5,000 to at least 50,000.[49] Complex loci involve numbers of base pairs which could potentially be included in single bands on the basis of these estimates. Thus, it is reasonable to conclude that some chromomeres do in fact contain multiple cistrons which code for different, but functionally related, polypeptides. There is also evidence that some chromomeres contain multiple copies of the same gene. Thus, Wimber and Steffenson[64] have located all of the genes for the synthesis of 5s rRNA in *Drosophila* in region 56 EF of the second chromosome. Since the entire region contains about 20 bands and there are approximately 100 copies of the 5s genes in *Drosophila,*[4] it follows that multiple copies of the gene are present in one or more bands. A similar conclusion also results from a consideration of results obtained by hybridizing histone mRNA in situ to *Drosophila* DNA. Hybrids are formed in a limited region involving bands 39E to 40A of chromosome 2.[65] While the degree of redundancy for histone mRNAs in *Drosophila* has not yet been reported, if one assumes that the percentage of histone sequences involved in *Drosophila* is similar to that of sea urchins, one would expect at least 200 copies of histone genes in the *Drosophila* haploid genome. Region 39E to 40A contains about 16 bands, insufficient to code for the number of cistrons involved using a one cistron: one band relationship. Indeed, because most histones have molecular weights in the range of 10,000 and thus contain about 100 amino acids, the possibility that most of the histone genes could be contained in a single band cannot be discounted. In any case, multiple copies of genes coding for proteins can be included in one chromomere.

In summary, each band (chromomere, locus) contains one or more structural genes. The structural genes can be identical as in the case of the 5s RNA genes, or apparently different, as we presume for the *rudimentary* locus. The molecular sizes of structural genes, as estimated by intragenic recombination, are consistent with the production of polypeptides 300 to 1,000 amino acids long. The evidence at hand indicates that most loci contain single cistrons, although the possibility that neutral genes are also present cannot be discounted. Assuming a single structural gene is present per chromomere only about 3% of the DNA is used to code for structural information in a simple locus. Although some chromomeres apparently contain

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multiple cistrons the number of such chromomeres appears limited. Hochman[22] found only 1 complex locus out of about 40 loci on the fourth chromosome. Also, with the exception of the *white* locus, there is no evidence of the existence of complex loci in the *zeste/white* region studied by Judd and his co-workers. Hence, it appears that only about 5% of the DNA in the *Drosophila* genome codes for structural genes.

For unicistronic loci there is no strong evidence that any of the mutant sites recovered involves regulatory functions. Since a site which binds a regulatory protein need only be composed of 14 nucleotide pairs in order to be potentially unique in the *Drosophila* genome ($4^{14} = 2.69 \times 10^8$ combinations of base pairs which is more than the *Drosophila* genome contains), it is possible that such regulatory sites will be hard to identify using current techniques. If the structural gene contains 1,000 base pairs and the regulatory site only 10, assuming equal mutagenicity, 100 mutations in the structural gene would be recovered for every one in the regulatory gene. Alternatively, if the regulatory sites within a chromomere are repetitious, and site mutations in one gene do not impair the overall function of the regulatory sites, it may be effectively impossible to recover such regulatory mutants. This is not to say that the fine structure mapping data for simple loci eliminate the possibility that *cis*-dominant regulatory sites have been recovered. We have noted above, and wish to emphasize again here, that the fine stuctural data, for a variety of reasons, do not exclude the possibility that the mutant sites recovered at a given simple locus reside in contiguous genes. This possibility can only be eliminated when fine structure mapping is accompanied by amino acid sequencing of known protein products. With regard to complex, multicistronic loci, there is some possibility that regulatory mutants have been found. Most of the noncomplementing alleles at the *r* locus are located to one side and may be regulatory in nature. Of course, these could also be polar mutations, and hence not regulatory. In addition, *Contrabithorax,* in the *bithorax* locus, has been interpreted by Lewis[43] as a *cis*-dominant regulatory mutant equivalent to the operator-constitutive mutants found in prokaryotes.

Despite the fact that some chromomeres contain multiple cistrons each chromomere is involved with a common function. Hence, there are essentially 5,000 functions encoded in the *Drosophila* genome. If, as we will discuss below, the chromomere is not only the unit of function but also the unit of regulation (at least at the genetic level), then the question about the number of genes is a trivial one insofar as development is concerned. What is of prime importance is the number of regulatory elements. It is particularly important to know whether individual cistrons within a chromomere can be individually transcribed and hence individually regulated or whether all must be transcribed together. This subject, and others, will be discussed in the following section.

MOLECULAR BIOLOGY OF CHROMATIN

The results of biochemical studies on the transcription of RNA together with the genetic evidence discussed in the previous section give some insights into the size and structure of the transcriptional unit in *Drosophila.* In eukaryotes, in general, the end product of transcription falls into four classes: rRNA, tRNA, mRNA, and nRNA (heterogeneous nuclear RNA). It is well established that rRNA is transcribed as a high molecular weight precursor which is cleaved in a nonconservative manner to yield the 28 and 18S rRNAs. (The tRNAs probably have a larger precursor as well).[66] Transcription of mRNA has proved difficult to study but evidence is now accumulating which indicates nRNA is messenger precursor.[67-72] The unit of transcription is very large, 20 to 100S or about 10^6 to 2.5×10^7 D and about 3×10^3 to 8×10^4 bases long. The size of the primary RNA transcript correlates well with the range of DNA content in the chromomeres (the average being about 20- to 30,000 base pairs with a range from 5,000 to at least 50,000).[40] Even more important, for the one Balbiani ring in *Chironomus tentans* which has been examined, the size of the RNA transcript indicates that an entire band is transcribed as a single unit.[73] Whether this large transcriptional unit is necessary for controlling transcription or a secondary effect of some other aspect of chromosome function is unknown.

Since the size of cytoplasmic mRNA is generally consistent with a monocistronic messenger[72, 74-76] the RNA transcript must contain sequences which are removed during processing. In *Drosophila* about 20 to 30% of the genome is transcribed at each developmental stage[77] or in a particular tissue.[78] Yet, we estimate above that

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only about 5% of the DNA codes for structural genes. If true, then it is clear that much of the primary RNA transcript is never translated but lost during the processing of nRNA to form mRNA. Even RNA synthesized from multicistronic chromomeres is reduced to monocistronic mRNA, at least in the case of chromomeres containing repeated homogeneous sequences. As we have noted before, multiple copies of histone genes must be contained within a single chromomere, and yet the polysomal histone mRNA is only about 300 nucleotides long.[1,79] Whether the production of unicistronic histone mRNA results from processing of a high molecular weight precursor or direct transcription of low molecular weight material is apparently not known. Histone mRNA is peculiar in that it is the only known mRNA which lacks poly-A,[67,79] probably reflecting a different type of processing or absence of processing[67] and suggesting that histone mRNAs are atypical.

From a variety of information some ideas about the nature of the DNA being transcribed can be formulated. In recent years development of techniques for DNA-DNA and RNA-DNA hybridization[80] have led to resolution of DNA into classes based on redundancy of sequence: 1) highly reiterated simple sequences;[81,82] 2) moderately reiterated sequences composed of either homogeneous sequences of "families" of heterogeneous but similar sequences; 3) unique sequences. The highly repetitious sequences are found almost exclusively in constitutive heterochromatin and are probably not transcribed.[83-85] The fraction of chromatin believed to be active in transcription is made up of unique sequences interspersed with moderately reiterated sequences.[77,86-88]

One possible regulatory device to facilitate synthesis of large amounts of particular proteins in somatic cells would be to increase the number of structural genes for these proteins in appropriate tissues, i.e., to amplify appropriate cistrons. There is no evidence of amplification of structural genes for proteins in somatic tissues. Cytological studies on giant polytene chromosomes show no specific amplification of genetic material in specialized tissues.[19] In the few cases where it has been possible to look for amplification of sequences of specific structural proteins produced in large amounts in specialized cells, no amplification has been found.[89-91] Sequences for several proteins made in large quantities are present in fewer than ten copies per genome and perhaps only one.[89-94] Kafatos[95,96] argues that gene amplification is unnecessary to produce a particular protein in amounts up to 50% of the total cell protein. The only documented case of gene amplification is rRNA specific DNA in amphibian oocytes. Hence, some unique sequences must be structural genes coding for mRNAs. About 80% of the DNA in *D. melanogaster* is unique,[5] yet we estimate that only about 5% of the DNA codes for structural genes. If this estimate is correct, then many of the unique sequences have another function.

Repetitious DNA is transcribed;[97-99] indeed most RNA-DNA hybridization data pertain only to the repetitious sequences.[100] The RNA transcript from a single Balbiani ring in *Chironomous* has been found to contain repeated sequences.[101,102] In *Drosophila melanogaster* much of the DNA in the chromomeres of polytene chromosomes is nonrepetitive.[88] It is estimated, however, that the repetitive sequences are distributed throughout the DNA with, on the average, a repetitive sequence about 100 to 150 nucleotide pairs long being associated with a unique sequence about 750 base pairs long.[88] In view of the size of the RNA transcript and the average distribution of repeated sequences it is not surprising that repeated DNA sequences are transcribed. Most of these transcribed sequences must be part of the precursor of mRNA which is degraded in the nucleus and not translated.[68,69,97,103,104] If only about 5% of *Drosophila* DNA codes for structural genes, information about the average distribution of the unique and repetitive sequences may not be meaningful in inferring the immediate environment of structural genes. The function of the repeated sequences remains to be established. There are chromosomal functions which might utilize some of the repeated sequences. The following possibilities come to mind:

1. Attachment sites to the nuclear membrane might require DNA of a specified length but nonspecific sequence which would not be transcribed.

2. Species of similar base sequence might serve as recognition sites for sister chromatids in meiotic pairing.

3. Spacer DNA might serve as sites where recombination would be possible without loss of function of structural genes.

4. Condensation of chromatin might utilize specific association sites for the folding of the

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chromosome in metaphase, heterochromatization, or localized inactivation in control of transcription. These association sites might involve an allosteric protein which recognizes similar sequences[105] or a "crystallization" of similar structures, the degree of chromatin packing being dependent upon the homogeneity of base sequence.[106]

Any of these proposals would account for the spacing of the redundant sequences. A recent suggestion[107] that families of repeated sequences are chromomere specific (i.e., each chromomere contains a group of similar sequences not found in any other chromomere) is consistent with any of the above possibilities.

The regulation of transcription is generally believed to be under the control of chromosomal proteins. Chromatin consists of a DNA molecule covered by a variety of proteins bound by different affinities.[108-115] Chromosomal proteins have been divided into two classes, histones and nonhistone, or acidic, proteins.

The histones found in all higher eukaryotic organisms fall into five major electrophoretic groups. They are characterized by their high content of basic amino acids and the fact that some of the amino acids are modified after protein synthesis.[112,116] The many studies seeking changes in histones under various conditions have found some differences,[117-119] but have not solved the problem of the histone function or the mechanism of action. The ubiquity of histones and their conservation during evolution strongly suggest they play some fundamental role in the structure or function of the chromosome. Their relative simplicity would seem to rule out the possibility of fine regulation of transcription.

The elevated template activity after the removal of histones and the inhibition of RNA synthesis on DNA or chromatin in vitro when histones are added led to the suggestion that histones are nonspecific repressors of transcription.[100,115] Although these in vitro results are well established the function of histones as repressors in vivo is still unresolved.[114,120-122] There is evidence that histones may be involved in maintaining chromosome structure[116,122-125] and in DNA replication.[126,127] Since diversity in the composition and behavior of the histones has been found[119, 121,123,128-131] it is possible that this arbitrary classification – histones – may include proteins with differing, but equally vital, function.

The isolation and characterization of the diverse class of nonhistone or acidic chromosomal proteins have been more difficult. The analogy with prokaryotic repressors and the lack of other candidates suggest the acidic proteins may be involved in the fine regulation of gene activity. Accumulating experimental evidence supports this conclusion. Acidic proteins have been implicated in specificity of transcription, at least in vitro,[100,115,120,132,133] the control of cell growth,[134,135] puffing in polytene chromosomes,[136] and hormone action.[137-139] Continued technical refinements should yield more insight into the properties and functions of nonhistone chromosomal proteins.

Not all chromatin is transcriptionally active at one time. Fractionation of chromatin has permitted the partial characterization of active (disperse) and inactive (condensed) components. Active and inactive chromatin have the same DNA/histone ratio[140-143] and proportion of repeated sequences.[140] The active fraction contains most of the RNA,[140,143] more nonhistone protein,[143] and is active in RNA synthesis. The DNA histone binding may differ because the thermal stability of the DNA is lower in the active fraction than in the inactive fraction.[143]

In special cases, areas active in transcription can be visualized. The polytene chromosomes of the Diptera offer one such opportunity in which puffs active in RNA synthesis are formed by the unfolding of the chromosomal material. The growing RNA chains become complexed with nonhistone proteins before leaving the chromosome.[144] Another example of apparent transcriptional activity is the "lampbrush" loops of the Y chromosome of *D. hydei* whose appearance during spermiogenesis is correlated with the function of male fertility factors.[145] "Lampbrush" chromosomes are also found in amphibian oocytes where RNA polymerase molecules and growing RNA chains complexed with proteins can be seen along the protein covered DNA loop.[146] Recently alkali-urea dispersion of polytene chromosomes of *D. melanogaster* has been found to induce a "lampbrush"-like organization into these chromosomes. The chromomeric DNA appears to protrude as loops from a compact central axis.[147-149] A core stabilized by proteins with the transcribable regions becoming distended loops during transcriptional activity has

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been suggested as a general model of chromosome organization.[125,150]

In summary, polytene chromosomes in Diptera provide cytological evidence of the structural organization of the genome, a linear sequence of DNA rich chromomeres. The puffs of chromomeres, where individual DNA-protein fibers are disperse, are areas of transcriptional activity. The size of the chromomere corresponds to the size of the RNA transcript in the one case where the product has been isolated. The range of DNA content of chromomeres is in agreement with the size of the messenger precursor RNA in other systems. A small portion of this large transcript contains the sequence to be translated. The remainder of the RNA must contain some repetitious sequences and, in *Drosophila* at least, apparently a large portion of nontranslated unique sequences. While several models have been proposed to explain the regulation of transcription and the nature of the RNA transcript,[71,151,152] a detailed discussion of this aspect of regulation is beyond the scope of this review. Our interpretation of the present evidence is that the chromomere is the unit of transcription. There is one transcript/chromomere and usually one, or in some cases several, structural genes/transcript. Thus, in *Drosophila melanogaster* the control of gene activity reduces to the control of about 5,000 transcriptional units.

THE ABSENCE OF SUPRACHROMOMERIC REGULATION

The evidence presented above indicates that the chromomere is the unit of transcription and regulation in eukaryotes. In *D. melanogaster* some chromomeres are multicistronic (about 2.5%), but most appear to be unicistronic. Both unicistronic and multicistronic chromomeres seem to be involved with single functions. One wonders if the regulatory strategy of the genome is limited to chromomeres, or if there are suprachromomeric regulatory entities. It is also interesting to ask: Is there any clear pattern in the distribution of specific chromomeres (functions) within the genome?

Insights into both of the above questions are provided by inspection of the genetic map of *Drosophila* with reference to loci specifying known biosynthetic activities or structural molecules (Figure 2). One characteristic which is clear is that the distribution of this group of genes is nonrandom. The loci are preferentially located near the centromeres and towards the telomeric ends of the chromosomes. It is not clear that such a nonrandom distribution is characteristic of all loci. Out of the 34 loci identified in Figure 2, 21 reside near the centromeres or telomeres and involve about 58 map units and 13 reside elsewhere and involve about 205 map units (not counting two telomeric ends not represented). The 21 loci residing near centromeres or telomeres involve about 1,750 bands (i.e., about 30 bands/m.u.) while the other 13 loci are associated with approximately 3,000 bands (again not counting the unrepresented telomeric regions) (about 15 bands/m.u.).[39] Assuming one genetic function per chromomere then there should be two loci recovered in the nonchromomeric and nontelomeric regions for every one locus recovered in the telomeric and centromeric regions. In fact, the opposite is found. For these loci there is about one locus in the nonchromomeric, nontelomeric regions for every two loci recovered in the chromomeric and telomeric regions. The Chi square value is about 6.7 which for one degree of freedom is significant at the 1% level. It appears that these genes are indeed concentrated in the centromeric and telomeric regions of the chromosomes.

Many, but not all, of the genes which map in these regions are clearly essential for the existence of the organism (rRNA cistrons, ribosomal protein cistrons, histone cistrons, 5S rRNA cistrons) or are involved in routine metabolism. These regions are characterized by comparatively low rates of recombination per unit physical distance (there are more bands per map unit than in the nonchromomeric, nontelomeric regions). Thus multicistronic and repetitious elements involved in the synthesis of rRNA, histones, and 5S rRNA would be protected against disruption by recombination. Furthermore, since there is evidence that recombinational events are mutagenic,[196,197] these essential genes, by virtue of the location in areas of low recombination, might also be protected against spontaneous mutation. Such protection indicates that these genes are conserved during evolution, an observation which is dramatically confirmed by the fact that histone mRNA from sea urchins hybridizes with a specific region of the *Drosophila* genome.[65] As such, one anticipates

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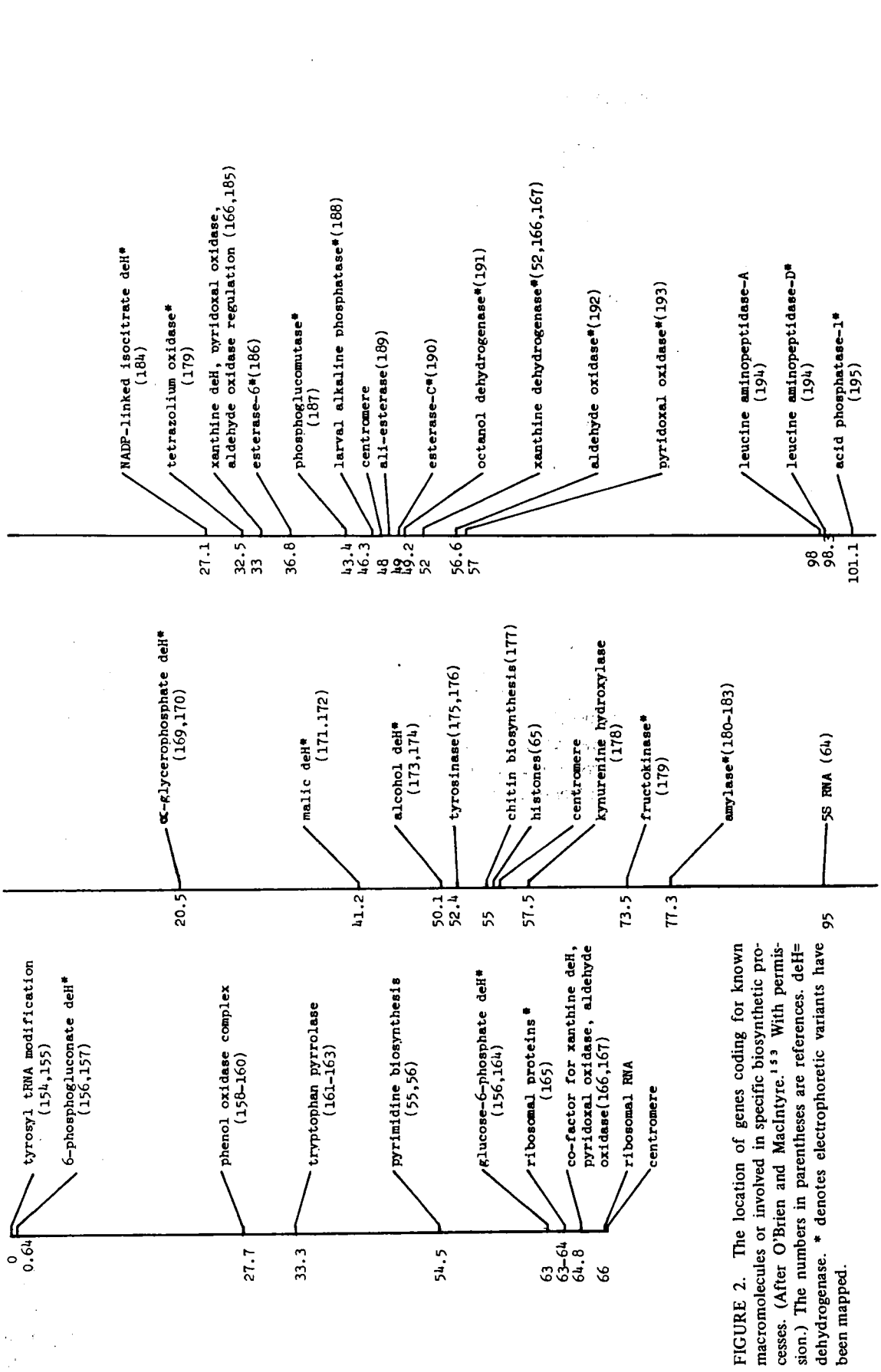

FIGURE 2. The location of genes coding for known macromolecules or involved in specific biosynthetic processes. (After O'Brien and MacIntyre.[193] With permission.) The numbers in parentheses are references. deH= dehydrogenase. * denotes electrophoretic variants have been mapped.

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that these genes are not involved in divergent evolutionary changes. One can argue that genes located in the conserved regions of the chromosomes are concerned primarily with the existence of the organism. Genes located in the noncentromeric, nontelomeric regions code for functions which result in divergent patterns of morphogenesis.

There is little support for the possible existence of suprachromomeric regulatory entities. Steffensen's observation[165] that seven electrophoretic variants of ribosomal proteins are associated with a single region of the genome (bands 19A-20A on the X chromosome) suggests that all core ribosomal proteins are encoded in this region. The total complement of 60 ribosomal proteins has a molecular weight of about 2×10^6 D,[165] corresponding to 20,000 amino acids. While the structural genes for these proteins might reside in several chromomeres, the 60,000 base pairs required to specify 20,000 amino acids could fit into a single large chromomere at the upper limit of the range specified by Rudkin.[49] Hence, there are at present no compelling data to indicate that the structural genes for ribosomal proteins are spread over a multichromomeric region.

A suggestion of suprachromomeric organization is provided by the locations of the structural genes for xanthine dehydrogenase, aldehyde oxidase, and pyridoxal oxidase. These genes are located on the third chromosome at map positions 52, 56.6, and 57 (Figure 2), respectively. If the map positions are correct, all three enzymes are located in different chromomeres, although there is a remote possibility that the genes for aldehyde oxidase and pyridoxal oxidase are in the same chromomere (they should be about 170,000 base pairs apart and therefore located in different chromomeres). These three enzymes all require the cofactor (or polypeptide) specified by the *maroon-like* locus and all three are affected by the *low xanthine dehydrogenase* locus (*lxd*, at position 33 on the third chromosome). The likelihood of three enzymes, requiring the same cofactor, being located together by accident is extremely low. Thus, the existence of three loci with similar function in a limited region of the genome is suggestive of suprachromomeric regulatory organization. However, the map positions of the three loci may be an historical accident. The similar functions of the three enzymes suggest that they might have evolved from a single primordial gene. If the gene duplication leading to the appearance of the three enzymes occurred comparatively recently, then the three structural genes might be expected to be found in association with each other. Hence, their association is hardly compelling evidence for any suprachromomeric organization.

There are, of course, numerous examples clearly demonstrating the lack of suprachromomeric organization. Genes for proteins in common biosynthetic pathways are widely distributed throughout the genome. For example, the genes for tryptophan pyrrolase and kynurenine hydroxylase are located on separate chromosomes. The genes for phosphoglucomutase, fructokinase, α-glycerolphosphate dehydrogenase and glucose-6-phosphate dehydrogenase, all involving carbohydrate metabolism, are widely scattered throughout the genome. Four temperature sensitive conditional lethals mapping a short region of the X chromosome have widely different lethal phases.[198] Salivary puffs responding specifically to the insect molting hormone are also widely distributed.[199] Hence, there is no compelling evidence indicating any suprachromomeric regulatory elements as involved in the differential control of specific genes. (We are again excluding consideration of such phenomena as heterochromatization and dosage compensation.) Apparently the only situation in which multiple cistrons can be coordinately controlled in one regulatory element is when they are included in a single chromomere. Thus, the regulation in the development of *Drosophila* entails the coordination of roughly 5,000 different elements, the chromomeres. Any general theory of programming for *Drosophila* development must be cognizant of this condition.

HORMONAL CONTROL OF *DROSOPHILA* DEVELOPMENT

We wish now to examine in comparatively great detail one aspect of *Drosophila* development, the modes of action of insect hormones. Holometabolous insects such as *Drosophila* grow and differentiate through four distinct developmental forms – the embryo, the larva, the pupa, and the adult. Between the larval form and the adult there is a remarkable reorganization and reconstruction of the body, metamorphosis, with tissues and organs being destroyed and formed. This developmental

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program is under direct hormonal control. During larval life there is a series of molts. In a variety of insects the decision to form the same or different developmental type (i.e., to remain in the larval form or enter into metamorphosis) has been found to depend on the relative titers of two hormones. Ecdysone (Figure 3), a steroid hormone, induces molting and metamorphosis. If the second hormone, juvenile hormone (Figure 3) is also present, the insect forms another larva stage (or under certain experimental conditions, another pupa) rather than proceeding to the next developmental stage. Although the mechanism of hormonal control of insect development is not known, the interpretation of the evidence is that ecdysone induces molting and permits the activation of, or itself activates, the genetic program for the next stage of insect development. In the presence of juvenile hormone the insect is unable to switch to the new developmental program and instead forms another larva (or pupa).[200,201] Understanding how these hormones act should help to elucidate the genetic programming in insect development, as well as serve as a model for aspects of insect development which are not under direct hormonal control (e.g., the induction of determination).

Periods of intense puffing activity in polytene chromosomes, believed to reflect changes in gene activity, are correlated with molts in several insects. This observation led to a series of experiments which have demonstrated that ecdysone induces the formation of specific puffs and puffing patterns corresponding to those observed in normal development.[144,202,203] Differences from the normal developmental sequence are probably due to the difficulties of in vivo studies with hormone injected into whole animals. The initial dose is nonphysiologically high. Thereafter the dose is difficult to quantify since the hormone is metabolized and may be unevenly distributed throughout the body. Ashburner[199] has now succeeded in inducing a developmentally normal puffing pattern in cultured salivary glands of *Drosophila melanogaster* with β-ecdysone. This is a clear demonstration that β-ecdysone is a sufficient inducer of a specific developmental change in gene activity, as evidenced by alterations of puffing patterns. There is also evidence that ecdysone regulates other differentiation specific events in salivary glands.[203,204]

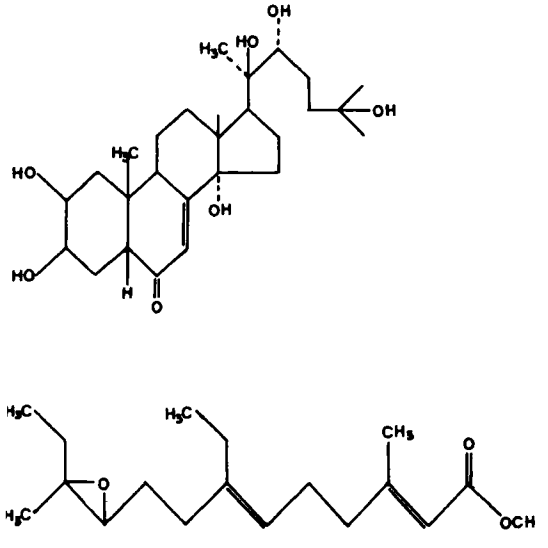

FIGURE 3. Structures of β-ecdysone (top) and juvenile hormone (bottom).

The mechanism of puff formation is unknown. In addition to ecdysone, a variety of nonphysiological agents or conditions induce or affect puffing (see References 144, 202, 203). Some of these agents may cause metabolic or physiological changes unrelated to development which elicit gene action as expressed by puffs. Others may affect the puffing process directly and be useful in understanding its mechanism. But ecdysone, the only agent which evokes the normal differentiation specific puffing pattern, must be the true developmental stimulus.

Despite many years of study of puffing phenomena, the developmental significance of this pattern is not understood. Some puffs must be correlated to primary developmental changes. Others may be secondary developmental effects or merely reflect metabolic activities. Unless the function of specific puffs is known, it is difficult to say which are integral parts of the developmental program. The activity of the Balbiani rings in *Chironomus* salivary glands has been correlated with the production of secretory proteins.[205] In other cases it has been possible to divide puffs into early and late responses.[199] Early puffs respond within minutes after ecdysone treatment; the others begin to appear several hours later.[206] Protein synthesis appears to be required for the formation of the late puffs but not the early ones, suggesting that the products of the early puffs are necessary for the formation of the late puffs.[144,199,202,203] But it has not been shown that a specific protein

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resulting from early puff activity is required for late puff responses.

In cultured salivary glands of *D. melanogaster*, a specific developmental puffing response is seen within five minutes after addition of ecdysone.[207] This very rapid response of the genome to the hormone is one of the earliest hormone effects known. Puffing is thought to be an indication of active RNA synthesis.[208-210] Consistent with this is the finding that one of the first biochemical responses observed after administration of ecdysone is an increased rate of RNA synthesis.[211-215] Puffing and other hormonal effects are suppressed by inhibitors of RNA synthesis.[144,208-210,216] This evidence taken together leads to the conclusion that the genome is the primary site of hormone action. There are reports that ecdysone becomes concentrated in the nucleus of target cells at certain developmental stages.[217-219] But since autoradiography of soluble cellular components such as steroids can be extremely difficult, technical advances in this field may be necessary for a detailed autoradiographic study of ecdysone localization in target tissues.

If the primary action of ecdysone is at the level of the genome, the immediate question is its mode of action. Ecdysone is a steroid hormone, a class of molecules much studied in mammals. Results from these investigations should aid in designing and interpreting experiments in insects. In mammalian systems, the primary site of steroid hormone action is the genome. Steroid hormones have been shown to induce the synthesis of new, differentiation specific RNAs and proteins.[220-226] (We make no attempt to review the extensive literature on the mechanism of steroid hormone action. We cite only selected recent references. The reader is referred to several recent publications for a comprehensive review and summary of the literature.)[227-232]

The early events of the cell-hormone interaction are known in some detail. The hormone enters the cell where it combines with a specific cytoplasmic receptor, a protein which binds the hormone with high affinity.[233-236] The steroid-receptor complex moves to the nucleus where the hormone then accumulates.[237-239] Hormone has been found bound to chromatin[240-242] and recent work suggests it is bound to that portion of chromatin active in transcription.[140] Free hormone is incapable of specific interaction with the nuclear sites. The cytoplasmic receptor-steroid complex is required for nuclear binding.[241] The nature of the chromatin acceptor site is not yet established.[241-253]

Evidence is accumulating that the hormone receptors which have been characterized biochemically are indeed effectors of hormone response. In hepatoma tissue culture cells the binding affinities of various steroids for the cytoplasmic receptors are closely correlated with the effectiveness of these compounds as inducers of specific enzyme activities.[233] A mouse lymphoma cell line resistant to the cytolytic effects of glucocorticosteroids was found to have very low levels of receptor activity for these steroids.[254] Mice carrying a mutation causing testicular feminization, an XY chromosomal constitution but an external female phenotype, have been shown to have a much lowered ability of cytoplasmic receptors to bind dihydrotestosterone, resulting in a lower nuclear uptake of the hormone.[255-256]

These findings taken with the biochemical studies characterizing the rapid hormone-receptor interaction lead to the conclusion that the hormone itself interacts directly with the genome and that the receptor system which has been identified is the molecular mechanism mediating the interaction. Other direct or indirect effects of steroid hormones are, of course, not excluded.[257-263] But the bulk of the present evidence compels the conclusion that the cytoplasmic receptor to chromatin pathway is the primary mechanism through which steroid hormones exert their effects on gene activity.

The action of ecdysone has been the subject of study for many years. The kinds of experiments described above investigating steroid action in mammalian systems require biological material, an adequate supply of reasonably pure tissue with a well characterized hormonal response, and a radioisotope labeled hormone of sufficient specific activity. These materials are now becoming available in insects and the search for specific ecdysone receptors in insect tissues is underway. Results of these initial investigations will be discussed below.

Understanding the details of ecdysone action has been complicated by a number of factors. Studies one would like to compare have been carried out in different species, even different orders, on different tissues and at different stages in the complicated life cycle. While one would expect the general mechanism to be the same in most cases, difference in detail could lead to

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pitfalls and confusion in interpreting data. There is also some confusion about which form of the hormone, α-ecdysone, β-ecdysone, or some other derivative, is the active or "true" hormone in different insects and different tissues.[264-271] Different analogues have been used in various experiments. In addition, the hormone dose used varies greatly in different studies. We will attempt to sort through these variables in search of the common unifying features which may be basic to the general mechanism of ecdysone action.

The results of studies on insect hormones before the availability of more sophisticated biochemical techniques provide some insights into ecdysone effects. As discussed above, ecdysone and its antagonist juvenile hormone are the keys to selecting and initiating the correct genetic program at the metamorphic stages of insect development. Among the earliest responses observed are formation of specific puffs in polytene chromosomes and increased RNA synthesis, indications of altered gene activity at the level of transcription.

Work from Williams' laboratory suggests an alternate mechanism may be acting in silkmoth spermatogenesis.[272-274] It is claimed that a macromolecular factor (MF) present in insect blood is required for spermatogenesis in vitro. In this in vitro system ecdysone and MF are required for intact testis, but MF alone induces sperm maturation in dissected spermatocytes. Rupture of the testis wall will substitute for the presence of ecdysone. This is contrary to the finding that ecdysone is required for sperm maturation in both intact testes and "naked" spermatocysts in the slug moth.[275] Since different genera of moths, different hormones, and different concentrations of hormone were used, this conflicting data over the requirement or lack of requirement of ecdysone for spermatogenesis must remain unresolved. In any case, MF is required in silkmoth spermatogenesis, although insect hemolymph can be replaced by mammalian sera. This suggests that MF may be a nonspecific factor and that it may in fact be a requirement in deficient culture media rather than the in vivo stimulus of differentiation. However, Kambysellis and Williams[274] argue, by analogy to mammalian systems, that spermiogenesis may be a special case. Ecdysone acts by its "usual" mechanism on the cells of the testis wall to bring about permeability changes which lead to sperm maturation. Such a permeability barrier between blood and testis has been found in mammalian testis.[274] Thus, in the silkmoth, sperm maturation may be a secondary effect of a primary hormone action on the testis wall.

In studying the mechanism of puff formation in polytene chromosomes, a variety of agents were found to affect puffing.[203] Two of these effectors with possible physiological activity are the insect hormone ecdysone and changes in intracellular ionic balance. This latter observation led to the proposal that ecdysone exerted its effect on puff formation indirectly by causing changes in the intranuclear ionic balance.[276] It is well established that a shift in the ionic balance of media causes changes in the puffing of isolated nuclei and chromosomes.[277-278] Although some hormone specific puffs appear, the pattern evoked does not correspond to that during ecdysone stimulation of normal development.[203] It is known that one effect of steroid hormones in general and ecdysone in particular is to cause changes in permeability of membranes.[211] Thus, it is not unreasonable that some puffs may be caused by changes in the intracellular ion balance, but this is unlikely to be the sole means by which ecdysone influences gene activity.

Experiments which have attempted to induce puffs in isolated nuclei with ecdysone have been unsuccessful[212,279] although some changes in RNA synthesis have been seen after ecdysone administration.[203,212,214,280] Rather than arguing against the direct action of ecdysone on the genome, this is exactly what would be predicted if ecdysone, like other steroid hormones, must be combined with a specific cytoplasmic receptor to exert its effects. Therefore, the significance of effects of "free" ecdysone on RNA synthesis in isolated nuclei is difficult to interpret.

There is now evidence that ecdysone itself interacts with the genome via a receptor system similar to that identified in mammals. The interaction of steroid hormones with their specific cytosol receptors is blocked by N-ethylmaleimide, an agent which irreversibly alkylates sulfydryl groups.[281,282] Ashburner[207] has found that this compound specifically inhibits the puffing response to β-ecdysone in *D. melanogaster* salivary glands, implicating protein receptors as mediators of the ecdysone induced puff formation. Emmerich has reported evidence for such a receptor for α-ecdysone in the salivary glands of *Drosophila hydei*.[283,284] The hormone becomes bound to cytoplasmic receptor proteins and moves

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to the nucleus in an energy dependent step. A portion of the hormone which is concentrated in the nucleus is reported to be tightly bound to the nonhistone proteins of the chromatin.

Drosophila salivary glands were a logical choice for this kind of investigation. This is a much studied tissue with a well characterized response to the hormone. A most important aspect of this response is the puffing of the polytene chromosomes which offer a near unique opportunity to employ cytochemical techniques to visualize and quantitate the hormone induced changes in gene activity. Drosophila salivary glands, however, are not embryonic but terminally differentiated tissue and respond to ecdysone by undergoing "terminal" differentiation in the literal sense. The cells synthesize and release the secretory proteins which attach the prepupa to the substrate and then die. In our own laboratory we have chosen to forego the advantages of salivary gland polytene chromosomes in favor of the imaginal discs, a tissue which, in response to ecdysone, embarks on a complicated morphogenetic program reminiscent of metamorphosis.

Early in the embryogenesis of holometabolous insects such as *Drosophila* small groups of cells are set aside which are destined to form specific structures in the adult. These disc shaped clusters of cells proliferate in the larva but make no known contribution to larval life.[285,286] They remain in this arrested state of development through the two larval molts until late in the third larval instar when the hormonal stimulus for the larval-pupal molt signals the dormant discs to commence the differentiation of adult structures. The hormonal signal for the larva-pupa molt, an increase in the titer of the molting hormone ecdysone while the titer of juvenile hormone remains low, causes salivary glands and other larval tissues to self-destruct, but stimulates imaginal tissues to differentiate and construct the adult fly. The hormone is a general stimulus which has profound effects on the differentiation and fate of insect tissues, but the character of the developmental program switched on or off by the hormone is a property of the responding tissue dependent on its developmental history and programming.

This programming, usually termed determination, is a well-known feature of imaginal disc differentiation. These cells which give no detectable sign of differentiation are nonetheless committed to carrying out a developmental program which will form a leg, a wing, or other specific portion of the adult. The secretion of ecdysone, in the absence of a corresponding increase in juvenile hormone titer, is the signal to begin to carry out these predetermined roles.

Imaginal discs isolated from *Drosophila melanogaster* undergo differentiation in vitro in a chemically defined culture medium. The initial step in morphogenesis is evagination in which changes in cell shape result in the formation of appendage primordia. This first step in normal differentiation occurs in vitro when the proper hormonal stimulus is applied. Essentially complete differentiation of imaginal discs has been achieved in vitro.[270,287,288] This permits the study under controlled conditions of both the mode of action of the hormone and the developmental responses of the discs. The early responses of the discs have been described in detail.[264] Briefly, ecdysone causes increases in RNA and protein synthesis which are essential for evagination. The continuous presence of the hormone is not required for the completion of the morphogenetic response. Hormone can be removed from the medium after 5 to 6 hr, at a time when there are no detectable morphogenetic changes, and evagination will proceed normally.[288]

We have begun to study the molecular basis of the hormone-cell interaction with mass isolated imaginal discs of *D. melanogaster* using ^{3}H-β-ecdysone. The characteristics of the uptake of β-ecdysone by discs in terms of kinetics, concentration dependence, and competition by ecdysone analogs appear to parallel the normal responses of the tissue to the hormone. The kinetics of uptake and release of β-ecdysone are similar to the kinetics of the change in rate of RNA synthesis following the addition and subsequent removal of the hormone from the culture media. Also, the effectiveness of analogs of ecdysone as competitors for uptake of ^{3}H-β-ecdysone parallels their activity as inducers of evagination. The recovery of an ecdysone-protein receptor complex from the cytoplasm of discs exposed to β-ecdysone has been demonstrated by three different assays. Further, ^{3}H-β-ecdysone has been found tightly bound to imaginal disc chromatin.[289] These preliminary results indicate that β-ecdysone binds with high affinity to specific receptors in imaginal discs and interacts directly with imaginal disc chromatin.

Juvenile hormone does not interfere with the uptake of β-ecdysone by imaginal discs[289] al-

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though it blocks the ecdysone induced increase in RNA synthesis.[265] Removal of juvenile hormone results in an immediate expression of the ecdysone-induced increase in RNA synthesis.[290] These results suggest that juvenile hormone does not prevent β-ecdysone from interacting with its receptor sites within disc cells, but blocks expression of ecdysone-induced changes in gene activity.

GENERAL MODEL OF GENE REGULATION

The intensive study of steroid hormones discussed in the preceding section has contributed greatly to our understanding of gene regulation. Although the basis of action of insect hormones is only beginning to be understood, there are some aspects of the apparent mode of ecdysone action which may be of general significance in the regulation of gene function during insect development. First, specific protein receptors seem to be required as mediators of hormone effects on gene activity. The hormone, by itself, does not appear capable of inducing tissue specific puffing patterns in isolated polytene chromosomes or nuclei. Although this failure may result in part from inadequate culture conditions, we believe that it is due primarily to the absence of the appropriate ecdysone-receptor complex. Because of the requirement of specific protein receptors for the action of ecdysone and other steroid hormones and the effects of nonhistone proteins on the transcriptional properties of chromatin in vitro, we believe it likely that proteins will be found to be the effectors of specific gene function. In many cases proteins must act in conjunction with low molecular weight molecules.

A second aspect and important attribute of the induction of gene function by ecdysone is the separation of responses into early and late ones. In both *Drosophila* and *Chironomus* there are puffs which appear very rapidly in response to hormone (within minutes) and others which appear substantially later (in hours).[199] Late responding puffs have never been found in the absence of the early ones. The early puffs can be induced in the presence of protein synthesis inhibitors, but continued protein synthesis is required for the appearance of later puffs.[291] These observations suggest that the products of the early puffs are involved directly, or indirectly, in the induction of the late puffs. The simplest hypothesis is that the protein product of the early genes is the effector of activity of the late genes. This pattern of sequential modulation of gene activity may be typical of the response of all tissues to effectors. Thus, effectors of gene activity may be divided into two classes: primary effectors, in the case of hormone action, those proteins already present in the cell at the time of exposure to hormone which direct a specific initial response; and secondary effectors, proteins synthesized in response to the primary effectors which are in turn effectors of other genes.

Such a mechanism for altering gene activities would provide a simple explanation for the variety of tissue specific developmental events initiated by a common morphogenetic signal. To be competent to respond to the signal to undergo differentiation a cell need contain only a few primary effector proteins. All other differentiation specific changes in gene activities or synthetic processes would be arrested until the correct stimulus initiated the developmental program. The production of such a primary effector might be the molecular basis of determination. The initial stimulus, e.g., a hormone, would be required during the first stages of differentiation, but might not be continually involved in the total set of responses which it triggers. Examples of the completion of ecdysone induced differentiation independent of the continued presence of hormone are metamorphosis in silkmoths[96] and the evagination of *Drosophila* imaginal discs.[288]

This model for gene regulation during development has several important implications. If gene activity is indeed regulated as suggested above, most chromomeres would respond to a specific secondary effector rather than a variety of different molecules. Thus, these chromomeres might have only a single regulatory site. Chromomeres containing the structural genes for the secondary effectors might have several recognition sites which would allow these chromomeres to respond to a variety of primary effectors. But since there are many more late puffs than early ones, the majority of chromomeres would contain only a single effector recognition site.

There are several reasons to believe that the number of transcriptional regulatory sites in a typical chromomere may be quite small. If our previous analysis is correct, the chromomeres which contain multiple copies of the genes for ribosomal proteins and histones should have little

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DNA which could serve a regulatory function. While the DNA content of most chromomeres is in excess of that required to code for known structural genes, the function of this extra DNA has not been determined. It has been suggested that the structural organization of the chromosome is such that the control of transcription requires a unit of certain minimal size.[52] Since the independent control of structural genes seems to require that they be in separate transcriptional units (chromomeres), single structural genes may be located in long stretches of nontranslated DNA. There is no reason to believe that most of this extra DNA encodes recognition sites for regulatory molecules. Consistent with the hypothesis that only a small portion of the DNA in the chromomere has a regulatory function is the failure of fine structure mapping studies to identify such sites. It is not surprising that regulatory sites within chromomeres have not been unequivocally identified in *Drosophila.* As we have argued before, they may be exceedingly difficult to find by traditional mutagenic experiments. The absence of multiple regulatory sites within chromomeres places an apparent constraint on the regulatory capabilities of the system. However, the extensive post-transcriptional modifications of RNA affords numerous opportunities for regulation which can easily compensate for any constraints found at the level of transcription.

Other than the early genes responding to ecdysone, one gene which might code for a secondary effector is *lxd* (*low xanthine dehydrogenase*)[166,185] which causes a simultaneous reduction in the level of xanthine dehydrogenase, aldehyde oxidase, and pyridoxal oxidase activity in crude extracts. An effector which is unable to induce the normal levels of transcription of these three genes would give such a result. The proposed model for the control of gene activity also provides an explanation for the compensatory regulation observed in the control of alcohol dehydrogenase in maize[292] and hemoglobin synthesis in *C. tentans.*[293] In the case of *Chironomus* a mutant site at or near the structural gene for one of the hemoglobins greatly increases the amount of that hemoglobin while decreasing the amount of another. Assuming a limited number of effector molecules, increased efficiency of binding of the effector to one site could result in decreased binding to another site. Thus, increased transcription of one chromomere could result in decreased transcription of another.

There is no reason to assume that all chromomeres contain structural genes for effectors or that each chromomere necessarily responds to a different effector. A study conducted several years ago by Berendes[294] on puffing patterns in polytene cells of the gut, malphigian tubules, and salivary glands showed that of 110 puffs, 69% were found in all tissues. Only 14 puffs were tissue specific (3 in malphigian tubules, 4 in the gut, and 7 in the salivary glands). The remaining puffs were found in 2 of the 3 tissues. A substantial number of the puffs in malphigian tubules were present with similar intensities at different developmental stages. These observations indicate that many chromomeres are transcribed in a nontissue specific manner during most of development. Regulation of these nontissue specific puffs may be different from that of tissue specific puffs. Such puffs may be unresponsive to hormones or other specific developmental stimuli. This type of "constitutive" chromomere may contain a structural gene, or genes, a site for binding RNA polymerase (with different efficiencies under different conditions), but no other site which can differentially affect the transcription of the structural gene(s).

COMMENTS ON THE GENETICS OF PROGRAMMING

Following fertilization in *D. melanogaster* there is a series of synchronous nuclear divisions. After about 1.5 hr at 25°C the majority of the nuclei migrate to the egg cortex where membranes are formed and a cellular blastoderm is produced. The blastoderm cells ultimately give rise to both larval and adult tissues.

The cleavage nuclei are capable of developing into any structure in the organism.[295] Programming of the nuclei to form particular structures occurs in association with cellularization[296] and is dependent on nuclei reaching appropriate regions of the egg cortex. Failure of nuclei to reach a particular portion of the cortex results in loss of specific structures. This has been demonstrated in *Cecidomyidae* for the polar regions (no germ cells being formed)[297] and is apparently true for other regions of the cortex as well. At the time of programming there are about

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3,200 to 3,500 blastoderm cells.[298] Assuming about 15 cells per embryonic Anlage[296] and 25 different adult Anlagen (6 for legs, 2 for eyes, imaginal rings for internal organs, etc.) at least 375 cells become committed to develop into adult structures, along with some which develop into both larval and adult structures. But this is the minimal number of cells involved. The estimate of cell number per Anlage is based on the estimate of the number of cells which produce clones surviving into the adult. It is conceivable that there are extra cells present initially which do not give rise to surviving cell lines. These cells may perform a "back-up" role and assure that there are enough cells in the Anlage to produce the ultimate differentiated structure. In addition, the number of Anlagen may be underestimated. There may be separate Anlagen for the musculature in each appendage, or several giving rise to the abdominal hypodermis which have not been included in the above calculation. A reasonable estimate is that at least 15 to 20% of the cells present in the embryonic blastoderm are specifically programmed to form adult structures. Presumably a similar proportion of the cortical region is devoted to programming these cells. The chemical nature of the cortical substances responsible for this programming is not known. In the polar region there are granules which are apparently required for the subsequent formation of germ cells.[297] These granules are composed of RNA and protein.[299]

Throughout development there is a progressive commitment of the cells to particular morphogenetic pathways. At the time of blastoderm formation there are two separate populations of cells destined to form the larval and imaginal structures. The imaginal primordial cells are already committed to give rise to either anterior or posterior tissues.[300] But the full programming for adult structures is not complete until much later in development. In the larva the different embryonic Anlagen are separated in discrete packages of cells, the imaginal discs. Schubiger[301] has evidence for a specific population of cells in leg imaginal discs which in third instar larvae retain the capacity to regenerate an entire leg disc or to give rise to structures found in other discs. It is also known that some homoeotic genes, genes which cause an appendage to be converted specifically from its usual form into another (e.g., antenna to leg), have their effect during the third instar.[302-304]

There is tenuous evidence that homoeotic genes act via protein products. Some alleles of the homoeotic mutants *bithorax* and *bithoraxoid* are suppressible by suppressor genes.[39] In bacteria and in yeast suppression often occurs during translation. Therefore, the suppressibility of these alleles suggests the existence of a protein product. However, in *Drosophila* suppression may occur after translation. A suppressor of *vermilion* appears to restore tryptophan pyrrolase activity via the interaction of nonmodified tRNA with the inactive enzyme.[305] Further tenuous evidence implicating a protein is the fact that at least one homoeotic allele is temperature sensitive. Since temperature sensitivity probably involves the thermal denaturation of an altered polypeptide, the identification of a temperature sensitive allele of the homoeotic mutant *spineless-aristapedia*[304] suggests the involvement of a protein product. Finally, one of the outstanding characteristics of programming in the imaginal cells of *Drosophila* is that it is inherited through many cell divisions.[296] In different cell types particular genes must be able to retain states of function through numerous DNA replications.

A model for the induction and maintenance of programming of cells can be derived from the general model for gene regulation presented in the previous section. Nuclei respond to particular effectors in the egg cortex by becoming programmed rather than by initiating gene function leading to immediate cellular differentiation. This response presumably involves the activation of chromomeres whose protein products effect a heritable "repression" or "derepression" of appropriate chromomeres. One can imagine one gene product which "permanently" derepresses its own chromomere and a set of "larval" chromomeres, and another gene product which derepresses its chromomere and a set of "adult" chromomeres. The products of the "larval" or "adult" chromomeres could, in association with appropriate external stimuli, affect other chromomeres and thus initiate a cascade of events leading ultimately to cell populations which are competent to differentiate appropriately. There is no difference in substance between this type of response and that involving differentiation itself. At the level of the genome the programming of cells and the differentiation of cells could involve similar mechanisms. They would differ only in the nature of the gene products formed; differen-

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tiation involving those which act to change cellular functions, programming involving those which affect heritable chromosomal functions.

SUMMARY

The unit of transcription and regulation is the chromomere. There is no convincing evidence for the existence of continuous multichromomeric regulatory entities, nor for the presence of multicistronic chromomeres containing genes for polypeptides with discretely different functions. Thus, the genetic programming for development in *Drosophila* involves the coordinated regulation of approximately 5,000 different regulatory units. Studies on the mechanism of action of ecdysone reveal that chromomeric function is regulated by nonhistone proteins and that genomic responses are temporally divisible into early and late ones. These last data serve as a basis for a general model of gene regulation during development. It is suggested that genes responding rapidly to developmental stimuli code for proteins which affect the function of other chromomeres and thus initiate a chain of events, which when coupled with appropriate stimuli, result in the ongoing development of the organism.

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