An analysis of the Notch locus in *Drosophila melanogaster*

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INTRODUCTION AND LITERATURE REVIEW

The Structure of the Eucaryotic Chromosome

Biologists have been fascinated with the nature of the chromosome for over one hundred years. Early investigators noted the presence of compacted objects in the nuclei of both plant and animal cells undergoing division, but were unable to discern the significance of these structures until the late 1800's. Nageli (1884; see DuPraw, 1968) first suggested that some undefined substance must be transmitted via the egg and sperm to insure a stable, consistent system of inheritance. Soon thereafter, it was recognized that these differentially stained, "colored bodies" (hence, the Greek word, "chromosome") were somehow involved in the hereditary process. With the rediscovery of Mendel's observations at the turn of the 20th century and a more comprehensive appreciation for the cell cycle, the chromosome assumed a level of primary importance to both the geneticist and cytologist alike. So it remains today!

Subsequently, both groups undertook to determine the structure of the chromosome and its functional roles in genetic transmission and cell viability. Initially, the chromosome was thought to be comprised entirely of protein, but now-classic genetic experiments conducted by Avery, MacLeod, and McCarty (1944) and Hershey and Chase (1952),

among others, demonstrated unequivocal proof that the nucleic acid, deoxyribose nucleic acid (DNA), was, indeed, the hereditary material. However, and emphatically not to be ignored, protein has since been recognized as a significant structural, and potentially functional, component of the composite chromosome. Primarily, in most general terms, the mitotic chromosome is comprised of tightly coiled DNA, packaged in an enveloping coat of protein, both basic (histones) and acidic (nonhistone), which together constitute the chromatin material of the cell nucleus.

The role of interphase, chromosomal DNA in the protein-synthesizing machinery of the functioning cell has been intensely investigated since Watson and Crick (1953) first described its primary structural properties and prophetically suggested its probable involvement in the transcription process. On the other hand, the functional involvement of the enveloping proteins remains highly speculative. Their possible roles in DNA packaging, reflected in specific chromosome morphology, and in the selective regulation of site-specific DNA transcription, are but two of a multitude of functions that have been attributed to the protein components to date (for review, see DuPraw, 1970). Since the role of the protein covering assumes little importance in the discussion which follows, we merely recognize its significance in the elucidation of chromosome structure and

function.

Based, then, on the increasingly better understood chemical composition of the eucaryotic chromosome, much effort has been spent in attempts to determine the structural arrangement(s) of DNA and protein in the eucaryotic chromosome, both quantitatively and qualitatively. To this end, a remarkable, interdisciplinary combination of genetic, cytological, and biochemical techniques have been applied.

The Metaphase Chromosome

Considerable debate has been waged over the quantitative composition of DNA within the metaphase chromosome by cytologists using both light and electron microscopes. Early, two schools of interpretation developed: 1) those who felt that a single, continuous DNA molecule comprised a typical metaphase chromosome (the unineme model); and 2) those whose observations led them to conclude that the chromosome was comprised of more than a single molecule of DNA (the bi- or polyneme model). Light microscope analyses of chromosomal morphology of many different eucaryotes, plant and animal, during stages of cell division contributed little more than confusion to an already controvertible problem. The results of such studies of closely related organisms often led the same investigator to opposite conclusions. Although one cannot deny the possibility of

species-specific chromosome structure through evolutionary divergence, one would not expect so unique a characteristic when large groups of related organisms share so many other common properties.

Electron microscopic studies, with markedly greater resolving powers, although offering no substantive proof, have generated, as we will discuss shortly, support for the unit character of chromosome substructure.

That the metaphase chromosome is comprised of one or more DNA molecules has stirred much controversy. Support for either of the two interpretations has come from various investigative approaches and will be summarized as follows:

1) Autoradiographic analyses. The classic autoradiographic experiments performed by Taylor and his associates (1957) provided indirect proof for the semiconservative replication of the eucaryotic chromosome which is known to occur in bacterial systems (Meselson and Stahl, 1958). Furthermore, these same studies pointed to the uninemic character of the chromosomes of Vicia faba root cells since, without additional postulates, the observed pattern of labelled DNA segregants through successive generations would be difficult to rectify if each chromatid consisted of more than one molecule of DNA. Deviations from this expected pattern (e.g. the isolabelling and sister chromatid exchange phenomena) have been noted and are often

presented as evidence in support of the binemic model (Peacock, 1963; Ris, 1966). Nevertheless, no conclusive data exists which confirms the presence of subvisible half-chromatids in a typical metaphase chromosomes. DuPraw (1970) argues that both sister chromatid exchange and isolabelling are the consequence of a break-rejoin event between two longitudinally folded, single DNA fibers.

2) Electron microscope analyses. The electron microscopic examinations of whole-mount (unsectioned) and sectioned metaphase chromosome specimens have led many cytologists (DuPraw, 1968; Comings, 1972) to conclude that the chromatid is comprised of a single DNA fiber. This fiber is seen to assume and maintain an orderly, but tightly compacted, configuration with no free ends extruding from its lateral surfaces.

However, other investigators voice strong objections to this interpretation. For example, Sparvoli et al. (1965) and Osgood et al. (1964) offer their micrographs as support for an alternative conclusion. Further, Gay and her co-workers (1970) contend that some ganglionic cells of first and third instar larvae of Drosophila melanogaster contain multi-stranded chromosomes as indicated by the results of two-wavelength Feulgen microspectrophotometric analysis. Comings (1972), however, has rebutted the positions held by Gay et al. by arguing that these chromo-

somes may merely be in early stages of polytenization.

Thus, descriptions of metaphase chromosome ultrastructure based on cytological examinations are far from reliable. One continually feels an overwhelming apprehension that observations drawn from cytological examinations may merely reflect intrinsic artifacts of the techniques employed to obtain suitable preparations.

3) Biochemical analyses. The most striking evidence in support of the unineme model for chromosome structure has recently come from the studies of Petes and Fangman (1972) and Kavenoff and Zimm (1973). In the former study, the sedimentation properties of the nuclear DNA of the yeast Saccharomyces cerevisiae were used to calculate the average amount of DNA per chromosome. The estimates of this value are what would be expected if each yeast chromosome contained a single DNA duplex. In the latter study, Kavenoff and Zimm (1973) applied the viscoelastic techniques used with notable success in studies of bacterial molecules to determine the average DNA content of ordinary Drosophila chromosomes. The results of their measurements led these authors to conclude that the chromosome, "in its simplest form", contains a single molecule of DNA. In neither study was the presence of protein linkers, interspersed along the DNA backbone, indicated.

4) Genetic analyses. Previous to the presentation of

the biochemical evidence discussed above, Comings (1972) argued most convincingly in favor of the single-fiber chromosome concept from a predominantly genetic viewpoint. He cited the recognized properties of eucaryotic chromosomal DNA with respect to replication, mutation, and recombination as overwhelming obstacles to alternative interpretations. For example, the accepted explication of simple Mendelian segregation ratios becomes untenable in the absence of compensatory mechanisms which would be required to insure the stability of a multifibrillar chromosome.

Before proceeding to the discussion of an unusual class of somatic chromosomes more pertinent to this report, the following, currently most attractive, description of the metaphase chromosome is offered: one continuous, linear DNA molecule that extends from telomere to telomere, is intimately bound by a protein coat, and assumes a descriptive morphology, characteristically maintained from generation to generation to the exclusion of change.

Giant Polytene Chromosomes

The Frenchman Balbiani (1881; see DuPraw, 1970, for an interpretation of the original, published in French) was the first to report the existence of a class of exceptional, giant chromosomes in the larvae of the dipteran, Chironomus,

quite unlike those found in other tissues of the same organism. He described them as being unusually large, with a series of darkly staining bands along their length, distinctly separated by regions of lesser intensity. Giant chromosomes are now known to be present in the cells of the salivary glands, the lining of the midgut, and the rectum, in the larvae of several genera of dipterans, including Drosophila melanogaster (Demerec, 1950).

Swift (1962), employing Feulgen microspectrophotometric techniques, demonstrated that giant chromosomes were the product of a polytenization process (hence, the synonym, "polytene" chromosome) during larval development. These replications, without separation and subsequent cell division, result in an approximate 1024-fold increase (geometric doubling) in the amount of DNA per chromosome. Autoradiograph studies of the salivary gland chromosomes of Chironomus conducted by Beerman and Pelling (1965) indicated that polytene chromosomes consist of a parallel arrangement of chromatin fibers extending longitudinally from telomere to telomere. They also coincidentally concluded that the grain distribution displayed on the autoradiograms was representative of that to be expected if each chromatid were comprised of a single fiber.

As noted in the previous discussion, interphase chromatin undergoes a process of folding which ultimately

results in metaphase chromosomes of characteristic structure. A somewhat analogous packing process typifies the polytene chromosome. Rae's (1966) electron micrographs of unsectioned Drosophila polytene chromosomes convey a similar mode of concentrated fiber folding at discontinuous intervals along the chromosome's length. These regions of more densely compacted mass are referred to as chromomeres; compacted regions of individual fibers then appear to pair in register with homologous regions of their sister fibers. The interbands exhibit far less, if any, folding which promotes or facilitates the easy recognition of the specific, characteristic banding patterns so noted by Balbiani (1881).

Although more will be said later about the functional activities of the polytene chromosomes and their significance to genetic analyses, their morphological properties, as presently understood, may be summarized as follows: a polytenization process similar to that occasionally observed during abnormal cell division results in giant chromosomes with an approximate 1000-fold increase in DNA content. The DNA is, in turn, surrounded by a common chromosomal protein coat which, when viewed microscopically, represents a unit structure. A distinctive pattern of transverse banding is revealed which results from the synapsis of tightly folded homologous regions. Such polytene chromosomes thus provide replicatively magnified and synapsed interphase chromatin with which the cytologist may work.

Significance and Properties of Polytene Chromosomes

Painter (1933, 1934) is generally credited as the first to recognize the significance of the banding pattern of giant chromosomes in various tissues of numerous Dipterans. He prophetically noted, if not overstated, the potential applicability of this natural phenomenon as a tool in unraveling the mystery of the gene. Although the realization of Painter's predictions has yet to be achieved, his advice has not gone unheeded. The polytene chromosomes and their characteristic banding patterns do provide a singularly unique system in which the structure-function relationship of the eucaryotic chromosome may be studied (Beerman, 1972). This relationship, as we shall see, may provide a significant contribution to the understanding of vital metabolic activities of the organism as a whole, to which we strive. Furthermore, this constant and reliably discernible banding pattern is, itself, an inherited character which has enabled the cytogeneticist to better describe the primary importance of the chromosome in genetic continuity.

Painter (1933) first noted that there was often a one to one correlation between abnormal salivary gland chromosome banding patterns and the mutant phenotype expressed by the adult fly. In so doing, he introduced the method of cytological chromosome mapping which facilitated the

assignment of particular mutant "genes" to relatively specific locations on the chromosomes. Later, Bridges (1935, 1938) presented more richly detailed chromosome maps of the entire Drosophila genome. The amazing accuracy of Bridges' maps, constructed entirely by light microscope examination, has since been confirmed, with only minor exceptions, by electron microscope studies pioneered by Sorsa and Sorsa (1967) and continued extensively by Berendes (1970).

In noting the apposition between phenotypic expression and chromosomal banding, both Painter and Bridges proposed that one gene could be assigned to only one salivary band, and conversely, that one band specified one, and only one, gene. Such an interpretation has evolved into the present "one band-one gene" controversy and is the substance of the discussion which follows.

The Chromomere

As we have seen, the chromomere (or band) of the polytene chromosome in Drosophila represents the compacted mass of DNA fibers, sharply delimited from adjacent bands by fibers in a more relaxed state (the interband region). The controversy stirred by early cytogeneticists who assigned an abnormal genetic character to a specific band-interband has prompted geneticists of various disciplines to specify the structure-function relationship of the chromomere

inherently implied by such an association. To understand the significance of this comprehensive undertaking, a systematic discussion of the complexity of the chromomere (albeit the inexplicable confusion surrounding it) will be attempted. For the sake of convenience and organizational clarity, each aspect of the nature of the chromomere will be addressed individually, although it is imperative that one ultimately recognize the chromomere as a mere contributor to the overall stability of the functional activities required by the organism. In the ensuing discussion, the following unit, or multi-unit, characters of the chromomere will be described:

1) Replication
2) Transcription
3) Function
4) Intra- and Intercistronic Interactions

The Chromomere as a Unit of Replication

The evidence gathered by Taylor *et al.* (1957) in autoradiographic analysis of the replication process in *Vicia faba* indicated that eucaryotic chromosomes replicate semiconservatively. Considerations for the relatively large amounts of DNA to be replicated during a relatively short S-period in the eucaryotic cell cycle led to the early prediction that replication must proceed from multiple

sites of initiation. The basis for this reasoning may best be demonstrated by the following example: the average chromosome of Vicia faba is approximately 800 times longer than that of E. coli, and replicates in roughly 8 hours. Assuming the uninemy of the bean's chromatid, we see that replication proceeds 8 times faster in Vicia faba than in E. coli (Callan, 1973). Huberman and Riggs (1968) provided support for this hypothesis by showing that DNA replication in cultured mammalian cells proceeds bidirectionally from multiple initiation sites distributed along the entire length of the chromosome. They also noted that initiation sites were not equally spaced and did not appear to be synchronously regulated. In constructing a working model, Huberman and Riggs (1968) further suggested, without experimental support, that the two termini resulting from bidirectional growth be equidistant from the point of initiation. Nearly all of these early observations have been corroborated by others conducting similar investigations of eucaryotic replication (for review, see Callan, 1973).

Keyl and Pelling (1963, see Mulder et al., 1968, for discussion of original paper, published in German) studied the mechanism of DNA synthesis of the salivary chromosomes of hybrids generated by mating the dipterans, Chironomus thummi thummi and Chironomus thummi piger. Since thummi contains 27% more DNA than piger, hybrids of the two provide

a convenient system suitable not only for studying the replication required for polytenization (^{14}C and ^{3}H-thymidine pulse-chase techniques) but also for measuring the duration of synthesis in regions containing unequal amounts of DNA. Autoradiographic results indicated a discontinuous labelling pattern in addition to the marked difference in label intensity, which presumably reflects the regional DNA contents, characteristic of each subspecies progenitor. Furthermore, replication was observed to commence simultaneously but terminate differentially, dependent, again, on localized DNA content. From these results, Keyl and Pelling proposed that each chromomere was an independent unit of replication and that the duration required for replication was a function of the amount of DNA which localized therein.

From similar studies conducted with Drosophila hydei, Mulder et al. (1968) lent their support for the conclusions drawn by Keyl and Pelling (1963). Most noteworthy, they observed definite labelling tracks in the interchromomeric regions; but, due to inherent limitations of the autoradiographic techniques employed, they were unable to conclusively determine whether the replication of these connecting fibers were under the coordinate control of the adjacent band(s).

However, the conclusions drawn by both Keyl and Pelling

(1963) and Mulder et al. (1968) have not gone unchallenged. Howard and Plaut (1968) have examined DNA synthesis of the salivary chromosomes of Drosophila melanogaster. Their results have led them to conclude that each chromosome is subdivided into many coordinately controlled groups of chromomeres which may undergo replication independent (temporally) of other groups located on the same chromosome. Further, they extend the concept of independent, asynchronous replication to include coordinate control of chromomeric groups without regard to genomic localization. In support of their proposal, the asynchronous replication of "puffed" regions of the polytene chromosomes of Sciara coprophila (Gabrusewycz-Garcia, 1964) and the definitively late replication (if any) of the heterochromatic regions in the polytene chromosomes of Drosophila are cited.

To summarize the present understanding of polytene chromosome DNA replication is an arduous task. Conflicting results from experiments analyzing the synchrony of replication force restraint in offering conclusive arguments. The sensitivity of tests now being employed to this end (e.g. autoradiographic techniques) severely restricts the depth of examination required. Gaps in our understanding of polytene chromosome replication are many. For example, present knowledge provides no indication as to the actual sites of initiation and termination within a single replicon

(a unit of replication). Furthermore, it has yet to be shown that replication of polytene DNA is bidirectional as is observed in diploid cells of other eucaryotes. If indeed, replication is bidirectional and if each chromomere behaves as an independent replicon, the determination of the initiation site, when accomplished, will draw attention to the unit character of the chromomere and the regulatory controls it may exert on one or both adjacent interbands. Assuming for the moment that each chromomere represents an independent replicon, it is tempting to suppose that initiation begins at a point within the band itself (sequence specific for the required polymerase), proceeds bidirectionally, and includes equal or unequal lengths of DNA in both adjacent interbands. Such a system would conceiveably unify the chromomere as a unit of replication and include parts of adjacent interbands under its control.

Although disagreement about many subtle features of polytene chromosome replication does exist, most students of the process would agree that the chromomere does play a significant role in its own replication.

The Chromomere as a Unit of Transcription

Chromomere-transcript relationship

Puff formation Since the role of DNA in RNA synthesis was first implied (Watson and Crick, 1953), and as evidence accumulated in support of the unit chromomere

hypothesis, attention was turned to investigating the chromomere as the unit of transcription in Dipterans. Again, the polytene chromosomes provided a system in which the transcriptional activities at the sub-chromosome level could be observed. Poulson and Metz (1938) were the first to note characteristic disruptions in the polytene bands in cells at different stages of development in Sciara ocellaris. However, they attributed these changes, which have since been described as "puffs", to the differential response of particular cells to unspecified environmental factors.

Beerman (1952; for discussion of original paper, published in German, see Ashburner, 1970), after studying the spatial and temporal distribution of puffs in the polytene chromosomes of Chironomus tetans, proposed that such protrusions were the physical manifestations of the transcriptional activities of particular genes. He later pointed out (Beerman, 1956) that puffs appear to localize to particular bands and that puffing patterns are apparently both time and tissue specific. Pelling (1959) confirmed Beerman's (1952) early hypotheses by demonstrating the differential and selective uptake of labelled RNA precursors by chromomeres in a puffed state. The puffing, therefore, is merely a reflection of the relaxation of the banded chromatin being transcribed.

Indirect proof that puffs are the sites of DNA-depen-

dent RNA synthesis followed from the studies conducted by Ritossa and Pulitzer (1963). Actinomycin-D in low concentrations, a recognized inhibitor of eucaryotic RNA synthesis, was shown to block the incorporation of labelled RNA precursors at puffed regions on polytene chromosomes of Drosophila buckii.

That a puff is restricted to one (and only one) chromomere, which in turn is transcribed independent of other bands, has been elegantly demonstrated by Daneholt and Hosick (1973) in their analysis of "Balbiani Ring" (BR), or giant puff, number II of Chironomus tetans. The cytological assignment of BR-II to a particular chromomere presented no problems. After salivary glands are incubated for short periods in a solution containing labelled RNA precursors, BR-II can be physically separated by microdissection and the newly synthesized RNA isolated for chracterization. They found that the RNA transcript had a sedimentation value of 75S as determined in sucrose gradients. From estimates of the DNA content of BR-II (Daneholt and Edstrom, 1967), the authors concluded that the 75S RNA product closely approximates that expected if the total BR-II DNA had been transcribed. Lambert (1972) has hybridized this 75S RNA fraction with BR-II DNA and the sequence homology observed reinforces the position held by Daneholt and Hosick (1973), although the sensitivity of Lambert's tests precludes definitive con-

clusions being drawn. Lambert (1972) significantly noted, however, that the original 75S transcript was transported intact, and apparently unchanged, to the cytoplasm.

These results and the conclusions drawn from them are difficult to interpret when an abundance of evidence indicates that a large proportion of the original transcript is cleaved and undergoes further processing in the nucleus before being transported to the cytoplasm (see Georgiev et al., 1973). In addition, the size of the BR-II transcript (75S) is considerably larger than expected for either ribosomal or transfer RNA. And finally the stability of BR-II RNA, as evidenced by its accumulation in the cytoplasm (Lambert, 1972), appears unprecedented when compared to the average turnover rates of messenger RNA in a functionally active cell. However, one must recognize the possibility that BR-II does produce a large, stable transcript, analogous to the polycistronic messengers observed in lower organisms.

Additional support, from yet another approach, for the unit character of the chromomere in transcription, has come from the electron microscope analyses of lampbrush chromosomes of salamander oocytes by Miller and Beatty (1969). Their micrographs of rRNA synthesis and the determined size of the molecules produced, suggest that the lampbrush loop DNA constitutes one or a few genes and probably a single unit of transcription (Pelling, 1972).

Before turning to a more comprehensive treatment of the

role of the chromomere in protein synthesis, a few prominent observations emanate from the extensive analyses of the puffs which protrude from the salivary chromosomes of numerous Dipterans. First, puffs appear to represent the unfolding of compacted fibers which facilitates the transcription of the region defined by the puff. Second, a particular puff can be seen to initiate and localize within a single chromomere at specific times during development. Third, that RNA-precursor uptake is indigenous to puffed regions, exclusively, would indicate that the chromomere is the site of actual RNA synthesis. And fourth, indirect evidence indicates that an entire chromomere is transcribed as reflected by the size of the RNA molecule produced. However, none of the evidence cited in these studies implies that the cytoplasmic mRNA serves as the template for more than one polypeptide product.

In the discussion which follows, we will turn our attention to the characterization of chromomeric DNA, from which puff formation and transcriptional activities are thought to originate. If the chromomere is the transcriptional unit of the Drosophila chromosome, as has been proposed, one ultimately asks how the composition of the chromomere is finally resolved by its protein product(s).

From a glance at Bridges' early cytological maps (1935, 1938), one quickly realizes that the dimensions of an individual band are not easily defined; to do so requires

indirect estimates of its DNA composition and breadth. Morphologically, bands are generally characterized as large, intermediate, or small; one would expect these differences to be a reflection of the amount of DNA comprising them. If one measures the amount of DNA per chromosome and divides by the number of bands which it possesses (Bridges, 1935, 1938; Berendes, 1970), approximate amounts of DNA per band can then be derived. Such was the reasoning invoked by Rudkin (1961) to estimate the DNA content of Drosophila chromosomes by ultraviolet absorption techniques. He found the average band per chromatid to contain 5×10^{-7} picograms of DNA or roughly 4×10^{4} nucleotide pairs. From this mean value, he estimated that large bands contain 10^{5} base pairs while the smallest bands only 5×10^{3} base pairs of DNA.

The rather rigorous stretching of polytene chromosomes required for band recognition presents a distorted estimate of distances between bands and makes the determination of interband DNA content by the same approach considerably less reliable. However, the average interband length approximates .1 microns (u), equivalent to 1000-2000 nucleotide pairs. As we shall see shortly, this value assumes importance (Crick, 1971) when considerations are made for the amount of DNA required to produce polypeptides of average size in both procaryotes (Hayes, 1968) and eucaryotes (see Laird, 1973).

Thus, the conflict and confusion: if there is approxi-

mately thirty to forty (depending upon whose maps are used for band number estimates) times more DNA per chromomere than is required for the production of an average size protein, why then the abundance of DNA per chromomere? Three potentially testable interpretations may be rendered to satisfy these observations at face value. First, the majority of the chromomeric DNA (including the adjacent interband(s)) is of the structural variety and comprised of tandom repeats of a single nucleotide sequence. Callan and Lloyd (1960) originally proposed such a model to describe the organization of the lampbrush chromosomes of Triturus cristatus. Second, the chromomere is comprised of a series of nonrepetitive DNA sequences (between thirty and forty), from which is transcribed a multi-cistronic messenger, eventually resulting in the translation and synthesis of up to forty unique polypeptides. Such a system, including regulatory sequences, is immediately recognized as similar to the "lac" operon in E. coli (Jacob and Monod, 1961). And third, only a small fraction of the total chromomeric DNA is resolved as translationally functional, while the remainder acts in some enigmatic regulatory capacity as was first suggested by Britten and Davidson (1969).

Well, as will be seen later in this discussion, each school of thought has its many disciples, who have, in turn, proposed and expanded their respective models in an attempt

to rectify this seeming incongruity. However, a few steps in the overall process from DNA to protein have been elucidated, and whose description is pertinent to the validation of each interpretation.

Characterization of chromosomal DNA The first of these has been the categorization and characterization of DNA comprising the genome of Drosophila. Generally, chromosomal DNA falls into one of two classes: euchromatin and heterochromatin. Euchromatic DNA is thought to be the more functionally active, responsible for the production of RNA molecules; heterochromatin has been considered functionally inert but structurally prominent (e.g. centromeric regions). More recently, however, Pardue and co-workers (1970) have demonstrated by in situ hybridization that 18S and 28S ribosomal RNA cistrons localize within heterochromatic regions, which will require accommodation in distinctions in DNA put forth in the future.

The evaluation of the renaturation kinetics of Drosophila DNA has yielded a significant contribution to the understanding of chromomere organization (Laird, 1971; for review, see Laird, 1973 and Laird et al., 1973). Their results indicate that about 85-95% of Drosophila euchromatic DNA renatures at a rate expected for single-copy sequences. The remainder, intermediate-repetitive DNA, is thought to be randomly distributed throughout the genome; although its

specific function remains undetermined, it is believed to act as "spacer" sequences interspersed among the unique (Laird et al., 1973). Laird (1973) and Laird et al. (1973) also argue that these renaturation kinetics properties offer ample refutation to Thomas' (1973) results in support of the model set forth by Callan and Lloyd (1960). Laird's conclusion, if correct, would effectively eliminate this interpretation, then, from further consideration.

Transcript size The second essential feature, already briefly discussed, of the relationship of the chromomere to protein synthesis, is the size of the messenger RNA molecule. If the initial transcript were of a length that reflected only 1000 to 2000 nucleotides of DNA, then the possibility for multi-cistronic chromomeres would be precluded. This has not been demonstrated; on the contrary, recent evidence indicates that the initial RNA transcript is too large for the interband alone, or a similarly sized intrachromomeric sequence, to have been responsible for its production. Indeed, the entire sequence of nucleotides defined as the chromomere may be implicated, as has been recently suggested by Laird (1975). This suggestion, you will recall, is in agreement with conclusions drawn by Daneholt and Hosick (1973) regarding the properties of the transcript emanating from BR-II in Chironomus. How-

ever, the sensitivity of tests employed in either study does not resolve the involvement of the interband regions in transcription, as would be desired. If the entire chromomere is transcribed as is suspected, then few conclusive arguments can be made at the present concerning the fate of the initial product, since much evidence has accumulated which indicates that a certain amount of RNA processing occurs between the nuclear chromosome and the cytoplasmic translation apparati.

RNA processing The occurrence of very large pre-messenger RNA molecules (the so-called heterogeneous nuclear or "hn" RNA) which subsequently undergo enzymatic processing prior to cytoplasmic translocation was first reported by Georgiev and Mantieva (1962). Since their discovery, a great deal of interest has been manifested and energy expended in the analysis of this process, but only the results pertinent to this discussion will be briefly described (for review, see Lewin, 1975a, 1975b). Summarily, the following observations from studies of numerous cell types have been noted:

1) The initial RNA transcript (hn RNA) is a very large fragment of high molecular weight.

2) By a rapid nuclear mechanism, the hn RNA is selectively cleaved, the original 3'terminus polyadenylated for apparent conservation (i.e. protection from continued

cleavage), and transported from the nucleus to the cytoplasm. However, present evidence does not eliminate the possibility that sheared fragments may also undergo similar changes (Derman and Darnell, 1974).

3) The processed and transported RNA molecule (considered mRNA upon reaching the cytoplasm) is but a fraction of the length of the original hn RNA transcript.

4) Little is known about the fate of those sheared fragments of hn RNA which are not immediately polyadenylated and/or transported to the cytoplasm.

Gelbart et al. (1974) have recently concluded that the size of xanthine dehydrogenase, the enzyme product attributed to the "rosy" (ry) locus in Drosophila, reflects only a very small fraction of the chromomere(s) DNA being responsible for its specificity. From the known size of each subunit of the functional xanthine dehydrogenase molecule (an enzyme catalyzing the dehydrogenation of xanthine), these investigators reasoned that only about 3000 nucleotide pairs of DNA within the rosy locus coded for its synthesis. However, Gelbart and his colleagues have not yet succeeded in isolating and characterizing the hn RNA molecule from which the translational template was presumably derived. Unfortunately, and particularly relevant to this discussion, the cytological assignment of the rosy locus to a particular chromosome band (or bands) is far from exact.

Thus, as we have seen, the apparent independent transcription of an individual chromomere adds support for its unit character. However, our present understanding of transcription in eucaryotes provides little additional information regarding sub-chromomeric organization and composition.

The Chromomere as a Unit of Function

Gene number

The *Drosophila* chromomere is comprised of a large mass of DNA (average size = 30,000 nucleotide pairs) and appears to maintain a degree of autonomy in replication and transcription. Central to the issue of chromomeric unity has been the attempt to ascertain whether one chromomere is responsible for the coding of one, or more than one, polypeptide (disregarding r- and t-RNA specific DNA sequences from this consideration). Or put more simply, is the eucaryotic chromomere analogous to a single procaryotic cistron? The significance of this question intrigued early cytogeneticists and continues to be one of the most controversial, paramount issues in biology today.

Painter (1934), in his original paper on the banding pattern of polytene chromosomes in Drosophila, suggested, essentially, that each band represented one gene. Muller and Prokofyeva (1935) concurred with Painter's opinion

following their studies of a number of gross chromosomal rearrangements. Subsequently, many investigators have noted the association of a specific mutant phenotype with a particular band on the basis of deletion mapping and rearrangement breakpoint analyses (Mackensen, 1935; Demerec, 1941; Hannah, 1949; among others). Bridges (1935) also commented on this probability and proposed that the number of bands in a particular region, regardless of their size, was equal to the number of genes which localized there. More specifically, Bridges (1935) estimated the total gene number to be 3,540, corresponding to the number of recognizable bands on his early map; this figure was subsequently raised to 5,072 in revised maps published later (Bridges, 1938; Bridges, 1942). Although Bridges' figures may be slightly in error as suggested by later electron microscope examinations (for a review of these discrepancies, see Lefevre, 1974), his early maps of the entire Drosophila genome provide the best estimates of total band number available. His contention, however, that band number equals gene is not severely challenged by suggestions that his maps represent overestimates of band number (Berendes, 1970).

The most frequently employed means of calculating the number of independent genes has traditionally been by mutant saturation of a particular chromosome or a sharply defined chromosome region. Underlying such an approach are the

assumptions (still unproven) that all the DNA comprising a definite region is equally mutable and that all resulting abnormalities are equally detectable by common screening procedures.

Early investigators used lethal mutation induction and supposed saturation to estimate the number of genes in Drosophila (Alikhanian, 1937; Gowen and Gay, 1933). Although, in retrospect, one might consider many of their assumptions unfounded (Beerman, 1972), their calculations of total number of X-linked genes are remarkably similar to the number of bands recognized by Bridges (1938). More recently, extensive studies employing similar techniques, but accommodating the molecular properties of the chromosome, have been undertaken to better determine gene number.

The most ambitious of these, by far, has been the attempted mutant saturation of a small region of the X chromosome by Judd, Shen, and Kaufman (1972). Employing various means of mutant induction (e.g. X-ray, ethyl methanesulfonate, and N-methyl-N'-nitro-N-nitrosoguanidine), they isolated 121 lethal, semi-lethal, or gross morphological mutants which were later shown to map in a small region defined by salivary bands 3A1 to 3C2, inclusive. By means of deletion-duplication mapping and complementation tests, Judd and his associates determined that each of the 121 isolates could be assigned to one of 16 linear comple-

mentation groups between "zeste" and "white" on the genetic map. Since more than one new mutant could be assigned to each complementation group (and many contained several apparent alleles), Judd et al. concluded that the 3A1-3C2 region had been saturated with lethal or semi-lethal mutations. Most significant to this discussion is their pointed observation that the number of complementation groups (16) is identical to the number of chromomeres located within this segment. Judd et al. conclude from these results that the chromomere is the simplest chromosome unit which contains the information for a single function. They further offer their results in support of the one band-one gene concept of eucaryotic chromosome organization.

Additional support for this position has come from Hochman's (1971) analysis of chromosome IV of D. melanogaster. Although his investigation has not been completed, he has shown that 33 loci are capable of mutating to lethality (chemical, X-ray, and spontaneous) on the small 4th chromosome. He estimates that there are probably no more than 40 essential loci on chromosome IV which approaches the number of bands (50) present (see Slizynska, 1944; Hochman, 1971).

Lifschytz and Falk (1968, 1969) have conducted a similar analysis of a small region of the X chromosome, comparable in size to the 4th autosome studied by Hochman

(1971). The combined results of their studies indicate that the 105 induced lethals (35 X-ray and 70 chemically induced) could be assigned to 34 complementation groups. This figure is in close agreement with the number of bands assigned to the proximal segment of the X by Schalet et al. (1970).

Before turning our attention to proposed models for chromosome organization, it may be appropriate to consider a few of the objections that have been voiced in opposition to conclusions drawn by the mutant-saturation workers described above.

O'Brien (1973), for one, has taken notable exception to conclusions drawn from studies performed by, or similar to those of Judd and his associates. First, he argues that the mutant screens were devised to facilitate the easy detection of lethal, semi-lethal or severe anatomical aberrations. As a result, many loci which code for non-vital functions, but localize within the same region, may have escaped detection. As a basis for this argument, he has assembled a list consisting of fourteen structural genes in Drosophila for which "null" alleles are known. Of these loci, in only one, the "bobbed" locus, does a alteration to the null form result in lethality. If such were the case at many more loci, then, O'Brien asserts, the screening techniques employed would necessarily fail to detect their

existence, resulting in an underestimate of total gene number. Furthermore, the undetected localization of such non-vital genes in a particular region would certainly indicate that the region had not been saturated, as might have been concluded. Lefevre (1974) has challenged this argument with his interpretation of the voluminous data on chromosomal rearrangement breakpoints and the phenotypic consequences which result. He notes that such breakpoints are associated with either a detectable mutant effect (including lethality) or no apparent effect at all; in no case, to his knowledge, do breaks involving a particular band cause both effects. Lefevre maintains that such dual effects would be expected if the chromomere were comprised of units of both vital and neutral function as suggested by O'Brien (1973).

Davidson and Britten (1973) also take exception to the interpretations of Judd et al. (1972) and present a somewhat novel view of the complementation group. They suggest that this genetic unit may also represent a regulatory element which controls the activity of many structural genes. Such a regulatory system might be contiguous with its structural genes (i.e. cis-dominant) or localize elsewhere in the genome and only appear to map at the same mutant site. Mutation to a regulatory element may, in fact, result in lethality if many coordinated informational sites are

rendered nonfunctional by the debilitating effects of the alteration. This explanation might also account for many of the diverse pleiotropic effects commonly attributed to the mutation of a single strucutral gene.

Others have objected to the one band-one gene correlation on grounds that much difficulty is often encountered in determining the exact number of bands for a particular region (see Davidson and Britten, 1973). The uncertainty in designating particular, sub-light microscopic bands as gene loci is best exemplified by the work of Sorsa, Green, and Beerman (1973) at "white" and Welshons (1974) at "Notch".

A final objection, and probably the most common, to the one band-one gene hypothesis is the requirement to justify and characterize the copious quantities of DNA per chromomere, accepting the estimates by Rudkin (1961) as correct. That most of the DNA is functional, in one way or another, is to be expected when considerations are given to selective pressures which would presumably act to eliminate superfluous, energy-consuming material. Scientific folklore, with its appreciation for the conservative propensity of "mother nature", would certainly question a chromomere being comprised of large, constitutive amounts of nonfunctional DNA. As will be seen in the section which follows, numerous models have been proposed in attempts to assign meaningful roles to the chromomeric DNA.

Thus, after considering the voluminous evidence in support of the unit function of the chromomere and the accompanying objections raised in opposition, we are still faced with the confusing, but intriguing, dilemma for which no clear-cut explanation exists. Attempts, in the form of explanatory models, have been made to render compatible those observations defining this paradox.

General models for chromomere organization

As knowledge of the molecular composition of the eucaryotic chromosome expanded and information describing its many functional characteristics were assimilated, interest focused on the chromomere as the central unit in replication, transcription, and function. So too, endeavors to view the chromomere as a comprehensive unit have resulted in an abundance of models to characterize its organization. These models deal primarily with transcription, and those mechanisms presumably required to regulate it.

Subsequent to the demonstration of a coordinated system of multi-cistronic control and function in bacteria (Jacob and Monod, 1961), a flood of enthusiasm surfaced in favor of a similar system for higher organisms. That eucaryotic genes might be organized into operon-like complexes seemed reasonable; on the other hand, the existence of many chromosomes and a developmental specificity unshared by the bacteria,

would, probably, require a somewhat different organization. Nonetheless, the existence of a "super-operon" has yet to be demonstrated in eucaryotes although most of the models to be discussed hint at a similar level of complexity.

The earliest model to be proposed was that of Callan and Lloyd (1960). They suggested that the chromomere was comprised of tandemly repeated, identical sequences of DNA ("slaves") which would specify the same gene product, and which were regulated by a common "master" sequence. The master sequence would direct the transcriptional activities of its slaves and rectify most of the deleterious changes incurred by them as a result of mutation or recombination. This interpretation has been summarily dismissed in view of the results of renaturation kinetics studies which indicate that the majority of Drosophila DNA is comprised of unique sequences.

The model of Britten and Davidson (1969, 1971) followed chronologically and offered both a novel interpretation of gene regulation, and a system whose potential for testing continues to expand. Central to their paradigm is a mechanism for the complex coordinate control of small numbers of non-contiguous structural genes (i.e. protein-coding cistrons). Their model is summarized as follows: first, an external developmental stimulus sensitizes specific DNA sequences called "sensor" genes which in turn activate

another sequence, the "integrator" gene. Upon activation, the integrator genes produce an "activator" RNA molecule whose sequence specificity is required for the subsequent reaction with the "receptor" sequence or gene (analogous to the "operator" region in the operon complex). The receptor gene is structurally linked to the "producer" genes (i.e. structural, a la Jacob-Monod, 1961) and facilitates the transcription of its producer gene when complexed with the activator molecule, be it RNA, or a protein molecule as more recently suggested (Davidson and Britten, 1973). Only producer sequences are required to be unique in the Britten-Davidson scheme which might account for a multiplicity of coordinately controlled metabolic activities. Thus, one or many producer genes might be activated in response to a common developmental signal. The possible roles of the chromomere, itself, are many in this proposal. Each chromomere might possess one or all of the types of "genes" required in the total operation.

Georgiev (1969) has proposed another operon-type model which is strikingly similar to that of Britten and Davidson (1969, 1971). He describes the chromomere as being divided into two primary "zones": promoter - proximal acceptor (or noninformational) zone and promoter-distal structural (or informational) zone. The noninformational zone would contain one or more control sequences (operators or promoters),

either different or identical, which would react with specific regulatory proteins to prescribe the transcriptional activities of the adjacent informational zone. Selected structural genes might also code for the regulatory proteins required in this system. Considerations for the large primary transcript (hn RNA) and the possible roles of its degradation products are included in Georgiev's interpretation. The feature that distinguishes this model from the Britten-Davidson model (1969, 1971) is the close linkage of regulatory and structural elements within a single functional unit (the chromomere?).

The chromomere model most distinctly different from the others in its interpretations is probably that proposed by Crick (1971). Crick suggested this explanation, primarily, to accommodate the vast amount of cytogenetic data compiled from studies of gene-band localization. Its most radically unique feature is the assignment of the structural gene (the so-called "fibrous coding" DNA) to the interband. The band itself is assigned a totally regulatory function in much the same capacity as controlling sequences in any operon-type model. The supercoiled loops of the band would unravel over a distance of several hundred bases (puffs?) to facilitate the proper binding of regulatory proteins (perhaps histones). However, Crick does not amplify the actual mechanisms of control that might be operating. The attractive aspect of Crick's model is the functional role

played by interband DNA which much cytogenetic data would suggest (Welshons, 1974); on the other hand, one might seriously question whether the entire band (about 30,000 nucleotide pairs) is required for regulation in the rather simple manner he prescribes.

Admittedly, none of the models for chromomere organization described above can accommodate the many observations gathered from numerous lines of approach. It is to be appreciated, however, that models of this sort do generate new ideas and provide both useful clues and testable hypotheses from which to proceed. The "goodness of fit" of experimental results discussed in this essay to the suggested models will be considered later (see Discussion).

Analyses of Complex Loci

The fine structure analysis of "complex loci" in Drosophila is one cytogenetic technique now being employed which offers promise in testing the validity of particular tenets of each of the aforementioned models (and others) for chromomere organization. Similar fine structure studies of the genomes of lower forms (e.g. in the viruses and bacteria; see Benzer, 1961) have led to the high resolution of locus "topography" and an improved understanding of the coordinate control of developmental functions.

The concept of a "complex locus" (first described by

Dunn; see Lewis, 1963) has come to represent a specific chromosome region occupied by seemingly unrelated functional groups. Mutants which map within such a locus appear to effect different morphological changes during the same or different stages in organismal development. Furthermore, these mutants exhibit a variety of patterns of complementation in common "cis-trans" tests and generally tend to cluster into "subloci" on this basis. Related mutants of this type were once called "pseudoalleles" (i.e. phenotypically allelic but separable by recombination, when this was the primary criterion for classification) but the unequivocal demonstration of intragenic recombination made this description unfavorable. Upon the suggestions of Lewis (1964), the terms "pseudoallelism" and "pseudoallelic series", when applied to the complex loci, now imply the functional complexity associated with a particular, chromosomal region. Respect for Lewis' suggestion will be shown when using these terms in the discussion which follows. Additionally, the terms "locus" and "gene" will be used rather loosely to specify a location rather than a function.

Several complex loci in Drosophila melanogaster have been and continue to be investigated. The properties of three X-linked, including Notch, and one autosomal complex loci will be briefly discussed (for more complete phenotypic descriptions of each, see Lindsley and Grell, 1968).

The Notch complex

The Notch phenotype was first reported and characterized as a sex-linked, recessive lethal mutation (i.e. lethal in hemizygous males or homozygous females) by Mohr (1919). Slizynska (1938) subsequently examined many Notches associated with cytological deficiencies, including the original, N^{8}, and determined that Notch localized at or near salivary band 3C7 on Bridges map (1938) of the X chromosome. She concluded that the Notch mutation was an amorphic type, resulting from the visible deletion of 3C7. As we shall see, this observation was a premature oversimplification.

Since that time, both recessive lethal and recessive visible Notch pseudoalleles have been isolated and assigned to this locus on the basis of complementation tests and pseudodominant expressions (Welshons and von Halle, 1962). Of the recessive lethals (usually dominant for the Notch phenotype in N^{x}/N^{+} heterozygotes), there appear to be three types. First, the typical Notch, with serrated wing margins and assorted pleiotropic effects, which are, without exception, lethal in an hemizygous male. Second, the "Abruptex" mutations which cause premature termination of one or more wing veins, a distorted bristle pattern, and may or may not be lethal when hemizygous. And third, the non-Notched recessive lethals (e.g. l^{NB}) whose phenotype in heterozygous females is indistinguishable from the wild-type.

The recessive visible pseudoalleles (typical recessive mutants) on the other hand, can be divided into two, or perhaps three, classes on the basis of whether the eyes, the bristles, or the wings are abnormal in the homozygous or hemizygous condition. First, the "facet" series of closely linked alleles are generally characterized phenotypically by the rough or glossy eye facets which result. Second, the "notchoid" series of alleles cause the thickening of wing veins and, most frequently, the nicking of wing margins similar to the dominant N's. The third possible class, presently comprised of a single allele, split (spl), is phenotypically aberrant for both eye texture and thoracic bristle distribution. As will be discussed in greater detail in the body of this paper (see Discussion), two exceptions to the genic distribution of like-alleles do exist (see Figure 1 for the locations of various alleles on the Notch map).

Generally, a correlation can be drawn between allele map location and intragenic complementation behavior. Interestingly, nearly complete complementation is observed (within the visual resolving powers of the observer) between alleles of the two major classes (eye and wing), while non-complementation is generally the case between two alleles of the same series. For example, the facet-glossy (fa^{g})/ notchoid (nd) heterozygote appears phenotypically normal;

however, a $\underline{nd}/\underline{nd^2}$ heterozygote expresses a strong mutant phenotype. The exceptional spl allele appears to complement all other recessive visible members of the N series.

On the other hand, interactions of recessive lethal N-type alleles and the recessive visibles in heterozygotes are generally more consistent: the pseudodominant expression (and frequent enhancement) of the recessive visible is observed, in addition to the dominant Notch affect. In no case is complementation observed between the two. The lethality of one particular heterozygote, $\underline{N^x}/\underline{fa^{no}}$, has provided a useful genetic tool in the fine structure analysis of the locus.

The interallelic complementation of Abruptex (Ax) heterozygotes defies simple definition. These interactions have been the object of recent investigations and will be discussed later.

The results of complementation tests and recombination analyses involving Notch pseudoalleles led Welshons (1965, 1974) to suggest that the locus was comprised of a single cistron which could be assigned to salivary band 3C7 and/or its adjacent interband(s). Although many of the cytogenetic observations are beyond dispute, many equivocal features of the data could lead to alternative interpretations.

The bithorax complex

The "bithorax" pseudoallelic series, located on chromosome III, is comprised of five groups of phenotypically distinguishable mutants, both recessive lethal and recessive visible, which apparently effect different segments of the developing embryo (Lewis, 1963, 1964). The primary mode of mutation is the transformation of one segment (or organ) into a different, but embryologically similar one. For example, Lewis (1964) has demonstrated, by the appropriate genetic construction of specific intra-locus combinations, that it is possible to transform the normally vestigal secondary wings (the halteres) into structures very similar, if not identical, in appearance to the normal primary wings, resulting, quite literally, in a fly possessing two pairs of wings.

Lewis had originally grouped bithorax alleles into subloci on the basis of transformational characteristics; however, when these alleles were subjected to recombination analysis, a direct correlation between the two criteria was noted. Recombination tests revealed the same five subloci (a, B, C, d, e); all five were subsequently shown to map in the 89E region which contains two prominent doublet bands.

The complexities of intra-and inter-series interaction patterns (phenotypic) make for both difficult reading and interpretation. However, certain observations of Lewis'

extensive studies are worthy of note. First, the induction of chromosomal rearrangements involving the bithorax region results in two types of inaction: in one, all of the genes of the entire series appear to be inactivated; in the other, only the wild-type alleles of the "d" and "e" subloci are inactivated. Second, the results of complementation tests, when possible to distinguish phenotypes, indicate that, although five different groups seem to exist within the complex, an interdependence between groups is noted and a coordinate regulatory system is thought to exist.

The interpretation of these observations and others, including polarity effects, prompted Lewis (1967) to suggest that a favorable comparison could be drawn between the bithorax pseudoallelic series and the operon system known in bacteria. For instance, the polarization effects of specific alleles on other subloci and the total inactivation of the entire complex by a particular rearrangement (independent of position effect variegation) are certainly analogous to similar phenomena characteristic of alterations to an operon complex. However, the inability to more specifically assign the bithorax series to a particular salivary band or bands provides little contribution to the solution of the one gene-one band problem.

The rudimentary complex

The rudimentary series has been chosen for this discussion for two major reasons: first, the results of genetic recombination and complementation tests initially indicated that rudimentary is comprised of a single cistron; and second, more recent biochemical analyses point to its complexity and possible multicistronic composition. Rudimentary (r) is a sex-linked, recessive mutation which, in general, effects wing morphology and, often, causes female sterility (Lindsley and Grell, 1968).

Low-scale recombination tests of eighteen r alleles by Green (1963) showed a distribution into two peripheral groups, each containing six or more alleles, and one site distinguished by a single allele, located roughly midway between the two. The same clustering of alleles was seen in cis-trans tests; that is, members of one sublocus showed no complementation, while inter-group heterozygotes were partially complementary for both wing phenotype and female fertility. Thus, the complementation map was colinear with the recombination map, similar to that observed at Notch.

A more extensive, yet similar, study of the rudimentary locus was undertaken by Carlson (1971). His results confirmed in substance those of Green (1963) and Fahmy and Fahmy (1959). He further endorsed the mono-cistronic description of the rudimentary locus, citing the inconsistent

patterns of complementation exhibited. Carlson concluded that, by analogy to similar complementation shown in procaryotes, rudimentary was comprised of a single cistron.

Fristrom and his colleagues (Fristrom and Yund, 1973; Rawls and Fristrom, 1975) have cogently argued in opposition to the interpretation held by Green (1963) and Carlson (1971). They cite numerous molecular mechanisms which might accommodate the detailed genetic data.

Rudimentary mutants are now known to represent functional alterations in the de novo biosynthesis of pyrimidines (Norby, 1970; Falk and Nash, 1974). Rawls and Fristrom (1975) note that the rudimentary locus, as presently characterized, codes for at least three functional enzymatic products in the same synthetic pathway. Furthermore, there appears to be a relationship between enzyme specificity and the complementation group assignment of pseudoalleles; that is, members of the same complementation group effect the same enzyme. These authors propose that the rudimentary complex is comprised of at least three closely linked functionally related cistrons whose transcription is coordinately controlled. They further suggest that other complex loci may share this more sophisticated level of organization.

It should be noted that the cytological assignment of the rudimentary series is far from being informative at the present. However, the impending cytological localization of

r might provide an unequivocal choice between the two interpretations discussed above and a clue to the functional organization of complex loci in general.

The white complex

Detailed genetic analyses of both intra- and intergenic interactions involving white pseudoalleles illustrate the complexity of this locus. As we shall see in the next section, great efforts have been made to assign white to a specific cytological location. Therefore, recognition of the white series is pertinent to any considerations of the cytogenetics of Notch, to which this report addresses itself.

The original white mutation was the first deviation from wild-type to be isolated in the budding days of *Drosophila* genetics (Morgan, 1910). White alleles have since been characterized as sex-linked, recessive visibles which modify the pigmentation pattern of the adult eye. A great many alleles, expressing a variety of different eye phenotypes (see Lindsley and Grell, 1968), localize to the white locus. Fine structure analysis reveals that all white alleles appear to occupy five distinct subloci (Green, 1959; Judd, 1964).

In addition, Green (1959) studied the interesting interactions of thirty-six white alleles with other, non-allelic, eye mutants such as "zeste" (z) and "enhancer of white-eosin" (en-w^e), among others. He found, for example,

that certain w alleles suppress the expression of z homozygotes; that is, a fly, genotypically z +/z w^x, has either zeste or normal eyes, depending upon which white allele is present.

Green (1959) further noted the direct correlation between patterns of non-allelic phenotypic interactions and the distribution of the thirty-six alleles on the white map. First, all white alleles classified as dominant suppressors of zeste map to the right of the pivotal white-apricot (w^a) allele, while those exhibiting no interaction with zeste map to the left. No phenotypic overlap between the two classes was observed. Second, the classification of the same thirty-six with reference to en-w^e interactions showed a similar distribution; that is, all white alleles enhanced by en-w^e map to the right of w^a while the nonenhanced localize to the left.

Thus, Green concluded from these results that the white locus was probably comprised of two functionally and spatially distinct groups of pseudoalleles. This classification, he felt, was similar to the functional organization of the bithorax series (Lewis, 1963, 1964). Green further considered the two phenotypic groups a reflection of the alterations of two different gene products which functioned both in eye pigment deposition and in interactions with those of other loci.

Gene-Band Assignment

As alluded to in the previous discussions, the unequivocal assignment of a particular complex locus to a specific salivary band has yet to be achieved. Indeed, such a demonstration, coupled with knowledge of specific gene products, would contribute greatly to our understanding of the structure-function relationship. Needless to say, extensive efforts have been made to accomplish that intent.

Classically, these studies have been done at the level of light microscope resolution which, necessarily restricted by its limits, have yielded significant bounties (e.g. see Demerec, 1941). More modern electron microscope mapping techniques (Sorsa and Sorsa, 1967) have recently been employed to attain greater resolution. Sorsa, Green, and Beerman (1973) recently employed these techniques in a cytogenetic study of white. Prior to this study, the white locus has been variously assigned to band 3C2 or the doublet 3C2-3 on the basis of rearrangement breakpoint determination and deletion mapping (see Lefevre and Green, 1972). Sorsa *et al.* (1973) focused their study on the inversion chromosome, $\underline{In(1)z^{+64b9}}$, which effects a change in $\underline{w^{+}}$ function. They deduced that the functional description resulted from a loss or displacement of chromosome material in the 3C1-3C2 interval or that defined by a very faint band located there. The possibility that band 3C2 itself

involves $\underline{w^+}$ function is not ignored but probably only to an extent limited by the width of the faint band.

The cytological localization of the Notch locus, also found in the "3C" region of the X chromosome, has received much attention. Classical studies conducted by Slizynska (1938) pointed to the involvement of band 3C7 in $\underline{N^+}$ activities; deficiencies for 3C7 invariably result in the aberrant Notch phenotype. However, more recent cytogenetic studies of Notch emphasize the need to re-evaluate Notch data.

Briefly, Welshons (1974; and Welshons and Keppy, 1975) has noted that nonoverlapping deficiencies, whose breakpoints are either in the interbands to the left or right of 3C7, alter $\underline{N^+}$ function and behave, in complementation tests, as members of the Notch series. He notes that an interpretation of the cytological data, alone, would indicate a bilaterality of function associated with the interbands adjacent to 3C7. Such an interpretation, if correct, would be a striking departure from the unilateral band association observed at white and other loci, but Welshons (1974) cites evidence regarding the localization of the "vermilion" locus (Lefevre, 1969) in support of this position.

Development of the Problem

In the foregoing discussion, it has hopefully been shown that the elucidation of the complex structure-function

relationship of the Drosophila chromomere is far from complete. The in-depth analyses of singular aspects of chromomeric function illuminate the rather autonomous nature of this unit. However, when one considers the chromomere as comprehensively involved in various activities in the development and maintenance of the functioning organism, its independence is rapidly diminished.

Consideration has been given to the roles of the chromomere in such seemingly mutually exclusive processes as transcription, replication, and recombination. Lacking the experimental tools required to approach the chromomere as a comprehensive unit, investigators must, by necessity, focus on seemingly isolated aspects of the functional activities of the chromomere.

Thus, the thrust of recent investigations of the Notch locus and other complex loci in Drosophila melanogaster has been to better understand the functional unit(s) - chromomere relationship. To cope with the higher DNA content and the supposed greater complexity of the Drosophila genome as compared to representative procaryotes, students of the chromosome have employed many classical cytogenetic techniques to characterize a particular region. This approach early contributed to an understanding of bacterial gene structure-function and helped to distinguish between different concepts of the mechanics of gene action.

Hence, such was the reasoning invoked in applying gene fine structure techniques to the amplification of the organization of the Notch locus. The Notch locus, because of its noted complexity, offers a suitable system for considerations of intra-locus composition and the gene-band dilemma.

Although the limitations in any fine structure analysis are appreciated, it is felt that the elucidation and localization of any particular chromosomal unit may provide useful observations and suggest additional approaches toward the ultimate solution of the problem. It is certainly realized that the final explication will incorporate the results of cytogenetic examinations, imposed upon a detailed analysis of the gene product(s) associated with a specific region. Necessarily, the results from each approach must be compatible.

In this report, the genic localization of a number of Notch mutants will be described. These mutants include cytological deficiencies, recessive visibles, and mutagenically induced recessive lethal pseudoalleles.

MATERIALS AND METHODS

Culture Techniques

Genetic experiments

Cultures of *Drosophila melanogaster* were grown in quarter pint glass bottles on a medium consisting of a mixture of corn meal, sugar, yeast, and agar. Experimental cultures were maintained in a 25°C incubator (Percival Manufacturing Co.); progenitor and background stocks were maintained at room temperature and subcultured at regular fourteen day intervals. Schedules for subculturing parents in genetic experiments varied; an experience-guided appreciation of the fertility and viability of the female parents and of the anticipated progeny requirements directed these schedules. In addition, an effort was made to coordinate subculture schedules with other laboratory workers conducting similar experiments to maintain a degree of consistency upon which to make comparisons. On the average, however, subculturing followed a four, three, three, eight day routine to insure optimal culture conditions and maximum numbers of progeny. Virgin females to be used in genetic experiments were generally aged one or two days prior to being mated.

Cytological experiments

Cultures used for larvae production and subsequent polytene chromosome examination were grown on the same basic

medium but with supplemental dried bakers' yeast sprinkled on top to facilitate optimal egg-laying conditions; tissue squares were not inserted into the medium to allow easy larvae collection. Generally, the cultures were incubated for two days at 25°C, after which the parents were subcultured or discarded and the original bottles maintained thereafter at room temperature until third-instar larvae emerged for collection.

Cytological Techniques

Prior to detailed genetic analysis of Notch mutants, cytological preparations of salivary gland chromosomes were generally examined. As previously described, the Notch complex has been assigned to salivary band 3C7 on Bridges' (1938) map. To facilitate the maximum chromosome stretching required to consistently distinguish 3C7 from the adjoining doublet, 3C5-6, a method for chromosome preparation developed by Welshons (personal communication) was adopted and is described as follows. Plump, third-instar larvae were collected from cultures grown under optimal conditions and separated according to sex. Only male larvae were retained due to the rather distorted, and generally noninformative, banding pattern exhibited by females. The salivary glands were then dissected in a .75% saline solution under a binocular dissecting microscope. After removal, the glands

(one pair) were transferred to a siliconed slide, fixed in a drop of 50% acetic-alcohol for 3-5 seconds, rinsed in a drop of deionized water for 30-60 seconds, and transferred to a drop of 2% lactic-acetic orcein stain (synthetic orcein, Hartman-Leddon Co.). After staining the glands 30-60 seconds, a coverslip was gently applied and the glands disrupted and squashed. Since only temporary preparations were required, the coverslip was edged with rubber paraffin (1%) or clear fingernail polish, both of which satisfactorily preserved the preparations for one week. The salivary chromosomes were examined using a Zeiss Photomicroscope and photographed when required.

Genetic Techniques

Mechanics of experimental approach

The Notch locus is located 3.0 map units (per cent recombination) from the distal end of chromosome I (the X). Mapping at this locus are numerous recessive lethal and recessive visible pseudoalleles which effect a variety of morphological aberrations, including serrated wing margins (hence, "notch"), bristle number and distribution, facet texture of the eyes, wing vein termini, and "hairiness" of the legs. As noted in the Introduction, recessive visible mutants associated with similar morphological deviations tend to localize in clusters on the genic map.

Recombination experiments demonstrate that most alleles are separable and arranged in a linear sequence (see Figures 1 and 2 for maps of the Notch series). The frequency of crossing over between member alleles has been used to define the limits of the locus. These intragenic frequencies are further thought to be indicative of the relative amount of DNA comprising the locus (Lefevre, 1971).

The separation of recessive visible Notch alleles employs techniques similar to those used in typical intergenic crosses except that one must usually compensate for relatively close interallelic linkage and the possible reduction in the viability and fertility of the trans-heterozygous female. Furthermore, considerations must often be made for partial and/or complete interallelic complementation which might effectively prevent the recovery of both recombinant classes. For example, the phenotypes of two closely related alleles such as "facet" (fa) and "facet-3" (fa^3) (see Table 1 for descriptions of Notch alleles and nonallelic mutant markers included in genetic tests) are often indistinguishable. To distinguish a chromosome marked with one or the other from a chromosome carrying both (i.e. coupled) is often an impossibility. Such cases require the inclusion of phenotypically unambiguous flanker markers on each chromosome to confirm the occurrence of the crossover event and to provide a means for

distinguishing reciprocal and nonreciprocal exchanges.

The separation of recessive lethal N's from recessive visible alleles is usually achieved similarly. However, the commonly encountered reduction in viability and fertility (or even lethality) of the female heterozygote necessitates the introduction of a small duplication, including N^+ functions, which acts to cover the detrimental effects of the Notch mutations. This duplication, called $Dp(1;2)w^{51b7}$ and carrying the normal alleles of white, roughest, verticals, and diminutive, has been inserted into chromosome II and segregates autosomally (Lefevre, 1952).

The inclusion of this autosomal duplication is an absolute requirement in crosses designed to separate two recessive lethal N's due to the lethality, without exception, of such heterozygotes. Furthermore, in crosses of this sort, one of the N chromosomes is usually marked with w^a (a white allele located 1.5 units to the left of Notch) which is also covered by the autosomal duplication; the other N chromosome is appropriately marked with a non-Notch mutant located to the right (e.g. ruby and singed-3 are common proximal markers). Such flanker markers serve two purposes: first, they allow one to track the duplication through the cross; and second, their contribution to phenotypic expression allows easier screening of progeny and better recognition of recombinant chromosomes.

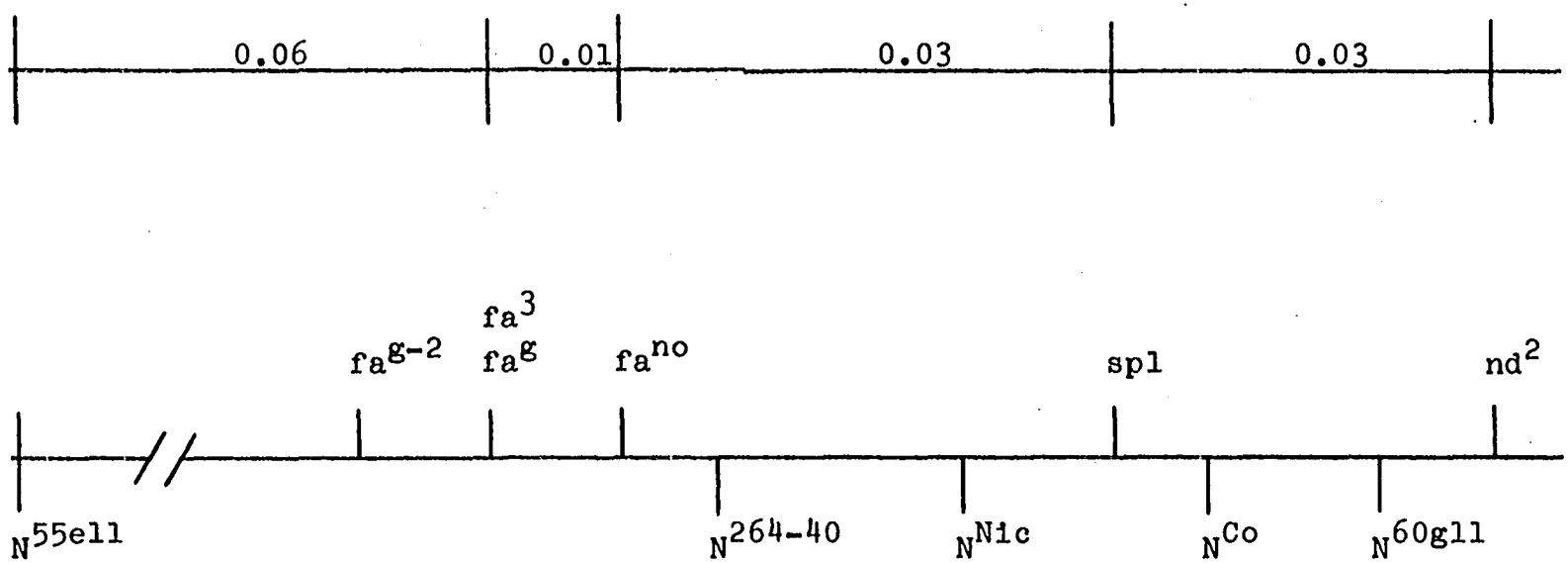


Figure 1. A simplified genetic map of the Notch locus from Welshons (1974), with fa^{g-2} and fa^{3} inserted. Recessive visible alleles are located above this line and recessive lethals below. Relative map distances are designated above.

Figure 2. Composite map of the Notch locus (Welshons, 1974, unpublished; Shellenbarger and Mohler, 1975; and this report). Recessive visible alleles are located above the line and recessive lethals below. Horizontal, parallel lines represent approximate locations of alleles not precisely positioned. A, B, and C columns of recessive lethal alleles indicate those alleles found in this report, Welshons (unpublished, personal communication) and Shellenbarger and Mohler (1975), respectively.

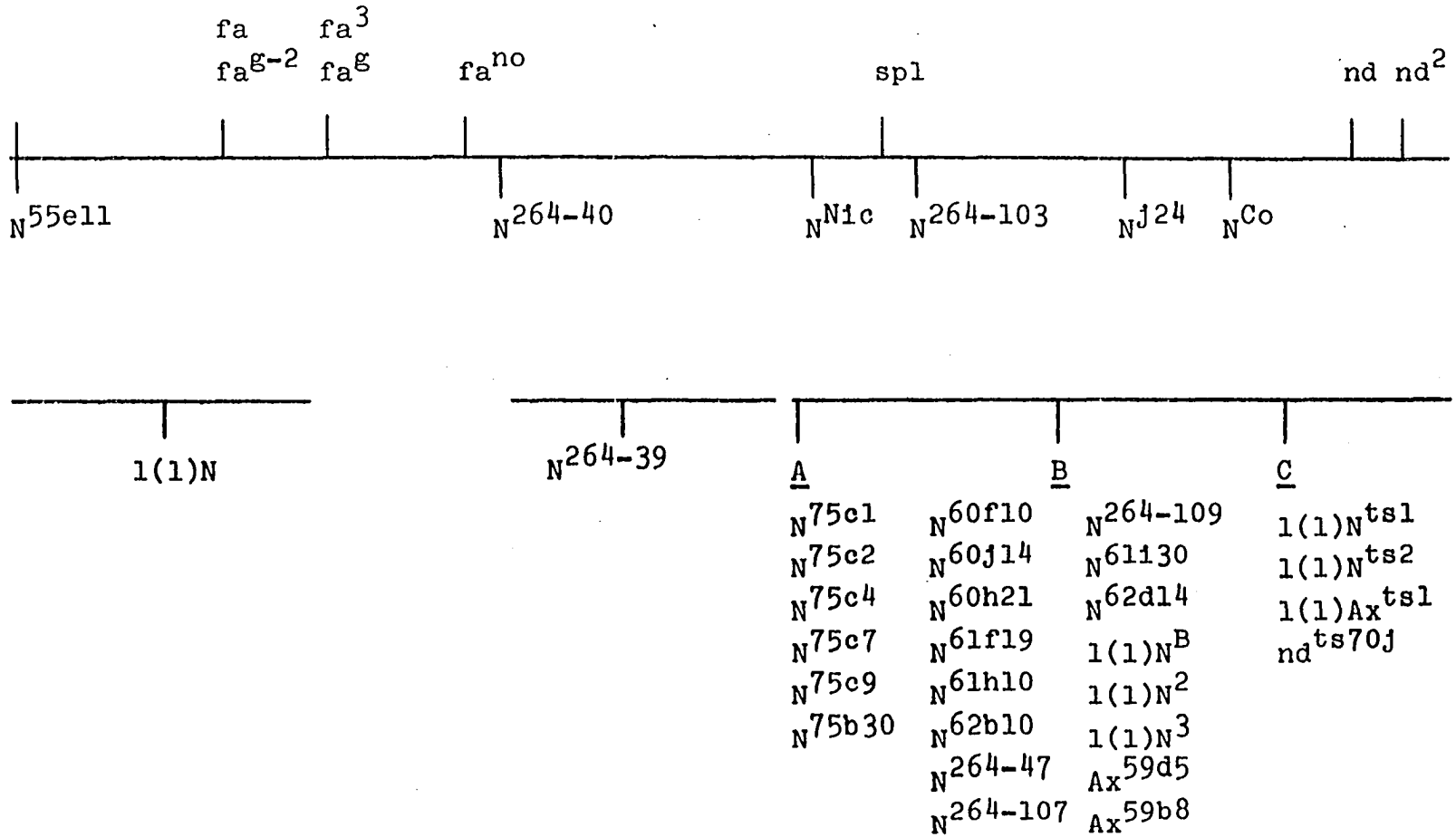

fa
fa^g-2
fa^3
fa^g
fa^no
spl
nd
nd^2
N^55e11
N^264-40
N^Nic
N^264-103
N^j24
N^Co
1(1)N
N^264-39
A
B
C
N^75c1
N^75c2
N^75c4
N^75c7
N^75c9
N^75b30
N^60f10
N^60j14
N^60h21
N^61f19
N^61h10
N^62b10
N^264-47
N^264-107
N^264-109
N^61i30
N^62d14
1(1)N^B
1(1)N^2
1(1)N^3
Ax^59d5
Ax^59b8
1(1)N^ts1
1(1)N^ts2
1(1)Ax^ts1
nd^ts70j

Table 1. Phenotypic description of X chromosome mutants employed in genetic tests or referred to in text.

Symbol	Name	Phenotype
Notch Mutants		
N	Notch	Recessive lethal; serrated wing margins, thickened wing veins, delta-like vein termini, distorted thoracic bristle distribution.
fa	facet	Recessive visible; moderately roughened eyes; pseudo-dominant effect with N.
fa^{g}	facet-glossy	Recessive visible; shiny, glossy eyes; eye pigment often unevenly distributed; pseudodominant effect with N.
fa^{no}	facet-notchoid	Recessive visible; wing margins nicked similar to N; no eye effect. Females heterozygous for fa^{no}/N almost always lethal.
spl	split	Recessive visible; roughened eyes; eyes usually more narrow than wild-type; thoracic bristles often doubled or absent; pseudo-dominant effect with N.
nd	notchoid	Recessive visible; wing margins nicked similar to N; no eye effect; pseudodominant effect indistinguishable because of phenotypic overlap with N.

Table 1 (Continued)

Symbol	Name	Phenotype
Non-Notch Mutants		
y	yellow	Recessive visible located 3.0 units to the left of N;yellow body color.
w^a	white-apricot	Recessive visible member of w pseudoallelic series located 1.5 units to the left of N; apricot eye color.
rb	ruby	Recessive visible located 4.5 units to the right of N; ruby eye color; white eyes in combination with w^a (i.e. $\frac{w^a\ rb}{w^a\ rb}$ = white eyes).
sn^3	singed-3	Recessive visible located 18 units to the right of N. Bristles twisted and shortened, giving a "singed" appearance.

Figure 3 illustrates a diagrammatic representation of the three experimental approaches employed in the separation of Notch alleles described above. It will be noted in Figure 3 that appropriately marked females, heterozygous for at least one N-type allele, are always crossed to males carrying, among other markers, the recessive visible allele, facet-notchoid (fa^{no}; see Table 1). The lethality or semi-lethality of N/fa^{no} heterozygous female progeny can be used to the definite advantage of reducing the number of viable progeny without measureably reducing the sensitivity of the test.

Determination of map distances Recombination experiments are designed primarily to demonstrate the presumed separability of two alleles; that is, one wishes to determine the genetic site of a particular mutation relative to the established positions of other alleles comprising the locus. The genotype of the subsequent recombinants, deduced from their phenotypic expression and appropriate progeny testing, specifies whether the allele being tested maps to the left or right of reference genic sites.

Secondarily, however, one may wish to estimate the relative distance between two alleles from the frequency in which crossing over occurs between them. The estimation of distances between two recessive visibles generally presents no problem because all progeny usually survive and, theoretically at least, the recovery of both classes of recombinants

Figure 3. Illustration of common crosses employed to separate two recessive visibles (A), a recessive visible and a recessive lethal (B), and two recessive lethals (C). Flanker markers might vary but are important to distinguish recombinant from parental chromosomes. The second Notch recessive visible allele in the males might also vary to facilitate the unambiguous detection of both male and female recombinants. Symbols: a^1 and a^2 = any two recessive visible heteroalleles, N = recessive lethal Notch, and Dp = autosomal duplication, Dp(1;2)51b7.

A

$$\frac{w^a\ a^1}{+\ +} \times \frac{+\ +}{a^2\ rb} \quad X \quad w^a\ a^{1\ or\ 2}\ rb$$

Screen Progeny for Recombinants

or

$$\frac{w^a\ +}{+\ a^1} \times \frac{a^2\ +}{+\ rb} \quad X \quad w^a\ a^{1\ or\ 2}\ rb$$

Screen Progeny for Recombinants

B

$$\frac{w^a\ N^x}{+\ +} \times \frac{+\ +}{a^1\ rb}\ (;\ \frac{Dp}{+}) \quad X \quad w^a\ fa\ fa^{no} \text{ or } w^a\ fa^{no}\ spl$$

Screen Progeny for Recombinants

or

$$\frac{w^a\ +}{+\ a^1} \times \frac{N^x\ +}{+\ rb}\ (;\ \frac{Dp}{+}) \quad X \quad w^a\ fa\ fa^{no} \text{ or } w^a\ fa^{no}\ spl$$

Screen Progeny for Recombinants

C

$$\frac{+\ N^x}{w^a\ +} \times \frac{+\ rb}{N^y\ +}\ ;\ \frac{Dp}{+} \quad X \quad w^a\ fa\ fa^{no} \text{ or } w^a\ fa^{no}\ spl$$

Screen Progeny for Recombinants

is possible. Adjustments in calculating recombination frequencies must be made, however, when phenotypic overlaps in progeny classes prevents the recovery of both types of recombinants.

Similar compensations for the lethality of hemizygous N males and heterozygous N/fa^{no} females must be made when determining the map distances in experiments involving recessive lethal N's. In addition, these lethal properties prevent, without exception, the detection of all double mutant recombinants. The number of progeny which survive, then, by virtue of the presence of the duplication, is used to estimate the number of tested chromosomes (i.e. total number of progeny in calculations). Appropriate adjustments are then made for inherent experimental shortcomings.

Determination of viable progeny number Viable, non-recombinant progeny, after being scored, were collected in a temporary morgue consisting of an 80% alcohol and detergent solution. Upon completion of an experiment, the preserved flies were water-washed thoroughly and placed in a drying oven (40-50°C) for about one week. A small number of dried flies (usually about 1,000) were hand-counted and weighed. The remaining uncounted flies were simply weighed and their estimated number determined by comparison to the number and weight of the counted flies. The total number of progeny

determined by this means was then used to calculate the recombination frequency between tested alleles.

Mutant Induction and Isolation

Before moving to an analysis of the actual recombination experiments conducted, it may be appropriate at this point to explain the techniques employed in the chemical induction and isolation of new recessive lethal Notch mutations. The mutant induction experiment was undertaken in an attempt to isolate N-type mutants that localize in the left-most half of the present genic map or in band 3C7, itself (see Figure 2; note that most N-type point mutants map to the right of split (spl)). Furthermore, it was wistfully hoped that a newly induced mutant might manifest itself in a somewhat unique genetic expression.

Two hundred 1- to 3-day old wild-type (Canton-S) males were treated with ethyl methanesulfonate (EMS) for approximately 24 hours according to the feeding method of Lewis and Bacher (1968). Ten treated males were mated to fifteen 1- to 2-day old virgin females homozygous for white-apricot (w^a) and split (spl; a recessive visible of the Notch series). Subsequently, the males were mated to new harems of fifteen virgin females every two days for a total of ten days and then discarded. Fertilized females were subcultured three

times and discarded.

F_1 female progeny were screened for any of the typical Notch phenotypes previously described and for the pseudo-dominant expression of spl to be expected in a N/spl heterozygote. Putative Notch isolates were then mated to wild-type males to establish the genetic basis for the observed abnormal phenotype; if so concluded, the F_2 females (N/+) were mated to the X chromosome balancer, y^2 M-5 (see Lindsley and Grell, 1968). The resultant balanced stocks were then maintained at room temperature for further genetic testing to be described in detail later. Figure 4 illustrates the mechanics of the mutant induction and isolation techniques employed.

Genetics of Notch Pseudoalleles

Genetics of left-side deficiencies

Welshons (1974) and Welshons and Keppy (1975) reported the preliminary results of ongoing genetic experiments involving four cytologically deficient chromosomes: Df(1)w^{67k30}, Df(1)N^{68f19}, Df(1)N^{66i25}, and Df(1)N^{62b1}. Only the latter three are associated with mutations to N; w^{67k30}, although deficient for the region adjacent to Notch, is apparently N^+.

Two of those effecting Notch, N^{68f19} and N^{66i25}, are thought to be deficient for salivary bands 3C2 to 3C6,

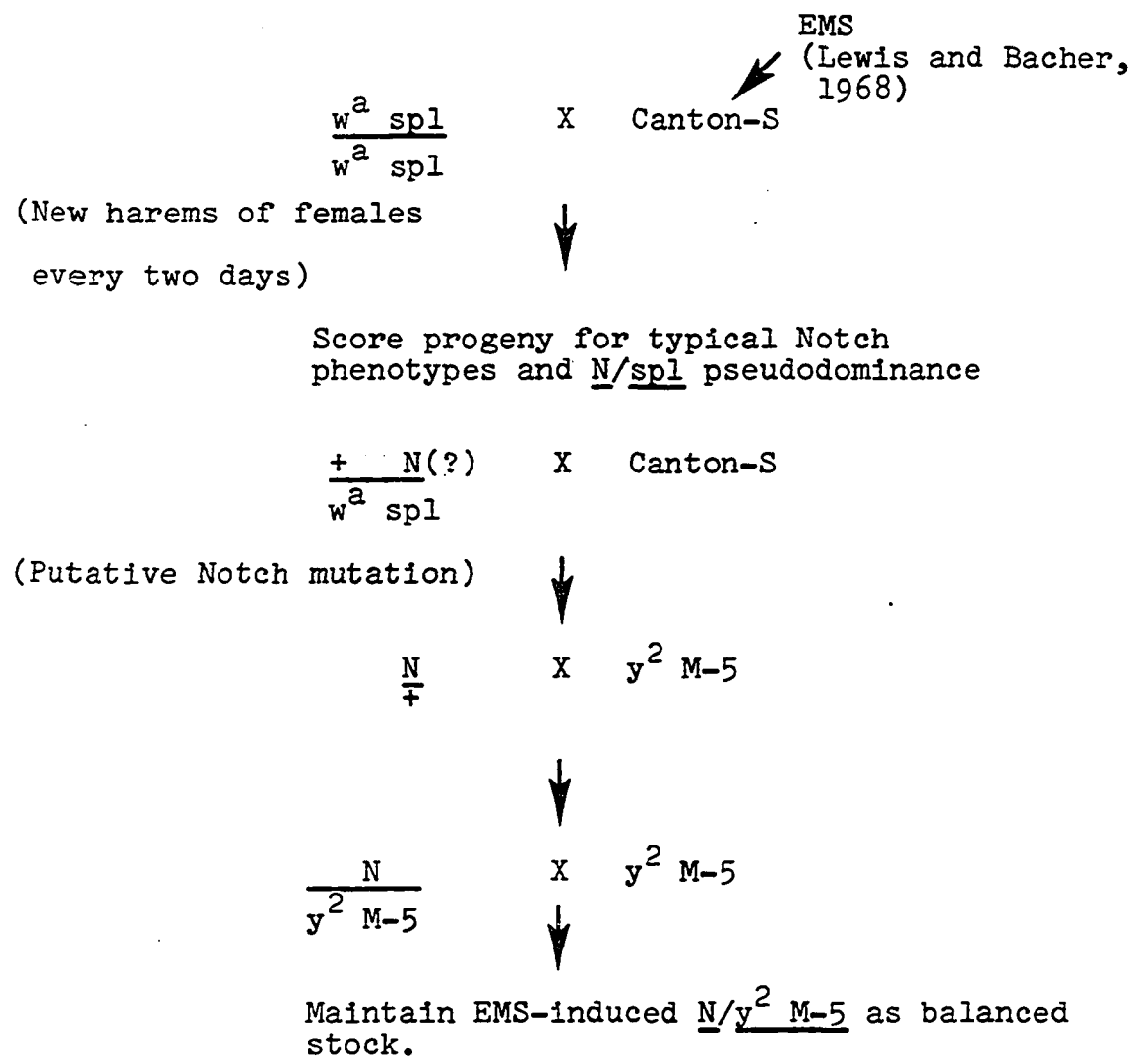


Figure 4. Illustration of technique employed for isolating and maintaining EMS-induced Notch mutants.

inclusive; Welshons (1974) concluded that band 3C7, classically thought to be the site of Notch, remained visible and apparently intact in cytological preparations of each chromosome. Such deficiencies, whose second breakpoint lies distal to Notch, have been described as "left-side" deficiencies. The third deficiency, N^{62b1}, is thought to represent a deletion including part of Notch and extending rightward (proximally) toward and including the "diminutive" locus (dm; located 1.6 units to the right of Notch). N^{62b1} has been appropriately described as a "right-side" deficiency.

In genetic tests, Welshons (1974) determined that both N^{68f19} and N^{66i25} were easily separable from two recessive lethal N's, N^{Co} and N^{60g11}, located in the right-most half of the Notch map (see Figure 1). However, as he extended the tests leftward across the genic map, only one of the two, N^{68f19}, could be separated from N^{264-40}. N^{68f19} and N^{66i25} were both tested with N^{55e11} but unsuccessfully separated from it. Yet, N^{68f19} was subsequently shown to recombine with N^{62b1}, located between N^{264-40} and N^{55e11} (see Results and Welshons, 1974).

In a similar series of tests involving the right-side deficiency, N^{62b1}, negative recombination results were obtained with all recessive visible tester sites. But, N^{62b1} did recombine, albeit at a low frequency, with N^{55e11}, presently considered the left-most tester site. These

results led Welshons (1974) to conclude that $\underline{N^{62b1}}$ was deficient for approximately 80% of the recombinational Notch unit.

Three significant, but equally perplexing, inferences are drawn from these investigations. First, the occurrence of two types of cytological deficiencies which infringe upon the Notch locus from opposite sides of band 3C7 result in the expression of the abnormal Notch phenotype. Second, the two left-side deficiencies, $\underline{N^{68f19}}$ and $\underline{N^{66i25}}$, although cytologically indistinguishable, can be distinguished genetically by recombination analyses with Notch tester sites. And third, the combined cytological and genetic tests involving $\underline{N^{68f19}}$ and $\underline{N^{66i25}}$, viewed comprehensively, indicate that these deletions appear to include parts of both interbands adjoining band 3C7 but do not visibly alter the band itself.

Mindful of the possible consequences of such inferences, it was decided that similar studies should be conducted with additional left-side deficiencies on hand. We felt that the delimitation of their breakpoints within the Notch locus would not only contribute to the interpretations of the previous studies involving similar deficiency chromosomes but, hopefully, lend to the determination of the site of $\underline{N^{+}}$ functional activity.

Deficiency chromosomes investigated Before turning to the consideration of the genetic techniques employed in the recombination analyses of the four deficiency chromosomes, a brief description of each is as follows:

1. $Df(1)N^{68f19}$ = Df L-3C7^{+}, N^{68f19}: Phenotypically w, rst, vt, and N (see Table 1). The Notch expression is similar to that of other recessive lethal N's. Cytologically deficient for salivary bands 3C2-3C6 inclusive (Welshons, 1974).
2. $Df(1)N^{66h26,12}$ = Df L-3C7^{+}, $N^{66h26,12}$: Phenotypically w, rst, vt, and N. The Notch expression is similar to that of other recessive lethal N's. Subsequently found to also carry the spl allele. Cytologically deficient for salivary bands 3C2-3C6 inclusive.
3. $Df(1)N^{66h26,31}$ = Df L-3C7^{+}, $N^{66h26,31}$: Phenotypically w, rst, vt, and N. The Notch expression is similar to that of other recessive lethal N's. Subsequently found to also carry the spl allele. Cytologically deficient for salivary bands 3C2-3C6 inclusive.
4. $Df(1)N^{66h10,1}$ = Df L-3C7^{+}, $N^{66h10,1}$: Phenotypically w, rst, vt, and N. The Notch expression is similar to that of other recessive lethal N's. Cytologically deficient for salivary bands 3C2-3C6 inclusive.

Each of the above N's was recovered from the w^8 mutable system (Green, 1967) and sent from M. M. Green's laboratory to W. J. Welshons who generously made them available for this study.

Recombination analyses of left-side deficiencies The mechanics of recombination experiments involving the four left-side deficiencies are summarized in Tables 2-6 (see Results) but will be briefly described here. In order to determine the extent to which each of these deficiencies include the Notch locus, recombination experiments were systematically conducted with N-type tester sites on the genic map. They were, from right to left, N^{60g11}, N^{264-40}, N^{62b1}, and N^{55e11}. Three of the above deficiencies were also tested with the recessive visible fa^g for reasons more obvious later.

Appropriately marked females (see Table 1 for description and location of non-Notch marker), trans-heterozygous for the Notch mutants being tested and carrying the autosomal duplication, $Dp(1;2)w^{51b7}$, were mated to either w^a fa fa^{no} or w^a fa^{no} spl males. Such a cross might be represented as w^- N^{DEF-L} + sn^3/w^a + N^Y rb; Dp/+ females by w^a fa fa^{no} males, with the left-side Notch being symbolized as N^{DEF-L}, the Notch tester site as N^Y, and the duplication as Dp.

If the deficiency terminates to the left of the tester

site (e.g. $\underline{N^{Y}}$), one would then expect to recover recombinant females phenotypically white-apricot (i.e. $\underline{w^{a}}$ + + $\underline{sn^{3}}$/ $\underline{w^{a}}$ $\underline{fa}$ $\underline{fa^{no}}$ +) in the absence of the duplication and males phenotypically white-apricot and singed (i.e. $\underline{w^{a}}$ + + $\underline{sn^{3}}$/Y). If the deficiency terminates to the right of the tester site, thus including chromosomal material ordinarily comprising this site, no recombinants are to be expected due to the nonexistence of homologous DNA required for the crossover event.

However, the inability to recover recombinant chromosomes generates no positive conclusions; that is, one might either conclude that the deletion includes the tester site and, therefore, extends to its right, or second, that the deletion does not include the tester site but terminates at a point closely linked to it. The latter would then be chance-dependent within the magnitude of the number of chromosomes being tested. Possible reasons for the reduction or inhibition of crossing over will be considered later.

Recombination analyses of recessive visibles As previously noted, recessive visible Notches which cause similar changes in the anatomy of the adult fly tend to cluster in subloci on the genic map. Two "eye" mutants, facet-3($\underline{fa^{3}}$) and facet-glossy-2 ($\underline{fa^{g-2}}$), originally symbolized as strawberry-62b ($\underline{swb^{62b}}$; see Lefevre and Kelly, 1972), were subjected to recombination tests to determine if each

mapped in the vicinity of other eye mutants (e.g. fa, fa^g). Both mutants behave as typical Notch recessive visible, point mutations and exhibit a pseudodominant effect when heterozygous (trans) with recessive lethal N's. Both complement recessive visible "wing" mutants (e.g. nd).

Facet-3(fa^3) was originally isolated as a spontaneous mutation by Welshons (personal communication). It may be characterized as expressing a slightly roughened eye, phenotypically similar to but more extreme than the fa allele which often overlaps the wild-type in appearance. Facet-3 is apparently dosage compensated; that is, there is little, if any, distinguishable difference in phenotypic expression between the homozygous female and the hemizygous male. fa^3 was tested with the fa^{no} allele to determine both the linear sequence of the two and the distance between them. The mechanics of the cross are found in Table 8 and are similar to those already described for separating recessive visibles.

Facet-glossy-2 (fa^{g-2}), an X-ray induced allele (see Lefevre and Kelly, 1972), was first characterized as a strawberry allele (non-Notch) because of its similar phenotype: rough, shiny, patched eyes that have been likened to the appearance of an overripe strawberry. However, its pseudodominant expression when heterozygous with N mutants and its noncomplementation and nearly identical phenotype with fa^g led to its assignment to the Notch series. Since

its phenotype is distinctly different than that of the fa^{swb} allele and nearly indistinguishable from fa^{g}, it was suggested that swb^{62b} should be designated as fa^{g-2} in the future. (Welshons, personal communication). Therefore, to further specify its position on the map, an attempt was made to separate fa^{g-2} from fa^{g}. Only one recombinant and two putative convertants of fa^{g} were recovered from the cross, w^{a} fa^{g-2} + +/+ + fa^{g} rb females X w^{a} fa^{g} rb males, which indicated that fa^{g-2} was located to the left of fa^{g}. An additional test was conducted to demonstrate the separability of fa^{g-2} and N^{55e11} and to reinforce the conclusion that fa^{g-2} and fa^{g} were closely linked (see Table 9 for the mechanics of the experiment).

Recombination analysis of EMS-induced N's. After successfully isolating a number of recessive lethal N's from the EMS-induction experiment, complementation tests involving selected recessive visible Notch alleles and each new N mutant were conducted. The results rendered their a priori assignment to the Notch locus correct. Recombination tests with the coupled fa^{g} and spl alleles were then made to determine the location of the new Notch alleles relative to the well-established positions of fa^{g} and spl. Females, genotypically N^{EMS-X}/y w^{a} fa^{g} spl rb, were mated to w^{a} fa^{g} fa^{no} rb males and the progeny screened for recombinants. Figure 5 illustrates the recombinant classes which

would result, dependent upon the location of the particular $\underline{N^{EMS}}$ relative to $\underline{fa^{g}}$ and $\underline{spl}$.

$$\frac{N^{EMS-X}}{y^2\ M-5} \quad X \quad y\ w^a\ fa^g\ spl\ rb$$

A $$\frac{+\ +\ N^{EMS-X}}{y\ w^a\ +} \times \frac{+\quad +\quad +}{fa^g\ spl\ rb} \quad X \quad w^a\ fa^g\ fa^{no}\ rb$$

Recombinant Chromosome: $y\ w^a + + + +$

B $$\frac{+\ +\ +}{y\ w^a\ fa^g} \times \frac{N^{EMS-X}\ +\quad +}{+\qquad spl\ rb} \quad X \quad w^a\ fa^g\ fa^{no}\ rb$$

Recombinant Chromosome: $+ + +\ spl\ rb$

C $$\frac{+\ +\ +\quad +}{y\ w^a\ fa^g\ spl} \times \frac{N^{EMS-X}\ +}{+\qquad rb} \quad X \quad w^a\ fa^g\ fa^{no}\ rb$$

Recombinant Chromosome: $+ + + + + rb$

Figure 5. Diagrammatic representation of typical cross to determine the positions of EMS-induced Notches (represented as N^{EMS-X} above) relative to the fixed sites, fa^g and spl. A, B, and C represent the three possible sites the EMS-induced Notches might occupy and the critical recombinant chromosomes which would result from a crossover event.

RESULTS

In an effort to maintain dialectic continuity and clarity, the results of experiments, whose mechanisms have been described, will be presented here in approximately the same sequence as was in the previous section (see Materials and Methods). Additionally, the results of recombination analyses involving each Notch tester site will be summarized in tabular form to accompany brief descriptions in the body of the text.

Cytology

Band distribution

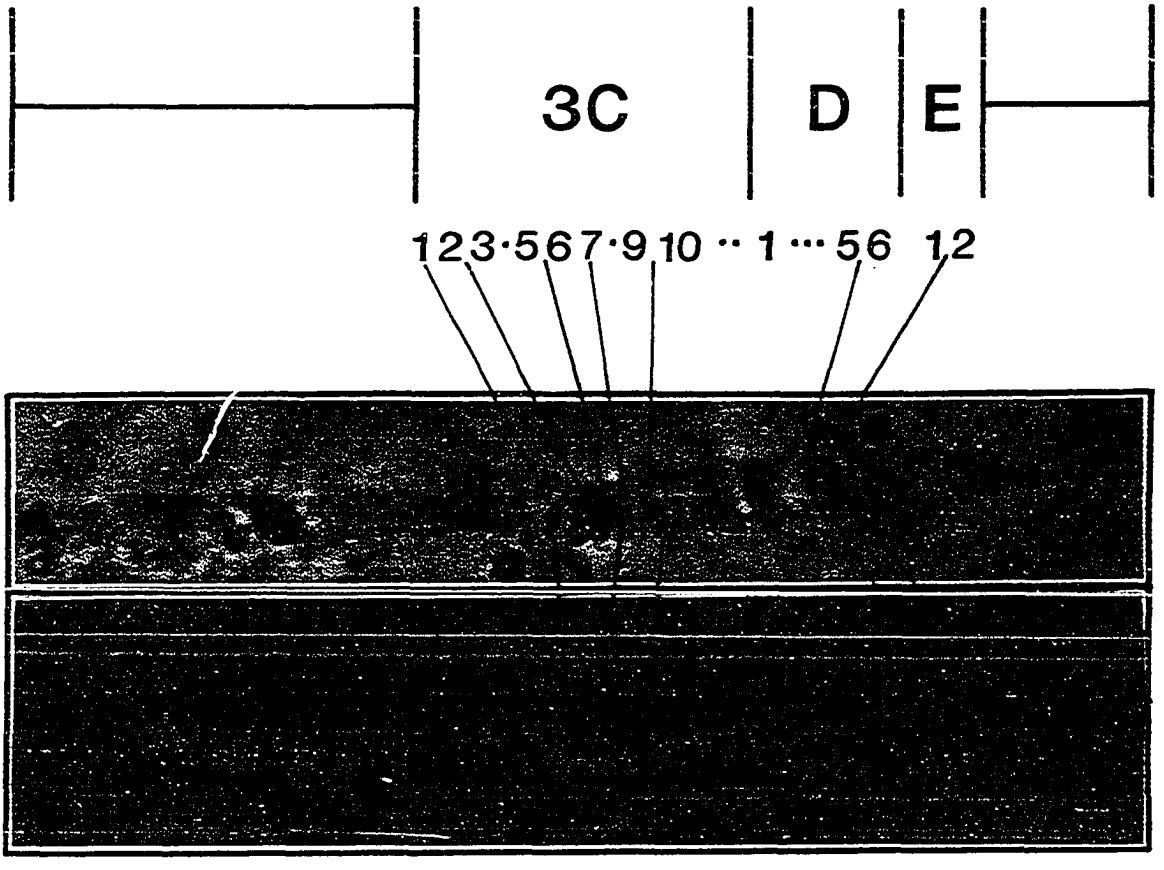
Cytology of deficiency chromosomes Cytological examinations of male salivary gland chromosomes of the left-side deficiencies, $N^{66h26,12}$, $N^{66h26,31}$, and $N^{66h10,1}$, revealed that each was deficient for a portion of the 3C region of the X chromosome (see Bridges, 1938). These deletions were interpreted to represent a loss of the conspicuous doublets 3C2-3 and 3C5-6; the banding pattern of each of the above is similar, if not identical in appearance to the cytology of the deficiency chromosomes, N^{68f19}, N^{66i25}, and w^{67k30} (the latter visibly deficient for 3C2-3 and 3C5-6 but phenotypically N^{+}), described by Welshons (1974). Bands 3C1, 3C7, and 3C9-10 are present and, at the resolving power of the light microscope,

apparently intact (Figure 6).

Accepting this interpretation of the cytology as a working model, continued efforts were made to discern a noticeable difference in band size between 3C1 and 3C7 in a great number of preparations, but proved unproductive. Both bands, however, appeared somewhat wider than usual in the deficiency chromosomes (see Figure 6 for comparison) but it has been suggested (Welshons, personal communication) that this appearance may be a reflection of the effect a deletion has on adjacent bands or an artifact of the technique employed. Subsequent attempts to strengthen this interpretation by examining the salivary chromosomes of females heterozygous for the deficiency were equally unproductive and provided no additional support. The significance of the interpretation of the cytology of all left-side deficiencies will be more readily apparent when the results of genetic tests are imposed upon it.

Cytology of recessive visibles Prior to conducting genetic tests involving the recessive visibles, $\underline{fa^{g-2}}$ and $\underline{fa^{3}}$, preparations of the salivary chromosomes of each were examined. The X chromosomes of both alleles appear normal when compared to the wild-type. Assuming that each behaved as simple point mutants, the cytologically normal banding pattern was not wholly unexpected, although an exceptional Notch recessive visible ($\underline{fa^{swb}}$) is now thought to be assoc-

Figure 6. Salivary gland preparations of a normal and a representative left-side deficiency chromosome. (a) The X chromosome of a wild-type (Canton-S) male. (b) The X chromosome of a $\underline{Df(1)N^{66h26,12}}$; $\underline{Dp/+}$ male; note the apparent increase in width of bands 3C1 and 3C7, compared to those in the wild-type. Other left-side deficiency Notches (e.g. $\underline{N^{66h26,31}}$ and $\underline{N^{66h10,1}}$) exhibit banding patterns virtually indistinguishable from that shown at right.


3C
D
E
1 2 3 · 5 6 7 · 9 10 ·· 1 ··· 5 6 1 2

iated with a visible deficiency (Welshons and Keppy, 1975).

EMS Mutant Induction

Approximately 25,000 F_1 females, heterozygous for an EMS treated X chromosome and an X chromosome carrying $\underline{w^a}$ and spl, were screened for mutants expressing the Notch phenotype. Approximately 250 expressed one or more of the traits usually associated with Notch (see Materials and Methods) but varied widely in the degree of expression. The majority of the initial isolates (about 130) were phenotypic mosaics for Notch characteristics; others, whose phenotype closely mimicked that of Notch, did not express the split pseudodominant effect expected in a N/spl heterozygote.

A rather interesting, though precedented observation (Jenkins, 1967), was noted while screening F_1 progeny and upon evaluating the results. It appeared that the number of putatively mutant flies (including other non-Notch abnormalities) was greatly reduced (to nearly 0) in cultures that had been originally fertilized by males eight days after having been treated with EMS. Though a number of speculative hypotheses can be set forth to satisfy this observation, no experimental evidence provides concrete explanation for this result.

After crossing the putative Notch F_1 females to wild-

type males, 48 F_2 females proved to be genetically Notch-like and were perpetrated for additional genetic analysis and cytological examination. Of these 48, 18 appeared to result from a mutational event at the Notch locus: fourteen were recessive lethal, phenotypically Notch; three were similar to Abruptex in expression; and one was male viable and phenotypically similar to the recessive visible notchoids (i.e. nd and nd^2).

Genetics of the Deficiency Chromosomes

The abnormal phenotypes expressed by the recessive lethals, N^{68f19}, $N^{66h26,12}$, $N^{66h26,31}$, and $N^{66h10,1}$, appear to result from deletions originating near, and including part of, the white locus and terminating at Notch. Recombination tests have been conducted with each of the above mutants to determine the extent to which each impinges upon Notch. Positive recombination results with intralocus tester sites indicate that the termination point of the deficiency falls to the left of the site being tested. Further tests with sites to the left of the previously tested site are then required, and so on. The results of these experiments are found in Tables 2-7 but will be briefly summarized here.

In the first series of experiments, $N^{66h26,12}$, $N^{66h26,31}$, and $N^{66h10,1}$, were tested with the right most N-

site, N^{60g11} (Table 2). Each was shown to recombine freely. In the first cross, w^{-} $N^{66h26,12}$ + + sn^{3}/w^{a} + N^{60g11} rb +; Dp/+ females (see clarification below) were mated to w^{a} fa^{no} spl males. Forty-six recombinant chromosomes, genotypically w^{a} spl sn^{3} or w^{a} sn^{3}, were recovered for a recombination frequency equal to .17% (it will be recalled that since only half the recombinants can be recovered; the realized number of recombinants must be doubled in the calculation of recombination frequency). Significantly, the progeny tests of the putative recombinant chromosomes recovered from this experiment revealed that the w^{-} $N^{66h26,12}$ sn^{3} chromosome also carried the recessive visible spl allele. Table 2 illustrates the corrected genotype of the deficiency chromosome and specifies the linear order of mutants comprising it. It will be noted that either w^{a} spl sn^{3} or w^{a} sn^{3} chromosomes can result from a crossover event between $N^{66h26,12}$ and N^{60g11}. This determination of the additional presence of spl eliminated the superfluous testing of $N^{66h26,12}$ with N^{Co} which had originally been planned (spl is located to the left of N^{Co}).

In the second cross, $N^{66h26,31}/N^{60g11}$ heterozygotes demonstrated similar ease in crossing over. Again, the recombinant chromosomes were genotypically either w^{a} spl sn^{3} or w^{a} sn^{3} as was seen in the $N^{66h26,12}/N^{60g11}$ test, indicating that $N^{66h26,31}$ also carried spl (see Table 2 for the com-

plete genotype of the parental female heterozygote). A total of 40 recombinant chromosomes were isolated in a sample of approximately 72,000 tested for a recombination value of .11%.

Similarly, the heterozygote $\underline{N^{66h10,1}}/\underline{N^{60g11}}$ yielded 34 $\underline{w^a}$ $\underline{+}$ $\underline{+}$ $\underline{+}$ recombinants out of 40,500 progeny for a recombination frequency equal to .084%, probably not significantly different than those of the other deficiencies. However, the $\underline{N^{66h10,1}}$ chromosome was distinguished by the absence of spl.

In the second series of tests, each of the three deficiencies was tested with $\underline{N^{264-40}}$, located about 0.06 units to the left of $\underline{N^{60g11}}$ and near the center of the map (Figure 1). Although the $\underline{N^{66h10,1}}$ chromosome did not carry spl, its demonstrated ease in crossing over with $\underline{N^{60g11}}$ in the previous test prompted the elimination of a test with $\underline{N^{Co}}$. The heterozygotes, $\underline{N^{66h26,12}}/\underline{N^{264-40}}$, $\underline{N^{66h26,31}}/\underline{N^{264-40}}$, and $\underline{N^{66h10,1}}/\underline{N^{264-40}}$ each exhibited recombination with frequencies equal to .036%, .05%, and .049%, respectively (Table 3). That each deficiency recombined with $\underline{N^{264-40}}$ is germane to the discussion of the comparative genetics of $\underline{N^{66i25}}$, another left-side deficiency.

Suspecting from the results of experiments described above that each deficiency terminated near the distal end of the genetic map, it was decided to attempt the separation

of each from the left-most site, $\underline{N^{55e11}}$. Unfortunately, negative results were obtained in each test: $\underline{N^{66h26,12}}/\underline{N^{55e11}}$: 0/54,800; $\underline{N^{66h26,31}}/\underline{N^{55e11}}$: 0/68,650; and $\underline{N^{66h10,1}}/\underline{N^{55e11}}$: 0/93,500 (Table 6). These negative results with $\underline{N^{55e11}}$ focused our attention on the direction subsequent experiments should take. However, as will be seen and discussed later, the results of the tests which follow would seriously confound the cytological interpretations already made.

Before turning to these studies immediately, it is appropriate at this point to diverge slightly to review the genetics of the cytologically similar left-side deficiencies, $\underline{N^{68f19}}$ and $\underline{N^{66i25}}$. It will be recalled that both deficiencies had been tested for recombination with $\underline{N^{264-40}}$ (Welshons, 1974): $\underline{N^{68f19}}/\underline{N^{264-40}}$ heterozygotes produced recombinants rather freely (.098%), while a similar cross involving $\underline{N^{66i25}}$ yielded no recombinants in 86,000 tested chromosomes. In subsequent experiments, Welshons (1974) was unable to separate neither $\underline{N^{68f19}}$ nor $\underline{N^{66i25}}$ from $\underline{N^{55e11}}$. Welshons (1974) suggested that $\underline{N^{68f19}}$ represented a deficiency which extended up to and possibly beyond a point in the Notch locus defined by $\underline{N^{55e11}}$. It has subsequently been shown that $\underline{N^{68f19}}$ recombines with $\underline{N^{62b1}}$ (Table 5; see below) which indicates, at least, that $\underline{N^{68f19}}$ does terminate to the left of $\underline{N^{62b1}}$.

In this 1974 publication, Welshons also reported the results of cytogenetic studies of the right-side deficiency, N^{62b1} (Df(1)3C8-3D5 = Df R-3C7^{+}). He concluded that N^{62b1} was deficient for approximately 80% of the Notch gene as defined by positive recombination with N^{55e11} (recombination frequency equals .023%) and negative recombination with all tester sites to the right.

After reviewing the above genetic data, it was decided to test N^{68f19} with N^{62b1}, reasoning that the two deficiencies should be nonoverlapping. Two recombinants out of a sample of 65,000 tested chromosomes were recovered for a recombination value of .006% (Table 5). Later, Welshons (Welshons and Keppy, 1975) repeated this experiment and obtained four recombinants/77,600 chromosomes (.010%). He noted that the frequency of recombination between N^{68f19} and N^{62b1} was less than expected if N^{68f19} localized near N^{55e11} when the distance separating N^{62b1} and N^{55e11} was considered. He pointed to the possibility that trans-heterozygous deficiencies could inhibit the rate of recombination between them as has been suggested (Lefevre and Moore, 1966; Lefevre, 1974). However, the positive recombination between N^{68f19} and N^{62b1} did confirm the suspicions that the two deficiencies were nonoverlapping.

Returning to experiments involving $N^{66h26,12}$, $N^{66h26,31}$, and $N^{66h10,1}$, the positive results obtained from N^{68f19}/N^{62b1}

heterozygotes in two tests suggested that the remaining three might also be separable from $\underline{N^{62b1}}$. Unfortunately, neither $\underline{N^{66h26,31}}$ nor $\underline{N^{6610,1}}$ yielded recombinants in 60,000 and 70,000 chromosomes, respectively. Slightly discouraged, but a bit reluctant (for reasons more apparent later) to draw a parallel between $\underline{N^{66h26,31}}$, $\underline{N^{66h10,1}}$, and $\underline{N^{66i25}}$, it was decided to test each, including $\underline{N^{66h26,12}}$, with $\underline{fa^{g}}$ (0.06 units to the right of $\underline{N^{55e11}}$). Each deficiency recombined freely (Table 4). Buoyed by these renewed successes, we decided to repeat, and enlarge, the $\underline{N^{DEF-L}}/\underline{N^{62b1}}$ experiments. Success, though limited! The heterozygote $\underline{N^{66h26,12}}/\underline{N^{62b1}}$ yielded two recombinants in an estimated 68,500 chromosomes for a value of .0058%. Likewise, two recombinants were recovered from the repeated $\underline{N^{66h26,31}}/\underline{N^{62b1}}$ experiment. When the number of tested chromosomes in the initial, unsuccessful test are included in the calculation of recombination frequency, a value of .003% is obtained (4/146,800; see Table 5). Neither figure would seem significantly different than that obtained from $\underline{N^{68f19}}/\underline{N^{62b1}}$ heterozygotes when possible inherent flaws and chance factors in such estimates are considered.

The combined efforts to separate $\underline{N^{66h10,1}}$ from $\underline{N^{62b1}}$ in two experiments have yielded no recombinants in nearly 170,000 tested chromosomes (Table 5). However, that $\underline{N^{66h10,1}}$ recombined with $\underline{fa^{g}}$ does distinguish $\underline{N^{66h10,1}}$ from

N^{66i25} which did not recombine with any tester site to the left of, and including, N^{264-40} in tests totalling nearly 155,000 chromosomes (compilation drawn from Welshons and Keppy, 1975).

A summary of all genetic tests involving the left-side deficiencies, $N^{66h26,12}$, $N^{66h26,31}$, and $N^{66h10,1}$ is found on Table 7.

Genetics of Recessive Visibles

To determine whether the recessive alleles, fa^{g-2} and fa^{3}, localized within the "eye-mutant" cluster (see Figures 1 and 2), recombination tests were conducted. In the first cross, fa^{g-2} was tested with its phenotypically related allele, fa^{g}. Only one wild-type recombinant was recovered in 61,000 chromosomes which indicated very close linkage (0.0032 units) and the placement of fa^{g-2} to the left of fa^{g} (Table 9). To support this assignment, fa^{g-2} was later shown to recombine with N^{55e11} at a recombination frequency equal to .048%, which value is not grossly different than would be expected (compare: N^{55e11}-fa^{g} = .06%; N^{55e11}-fa^{g-2} = .048%; fa^{g-2}-fa^{g} = .0032%). Thus, as had been predicted, fa^{g-2} mapped very close to the allele (fa^{g}) whose phenotypic effect is nearly identical.

Similar reasoning prompted testing fa^{3} with another

member of the facet-series, fa^{no}. In the cross, w^a fa^3 + +/ + + fa^{no} rb females mated to w^a fa^g fa^{no} rb males, 11 recombinants were recovered from 57,200 total progeny, so that the site of fa^3 was located 0.019 units to the left of fa^{no} (Table 8). Although fa^3 was not tested further, the recombination value obtained in this experiment would predict its position to be in the vicinity of the fa allele, to which it is phenotypically similar.

Genetics of EMS-Notches

The results of recombination experiments involving EMS-induced, recessive lethal Notches are found in Table 10 but will be summarized here.

Of the six mutants tested with the fa^g spl chromosome, three yielded recombinants: N^{75c1}, N^{75c7}, and N^{75c9}. Each localized to the right of spl at distances of 0.021, 0.05, and 0.019 units, respectively. It may be significant to note that the determined position of N^{75c7} is approximately 0.02 map units further to the right of spl than nd^2, heretofore the right-most site on the map, and a considerable distance further to the right than N^{60g11}, presently the most proximal recessive lethal (Figure 1). We do realize, however, that extending the recombinational length of the Notch locus is, probably, improvident until N^{75c7} has been tested with nd^2.

None of the remaining EMS-induced N's produced recombinants in this test. Based on these negative results, it is safe only to say that each is located at or near spl (i.e. to the left or right of spl but to the right of fa^g) but inseparable from it within the limits of such small experiments (approximately 20,000 surviving progeny in each). Of the new Notches tested, then, all appear to map within the right-most half of the locus, defined by all Notch mutations; this might have been predicted (see Figure 2).

While maintaining the EMS mutants in stock, an unusual observation was noted. In addition to expressing typical Notch characters (e.g. nicking of the wing margins), seven of the fourteen occasionally also mimic the phenotype of the Abruptex pseudoallele. Furthermore, the concomitant occurrence of both phenotypes results in an apparent enhancement of each: the wings of such flies are extremely serrated (including the lateral margins), the thoracic bristles more distorted, and the fifth longitudinal vein terminates more prematurely. Also, there is an apparent loss and distortion of the dorsal, abdominal bristles, quite unlike either the typical Notch or Abruptex variants. The significance of this dual expression has not been determined although its occurrence was not unpredicted (Welshons, personal communication).

Table 2. A summary of $\frac{N^{DEF-L}}{N^{60g11}}$ experiments.

$$\frac{w^- \quad N^{66h26,12} \quad spl \quad + \quad + \quad sn^3}{w^a \quad + \quad + \quad N^{60g11} \quad rb \quad +} \; ; \; \frac{Dp}{+} \times w^a \; fa^{no} \; spl$$

Surviving Fraction: 54,300 Recombinants: 46

Tested Chromosomes: 54,300 Recombination: 92/54,300 = 0.17%

$$\frac{w^- \quad N^{66h26,31} \quad spl \quad + \quad + \quad sn^3}{w^a \quad + \quad + \quad N^{60g11} \quad rb \quad +} \; ; \; \frac{Dp}{+} \times w^a \; fa \; fa^{no}$$

Surviving Fraction: 71,650 Recombinants: 40

Tested Chromosomes: 71,650 Recombination: 80/71,650 = 0.11%

$$\frac{w^- \quad N^{66h10,1} \quad + \quad + \quad}{w^a \quad + \quad N^{60g11} \quad rb} \; ; \; \frac{Dp}{+} \times w^a \; fa \; fa^{no}$$

Surviving Fraction: 40,500 Recombinants: 17

Tested Chromosomes: 40,500 Recombination: 34/40,500 = 0.084%

Table 3. A summary of $\frac{N^{DEF-L}}{N^{264-40}}$ experiments.

$$\frac{w^- \quad N^{66h26,12} \quad + \quad spl \quad + \quad sn^3}{w^a \quad + \quad N^{264-40} \quad + \quad rb \quad +} \; ; \; \frac{Dp}{+} \times w^a \; fa^{no} \; spl$$

Surviving Fraction: 66,500 Recombinants: 12

Tested Chromosomes: 66,500 Recombination: 24/66,500 = 0.036%

$$\frac{w^- \quad N^{66h26,31} \quad + \quad spl \quad + \quad sn^3}{w^a \quad + \quad N^{264-40} \quad + \quad rb \quad +} \; ; \; \frac{Dp}{+} \times w^a \; fa \; fa^{no}$$

Surviving Fraction: 57,350 Recombinants: 16

Tested Chromosomes: 57,350 Recombination: 32/57,350 = 0.05%

$$\frac{w^- \quad N^{66h10,1} \quad + \quad +}{w^a \quad + \quad N^{264-40} \quad rb} \; ; \; \frac{Dp}{+} \times w^a \; fa \; fa^{no}$$

Surviving Fraction: 48,500 Recombinants: 12

Tested Chromosomes: 48,500 Recombination: 24/48,500 = 0.049%

Table 4. A summary of $\underline{N^{DEF-L}}/\underline{fa^{g}}$ experiments.

$$\frac{w^{-}\ N^{66h26,12}\ +\ spl\ +\ sn^{3}}{w^{a}\ +\ fa^{g}\ +\ rb\ +} \quad X \quad w^{a}\ fa^{g}\ fa^{no}\ rb$$

Surviving Fraction: 42,250

Recombinants: 12

Recombination: 12/42,250 = 0.028%

$$\frac{w^{-}\ N^{66h26,31}\ +\ spl\ +\ sn^{3}}{w^{a}\ +\ fa^{g}\ +\ rb\ +} \quad X \quad w^{a}\ fa^{g}\ fa^{no}\ rb$$

Surviving Fraction: 44,700

Recombinants: 15

Recombination: 15/44,700 = 0.030%

$$\frac{w^{-}\ N^{66h10,1}\ +\ +}{w^{a}\ +\ fa^{g}\ rb} \quad X \quad w^{a}\ fa^{g}\ fa^{no}\ rb$$

Surviving Fraction: 43,100

Recombinants: 11

Recombination: 11/43,100 = 0.025%

Table 5. A summary of $\frac{N^{DEF-L}}{N^{62b1}}$ experiments.

$$\frac{+ \quad w^- \quad N^{66h26,12} \quad + \quad spl \quad + \quad sn^3}{y \quad w^a \quad + \quad N^{62b1} \quad spl^- \quad dm^- \quad +} \; ; \; \frac{Dp}{+} \; X \; w^a \; fa \; fa^{no}$$

Surviving Fraction: 68,520 Recombinants: 2

Tested Chromosomes: 68,520 Recombination: 4/68,520 = 0.0058%

$$\frac{+ \quad w^- \quad N^{66h26,31} \quad + \quad spl \quad + \quad sn^3}{y \quad w^a \quad + \quad N^{62b1} \quad spl^- \quad dm^- \quad +} \; ; \; \frac{Dp}{+} \; X \; w^a \; fa \; fa^{no}$$

Surviving Fraction: 146,800 Recombinants: 2

Tested Chromosomes: 146,800 Recombination: 4/146,800 = 0.003%

$$\frac{+ \quad w^- \quad N^{66h10,1} \quad + \quad +}{y \quad w^a \quad + \quad N^{62b1} \quad dm^-} \; ; \; \frac{Dp}{+} \; X \; w^a \; fa \; fa^{no}$$

Surviving Fraction: 168,600 Recombinants: 0

Tested Chromosomes: 168,600 Recombination: 0/168,600

$$\frac{y \quad w^- \quad N^{68f19} \quad + \quad +}{y \quad w^a \quad + \quad N^{62b1} \quad dm^-} \; ; \; \frac{Dp}{+} \; X \; w^a \; fa^g \; fa^{no} \; rb$$

Surviving Fraction: 65,100 Recombinants: 2

Tested Chromosomes: 65,100 Recombination: 4/65,100 = 0.006%

Table 6. Summary of $\frac{N^{DEF-L}}{N^{55e11}}$ experiments.

$$\frac{w^- \quad N^{66h26,12} \quad + \quad spl \quad + \quad sn^3}{w^a \quad + \quad N^{55e11} \quad + \quad rb \quad +} \; ; \; \frac{Dp}{+} \times w^a \; fa \; fa^{no}$$

Surviving Fraction: 54,800 Recombinants: 0

Tested Chromosomes: 54,800 Recombination: 0/54,800

$$\frac{w^- \quad N^{66h26,31} \quad + \quad spl \quad + \quad sn^3}{w^a \quad + \quad N^{55e11} \quad + \quad rb \quad +} \; ; \; \frac{Dp}{+} \times w^a \; fa \; fa^{no}$$

Surviving Fraction: 68,650 Recombinants: 0

Tested Chromosomes: 68,650 Recombination: 0/68,650

$$\frac{w^- \quad N^{66h10,1} \quad + \quad +}{w^a \quad + \quad N^{55e11} \quad rb} \; ; \; \frac{Dp}{+} \times w^a \; fa \; fa^{no}$$

Surviving Fraction: 93,500 Recombinants: 0

Tested Chromosomes: 93,500 Recombination: 0/93,500

Table 7. Summary of recombination values for the left-side deficiencies, $N^{66h26,12}$, $N^{66h26,31}$, and $N^{66h10,1}$, with intragenic tester sites.

	N^{55e11}	N^{62b1}	fa^{g}	N^{264-40}	N^{60g11}
$N^{66h26,12}$	0 0/54,800	0.006 4/68,520	0.028 12/42,250	0.036 24/66,500	0.170 92/54,300
$N^{66h26,31}$	0 0/68,650	0.003 4/146,800	0.030 15/44,700	0.050 32/57,350	0.110 80/71,650
$N^{66h10,1}$	0 0/93,500	0 0/168,600	0.025 11/43,100	0.049 24/48,500	0.084 34/40,500

Table 8. Summary of experiment involving $\underline{fa^3}$.

$\frac{w^a \quad fa^3 \quad + \quad +}{+ \quad + \quad fa^{no} \quad rb}$ X $w^a \; fa^g \; fa^{no} \; rb$			
Surviving Fraction:	57,200	Recombinants:	11
Tested Chromosomes:	57,200	Recombination:	11/57,200 = 0.019%

Table 9. Summary of experiments involving $\underline{fa^{g-2}}$

$\frac{w^a \quad fa^{g-2} \quad + \quad +}{+ \quad + \quad fa^g \quad rb}$ X $w^a \; fa^g \; rb$			
Surviving Fraction:	61,000	Recombinants:	1
Tested Chromosomes:	61,000	Recombination:	2/61,000 = 0.0032%
$\frac{w^a \quad N^{55e11} \quad + \quad rb}{+ \quad + \quad fa^{g-2} \quad +}$ X $w^a \; fa^g \; fa^{no} \; rb$			
Surviving Fraction:	25,000	Recombinants:	12
		Recombination:	12/25,000 = 0.048%

Table 10. Summary of experiments involving EMS-induced Notches and $\underline{fa^g}$, $\underline{spl}$ alleles.

$$\frac{+\ +\ +\ +\ N^{75c1}\ +}{y\ w^a\ fa^g\ spl\ +\ rb} \times w^a\ fa^g\ fa^{no}\ rb$$

Surviving Fraction: 18,850 Recombinants: 4

Recombination: 4/18,850 = 0.021%

$$\frac{+\ +\ +\ +\ N^{75c7}\ +}{y\ w^a\ fa^g\ spl\ +\ rb} \times w^a\ fa^g\ fa^{no}\ rb$$

Surviving Fraction: 19,650 Recombinants: 10

Recombination: 10/19,650 = 0.05%

$$\frac{+\ +\ +\ +\ N^{75c9}\ +}{y\ w^a\ fa^g\ spl\ +\ rb} \times w^a\ fa^g\ fa^{no}\ rb$$

Surviving Fraction: 15,800 Recombinants: 3

Recombination: 3/15,800 = 0.019%

Table 10 (Continued)

$$\frac{+ \quad + \quad + \quad + \quad N^{75b30} \quad +}{y \quad w^{a} \quad fa^{g} \quad spl \quad + \quad rb} \times \; w^{a}\ fa^{g}\ fa^{no}\ rb$$

Surviving Fraction: 20,500   Recombinants: 0

Recombination: 0/20,500

$$\frac{+ \quad + \quad + \quad + \quad N^{75c2} \quad +}{y \quad w^{a} \quad fa^{g} \quad spl \quad + \quad rb} \times \; w^{a}\ fa^{g}\ fa^{no}\ rb$$

Surviving Fraction: 21,650   Recombinants: 0

Recombination: 0/21,650

$$\frac{+ \quad + \quad \quad \quad + \quad N^{75c4} \quad +}{y \quad w^{a} \quad fa^{g} \quad spl \quad + \quad rb} \times \; w^{a}\ fa^{g}\ fa^{no}\ rb$$

Surviving Fraction: 16,600   Recombinants: 0

Recombination: 0/16,600

DISCUSSION

As we have seen in the foregoing discussions, a great deal of interest has been demonstrated in the analysis of functional units in higher organisms, especially the "complex loci" in Drosophila melanogaster. Countless investigations, including approaches of great diversity, have been conducted in hopes of elucidating the structure-function relationship of the Drosophila chromomere, including the adjacent interband regions. The conclusions drawn by classical cytogeneticists (e.g. those of Painter, 1934; Slizynska, 1938; among others) have focused more recent attention on the chromosome band, easily recognized in polytene chromosomes, as the structural component which functions somewhat autonomously in the metabolically active organism. That is to say, evidence gathered from the associations of genetically abnormal phenotypes and cytologically visible deficiencies led early workers to describe the band as the structural component which acted as the functional (i.e. cistronic) and replicational unit of the Drosophila chromosome.

The introduction of electron microscopic examinations has reinforced many of those early conclusions and has been an invaluable tool in the cytological determination of chromosome, particularly chromomere, substructure. However, the inherent limitations of purely cytogenetic techniques,

when applied to higher forms, prompted the employment of alternative approaches used similarly, and to great advantage, in the study of functionally less complex procaryotic systems. As might have been anticipated, results gathered from investigations using more sophisticated biochemical techniques have contributed much to our understanding of the eucaryotic chromosome. But, these results have also cast an aura of confusion over an already perplexing situation; these recent advances have necessitated the re-evaluation of early interpretations, and require their inclusion in explanations to be set forth in the future.

The problem being confronted by the student of chromomere structure-function can be simply demonstrated by briefly summarizing much of that described in the introduction of this report. Rudkin (1961) and Laird and McCarthy (1969) have shown that the amount of DNA per chromomere (including interbands) is considerably greater (on the average, 30X) than that required to produce a polypeptide product of average size in eucaryotes. Further, Laird and his colleagues (unpublished; see Laird, 1975) have recently demonstrated that the size of the initial transcription product (i.e. the hn RNA molecule) reflects the entire chromomere having been transcribed in Drosophila. However, from transcription through translation, the systematic processing (or lack of) and transporting of RNA has been less

well characterized (see Lewin, 1975a, 1975b). For instance, Gelbart et al. (1974) have recently shown that the size of the translation product coded by the rosy (ry) locus indicates that only about 10% (about 3000 nucleotide pairs) of the chromomeric DNA is required for product specificity.

Thus, the paradox: if, indeed, the chromomere represents a single cistron (presently the most widely accepted interpretation), why, then, is each chromomere comprised of so much DNA if only a fraction of the total is reflected in the final product? To be sure, the problem has been a bit overstated; when considerations are given to the probable requirements for complex regulatory systems, possible explanations become more tenable.

Mindful of these impositions placed on any interpretation of the structure-function relationship of the Drosophila chromomere, the ensuing discussion of the results of this study will address itself to these considerations. Special attention will be paid to the relevance of these results to the resolution of the chromomere, as applicable to the consideration of the one band-one gene hypothesis.

Cytology

Cytology of the left-side deficiencies

Welshons (1974) reported his observations of two Notch chromosomes which were cytogenetically deficient for salivary

bands 3C2-3C6, inclusive. A similar interpretation of the cytology of the left-side deficiencies, $\underline{N^{66h26,12}}$, $\underline{N^{66h26,31}}$, and $\underline{N^{66h10,1}}$, seems inescapable when the following observations are weighed: 1) accepting Welshons' (1974) interpretation of the cytology of both $\underline{N^{68f19}}$ and $\underline{N^{66i25}}$ as correct, a comparison of their banding patterns to those of $\underline{N^{66h26,12}}$, $\underline{N^{66h26,31}}$, and $\underline{N^{66h10,1}}$ reveals the same band number and arrangement; 2) a pseudodominant effect is expressed by recessive mutants which localize within the white-Notch interval (e.g. "roughest" or "verticals") when heterozygous with each of the left-side deficiencies (i.e. $\underline{N^{DEF-L}}/\underline{rst}$ or $\underline{N^{DEF-L}}/\underline{vt}$); 3) the dimensions of bands 3C1 and 3C7, very similar in preparations of normal chromosomes, are also strikingly alike in preparations of each of the left-side deficiency chromosomes; and 4) the bands normally seen to the left of 3C1 and to the right of 3C7, particularly 3C9-10, are present and apparently uneffected by the adjacent deletion.

To endorse an alternative interpretation in view of these observations becomes strenuous and would require the invocation of reasoning based on unlikely events (e.g. staggered deletions within a very small chromosomal region such as 3C). Thus, the interpretation of the cytological characteristics of the left-side deficiencies, to the exclusion of other genetic impositions, would lead to the

conclusion that the expression of Notch characteristics results from the elimination of material in the 3C6-3C7 interband and/or in a small portion of 3C7 itself. The detection of the latter event is probably beyond the resolution afforded by light microscopy.

This conclusion, coupled with the cytology of the right-side deficiency, $\underline{N^{62b1}}$(= Df(1)3C8-3D5), would suggest the involvement of both interbands adjacent to 3C7 in $\underline{N^{+}}$ function(s). Welshons (1974) noted this possibility and offered the cytology of chromosomal rearrangements affecting the vermilion locus (Lefevre, 1971) as another example of a bilateral band association in Drosophila. Welshons and Keppy (1975) have cautiously proposed that the chromosome might be comprised of a double fiber of DNA (i.e. binemic), which might rectify the bilaterality of 3C7 that has been observed. They offer electron micrographs showing the apparent binemic structure of Drosophila chromosomes presented by Sorsa and his co-workers (see Welshons and Keppy, 1975) as support for their interpretation. In this model, one longitudinal strand would represent the so-called "recombinational gene" and lie opposite the fiber associated with the chromomeric loop.

Difficulties surface with the supposition of such an interpretation, unless each strand is functionally independent of the other. For example, without additional

postulates, the interpretations of genetic data gathered from mutational and recombinational studies are severely strained when based on such a model. Furthermore, the functional autonomy of each strand, reflected in base sequence specificity, would be required if the conclusions drawn from investigations characterizing Drosophila DNA (e.g. Kavenoff and Zimm, 1973) are accepted as accurate. A number of other possible explanations, to be discussed later, have been put forth to satisfy these conditions but provide little more, if any, experimental support for such contentions.

Therefore, the cytology of the left-side deficiencies would seem to be their least equivocal feature when considered independent of their genetic behavior. It remains only to emphasize that a deletion which apparently includes either the 3C6-3C7 interband and/or a cytologically ill-defined portion of band 3C7 itself, results in the expression of Notch. This observation, it would seem, implies the important role played by this chromosome region in N^+ function.

Cytology of the recessive visibles

The normal banding pattern exhibited by the salivary gland chromosomes of the recessive visibles, fa^{g-2} and fa^3, comes as no significant surprise and requires little discussion. Only one recessive visible allele, fa^{swb}, which maps at the Notch

locus appears to result from a detectable cytological deficiency; in addition, the behavior of $\underline{fa^{swb}}$ in genetic tests belies rigid comparison with other recessive visibles beyond a similarity of phenotypic expression (Welshons and Keppy, 1975).

EMS Mutant Induction

Although little in the form of concrete explanation for the results of the mutant induction can be offered, a number of observations deserve attention. EMS, a monofunctional alkylating agent, was chosen as the mutagenic agent for inducing recessive lethal Notches because of its ease of administration (i.e. feeding rather then tediously injecting) and its recognized potency. Although most of the Notches that were recovered behave as simple point mutations, EMS is known to cause a variety of chromosome changes, including nucleotide substitutions as well as gross aberrations (e.g. deletions). The mechanism by which EMS effects such gross changes remains undetermined in light of its better understood activities and consequences at the single purine level.

The most perplexing observation noted was the loss of so many putative Notches after their initial isolation. As will be recalled (see Results), nearly 80% of the F_1 Notch-like females, whether whole-body or mosaic, produced phenotypically normal females (F_2) after being mated to wild-type

males. This inexplicable disappearance of abnormal phenotype of presumed genetic determination is apparently not unusual. A scan of reports dealing with similar induction experiments in Drosophila and other organisms reveals that many investigators have noted this occurrence, and others of an equally enigmatic nature. A number of plausible interpretations have been rendered to explain these observations; none, however, satisfactorily justifies endorsement at the present and must be considered speculative.

Genetics of the Left-Side Deficiencies

To briefly review the results of recombination tests involving the left-side deficiencies and N-type tester sites, we saw that three of the four, N^{68f19}, $N^{66h26,12}$, and $N^{66h26,31}$, were separable from all sites to the right of, and including, N^{62b1}. $N^{66h10,1}$ did not recombine with N^{62b1} but did so with N^{264-40}, located to the right. If the cytogenetics of N^{62b1}, which indicates that this right-side deficiency lacks 80% of the Notch recombinational unit (Welshons, 1974), is correct, then we would deduce from positive recombination results with three left-side deficiencies that the deletions to the left and right, are genetically as well as cytologically nonoverlapping. That is, chromosome material common to both N^{62b1} and the left-side deficiencies must exist in order that recombination

occur between them. None of the left-side deficiencies has recombined with $\underline{N^{55e11}}$. Hence, the combined results from both $\underline{N^{62b1}}$ and $\underline{N^{55e11}}$ tests would seem to indicate that $\underline{N^{68f19}}$, $\underline{N^{66h26,12}}$, and $\underline{N^{66h26,31}}$ each possess at least 80% of the Notch recombinational unit, defined by the distance separating $\underline{N^{62b1}}$ and $\underline{N^{55e11}}$ relative to the total map length.

The fourth left-side deficiency, $\underline{N^{66h10,1}}$, did not recombine with $\underline{N^{62b1}}$ in two experiments totalling some 170,000 tested chromosomes. Two (or more) alternative positions might be taken when considering these negative results.

First, one might treat the results at face value and conclude that $\underline{N^{66h10,1}}$ represents a deficiency extending into Notch beyond the site defined by $\underline{N^{62b1}}$, rendering the two deficiencies overlapping which, it is assumed, prevents recombination (i.e. homologous regions are thought to facilitate the pairing required for possible exchange). If this were the case, $\underline{N^{66h10,1}}$ could readily be compared to $\underline{N^{66i25}}$ (another left-side deficiency) which apparently terminates to the right of $\underline{N^{264-40}}$, which is located near the center of the Notch map.

Or second, one might attribute the negative recombination from $\underline{N^{66h10,1}}/\underline{N^{62b1}}$ heterozygotes to the effects of chance. Few recombinants were expected in the first place. It will be recalled that no recombinants were re-

covered in the first $\underline{N^{66h26,31}/N^{62b1}}$ test (60,000 tested chromosomes), but, upon repeating the experiment, only two were detected in a sample of 87,000 chromosomes. Additionally, $\underline{N^{66h26,12}/N^{62b1}}$ heterozygotes produced only two recombinants in a sample similar in size to each of the $\underline{N^{66h10,1}}$ experiments. Further support for this contention comes from a comparison of recombination values obtained in tests involving $\underline{N^{66h26,12}}$, $\underline{N^{66h26,31}}$, and $\underline{N^{66h10,1}}$ and tester sites to the right of $\underline{N^{62b1}}$ (see Table 7). When one considers these values relative to the numbers of chromosomes tested and the sensitivity of the techniques employed, the positive separation of $\underline{N^{66h10,1}}$ and $\underline{N^{62b1}}$ would be predicted.

Based on these reasons, the latter explanation for the negative results is favored. This interpretation, if correct, effectively eliminates the necessity to provide a speculative, and more complex than desired, explanation for $\underline{N^{66h10,1}}$, similar to that required by the cytogenetics of $\underline{N^{66i25}}$. In general, the evidence favoring this explanation appears strong enough for tentative acceptance; but, it is clear that results of experiments now being conducted to resolve some of the equivocal features of $\underline{N^{66i25}}$ (Welshons, personal communication) may necessitate the re-evaluation of the $\underline{N^{66h10,1}}$ data described here.

The negative recombination observed between all left-side deficiencies and $\underline{N^{55e11}}$ becomes less intelligible when

the cytology of the left-and right-side deficiencies is considered. Before giving further consideration to the apparent incompatibility of cytological and genetic interpretations, it may be helpful to discuss more fully the genetic data pertaining to N^{55e11} because its recombinational association with N^{62b1} might be similar to that of the left-side deficiencies.

Cytologically, N^{55e11}'s banding pattern has been described as normal. Genetic analyses indicate that it occupies the left-most (i.e. distal) site on the Notch map (Figure 1), possibly within band 3C7. N^{55e11} has been utilized as a pivotal tester site in recent recombination experiments to ascertain the extent to which various deficiencies include Notch and/or band 3C7. Consequently, a more specific assignment of N^{55e11} may be inferred from studies originally designed for other purposes.

For example, Welshons and Keppy (1975) and Keppy (1975) have recently reported the results of their cytogenetic analyses of the rather unique recessive visible, facet-strawberry (fa^{swb}), briefly alluded to earlier. It was concluded from cytological examination and reinforced by genetic tests that fa^{swb} represented a visible deletion within the 3C6-3C7 interband. Specifically, fa^{swb} was shown to be inseparable from N^{55e11} and to occupy a position to the right of the termination point of the deficiency, w^{67k30}

(= Df(1)3C2-3C6 but N^{+}). These results would seem to place N^{55e11} at the extreme left edge of 3C7 or possibly at the right edge of the 3C6-3C7 interval. The inability to separate the left-side deficiencies from N^{55e11} will be dealt with shortly in this context.

Returning to our consideration of the Notch deficiencies, the cytogenetics of N^{62b1} would place the recombinational unit of Notch specifically in the 3C7-3C8 interband. Hence, one would deduce that the left-side deficiencies should be a long distance (i.e. measured by recombination) away from N^{62b1}, if the cytological determinations of both classes of deficiencies are correct. However, the genetics of the left-side deficiencies do not bear this out, as a glance at Table 5 reveals: N^{68f19}, $N^{66h26,31}$, and $N^{66h26,31}$ each map within .005-.006 units to the left of N^{62b1}.

Now, an obvious question follows: why is not the expansive bulk of DNA comprising band 3C7 reflected in recombination values between sites on either side? Explanations for this dilemma generally focus on two possibilities: 1) the inhibitory effect on recombination by deletions adjacent to regions capable of exchange; and 2) the intrinsic involvement of the band itself in the crossover event. Each possibility will be considered individually, though it is realized that both might be contributing factors to a reduction in recombination frequency.

First, trans-heterozygous deficiencies may inhibit the pairing of homologous chromosome regions which is thought to mediate the exchange process. In the absence of such pairing (whether continuous or discontinuous) which one would expect where deletions exist, the rate of recombination is thought to be reduced. Lefevre and Moore (1966) concluded from their studies of large chromosome deletions (including large numbers of bands), that such deficiencies do have a noticeable, positive inhibitory impact on synapsis and crossing over in adjacent regions. However, the rather ambiguous recombination results gathered from experiments involving much smaller deletions (a few bands) adjacent to Notch tend to obfuscate the positive inhibition expected. Although this possible involvement should be appreciated, restraint must be applied to the immediate acceptance of this interpretation, tempting as it might be.

And second, interest has focused on the actual participatory role of the band in exchange events. For instance, Lefevre (1971) has demonstrated the apparently significant role played by heavy bands (i.e. greater DNA content) in increasing the frequency of recombination in a particular chromosomal region. That his proposal is valid can be exemplified by noting the correlation between map distances and band distribution (qualitative) in the adjacent region of the X chromosome: yellow to white and white to Notch.

Recombination tests indicate that the white locus is equidistant (1.5 units) between the two peripheral markers. However, Bridges' (1938) map of the X chromosome reveals that y is separated from w by a rather large number of bands of intermediate or narrow width while w and N are separated by only the two prominent doublets, 3C2-3 and 3C5-6. From similar observations, Lefevre (1974) infers that heavy bands may be the sites of strong attraction and homologous pairing which tend to facilitate an increase in crossing-over between sites adjacent to, but not necessarily involving, the bands themselves.

If this suggestion is correct, two considerations must be weighed with respect to the effect of adjacent "heterozygous deficiencies" on possible exchange events between them, in particular, the situation between left- and right-side deficiencies at Notch. First, the elimination of both the heavy doublets, 3C2-3 and 3C5-6, to the left of 3C7 (the left-side deficiencies) and the numerous lighter staining bands to the right ($\underline{N^{62b1}}$) might act to reduce recombination in the region separating them. But, the apparent presence of 3C7 in each type of deficiency would seem to provide the band to band attraction required, thus facilitating the exchange of genetic material common to both.

Finally, one might consider that the effects of heterozygous deficiencies and those of band quality on recombination

frequencies are not mutually exclusive. That is, the effects of deleted segments in reducing the rate of recombination may actually reflect the elimination of regional bands that promote, but do not participate in, the recombination event. This interpretation of band involvement in promoting, but not necessarily participating in, crossing-over might be applicable to the unexpected reduction in recombination noted between the left-side deficiencies (and perhaps $\underline{N^{55e11}}$ also) and $\underline{N^{62b1}}$.

Interpretations

From the foregoing discussion, then, the cytology and genetics of the left-side deficiencies appear, at face value, rather incompatible. However, a number of explanations come to mind that might conciliate their differences.

First, briefly alluded to above and suggested by Lefevre (1974), the chromomere (excluding interbands) might act solely as a synaptic factor in promoting the pairing apparently required for exchange, but whose DNA simply does not participate, per se, in such an event. This possibility might not be so unlikely when the structure of the meiotic chromosome at prophase I is considered. Although our understanding of synapsis and the associated role of the synaptinemal complex is far from complete, the likelihood of base to base pairing in such tightly compacted and coiled

regions as the chromomere appears remote. The base to base pairing that has been suggested by students of recombination might then occur between interband DNA which appears more diffuse in electron micrographs of prophase chromosomes (see DuPraw, 1970).

The distances separating sites on either side of the band, measured by recombination frequency, would not, then, reflect the bulk DNA comprising the band which is physically incapable of pairing and exchange. In the case of Notch, where 3C7 is thought to contain approximately 30,000 nucleotide pairs (Rudkin, 1965), the difficulties in justifying recombination frequencies between left- and right-side sites might be circumvented by such a model.

Second, and already discussed in some depth, is the inhibition of recombination by adjacent deletions as suggested by Lefevre and Moore (1966). It remains only to point out that their studies involved comparatively long deficiencies; the impact of much shorter deletions remains to be determined.

Third, the left-side Notches may represent deletions which initiate to the right of band 3C7, extend leftward toward white, and inexplicably retain the intact band in the process. The possible mechanisms required to explain such an event become more complicated than desired. As previously mentioned, Welshons and Keppy (1975) have also struggled with this problem and have suggested an interpretation based

on the binemic construction of the meiotic chromosome. In spite of available evidence in support of uninemic chromosome structure, this explanation remains viable because its application does satisfy many of the cytogenetic observations at Notch.

And fourth, each left-side N may, in addition to the cytological deletion to the left of 3C7, also carry an independent, but closely linked, mutation (point or subvisible deletion) to the right, as noted by Welshons (1974) in his interpretation of the cytogenetics of N^{68f19}. The presence of a second mutation would confound any interpretations based on the single deficiency assumption. Granted, the probability of this applying to all five left-side deficiencies that have been analyzed is remote, but should be considered, especially since the mutability system from which each originated (w^8; Green, 1967) remains so vague.

This discussion of hypothetical interpretations of the cytogenetics of the left-side Notches emphasizes the perplexity associated with our present understanding of chromosome substructure and mechanisms for recombination, upon which most explanatory models are constructed. Obviously these interpretations must be appreciated as such; however, they do suggest experimentally testable approaches which may eliminate one or more from consideration in the future.

To draw dogmatic conclusions from these results, many

of which are negative, of the cytogenetic analyses of the left-side Notches would seem improvident at the present. However, a few observations are pertinent to our appreciation of the structure and function of band 3C7 and $\underline{N^{+}}$ activities. These results, and accumulated evidence from similar studies (Welshons, 1974; Welshons and Keppy, 1975), would seem to argue, in general, for the cytogenetically bilateral nature of band 3C7, primarily in the interbands adjoining it. Cytological deletions of material to either the left or right of 3C7 result in the expression of Notch phenotype, and in most cases, recessive lethality. Genetically, the Notch series appears to localize in the 3C7-3C8 interband, assuming the cytogenetics of $\underline{N^{62b1}}$ are correct.

The recombination values obtained in genetic tests present the greatest obstacle to defining the limits to which left-side deficiencies infringe upon Notch. Until the band's involvement in recombination is better characterized, these values may be misleading.

Genetics of EMS-induced Notches

Recombination experiments were conducted to determine the location of six EMS-induced Notches, relative to the recessive visibles $\underline{fa^{g}}$ and $\underline{spl}$. The data in Table 10 indicate that each of these mutants localizes in the right-

most half of the genic map, defined by $\underline{fa^g}$'s roughly centric position. Three of the six were separable from spl and located to its right. The remaining three were inseparable from spl in relatively small samples of surviving progeny but whose positions to the right of $\underline{fa^g}$ could be inferred from the types of other intralocus recombinants observed during the course of the experiments. It is not unreasonable to predict that each of the three can be separated from spl and their sites more accurately determined in experiments of greater dimension.

Mindful of these results and cognizant of the apparent clustering of other recessive lethals to the right of $\underline{fa^g}$ (Figure 2), one seeks to determine what, if any, significance such a distribution has on descriptions of Notch organization. This observation raises several important questions regarding Notch structure and function.

First, if the Notch complex is comprised of more than one cistron as the allele distribution might suggest, are N-type mutations to the left of $\underline{fa^g}$ possible? That is to ask, could one expect to recover a recessive lethal, phenotypically dominant Notch (excluding $\underline{N^{55e11}}$ for the moment), as a result of a point mutation to the left of $\underline{fa^g}$ if the facet series is independent of a contiguous Notch series?

Or second, if Notch is simply one cistron involved in a variety of functional roles in development, does the cluster-

ing of recessive lethals merely reflect damage to a particular sequence of the resultant polypeptide? Visualized at the molecular level, do mutations, either missense or nonsense, in the right half of a single Notch gene, exclusively, suspend normal product activity?

Or third, is the clustering in the right half of the map evidence for the existence of so-called mutational "hot-spots" that were noted by Benzer (1961) in his studies of T2 bacteriophage? Contrary to evidence disputing their existence over longer regions on the Drosophila chromosome (Rudkin, 1965) this view suggests that recessive lethal mutations (spontaneous or induced) at Notch are restricted to specific sub-locus sites which are more susceptible to alteration than others. Considering the mechanics of mutation, this view seems unlikely; for example, EMS appears to indiscriminately methylate purines along a DNA strand. But, when the consequences of the mutational event are addressed, the clusters of phenotypically related alleles, observed at many complex loci including Notch, may argue in support of this interpretation. However, the apparent existence of sub-locus "hot-spots" may bear directly on the validity of the two alternative considerations of the Notch locus questioned above.

Before turning to the mono- vs. multifunctional nature of Notch suggested by the first two interpretations, two

additional aspects of recessive lethal clustering deserve attention. Firstly, it might be argued that the screening techniques employed in N isolation relies entirely upon the recognition of typical Notch characteristics and the subsequent realization of recessive lethality. This can not be denied; although, if the region to the left of fa^g is capable of mutating to a recessive lethal N-type, one would certainly expect to have isolated one by now. Perhaps more lethal sites, in addition to l(1)N, do exist between fa^g and N^{55e11}. A mutant screen, designed to detect such mutations without depending upon the Notch phenotype or the pseudodominant expression of a recessive visible allele, may facilitate their detection. Or, alternatively, the use of fa^g (or another allele of the facet series) in the screen may distinguish amorphic mutations to its left that might go undetected when recessive visibles to the right are employed. Whether this possibility can be realized remains to future investigations.

Secondly, Shellenbarger and Mohler (1975) have recently noted an interesting correlation between temperature-sensitive Notch pseudoalleles, either recessive lethal or viable, and their locations on the genic map (Figure 2 includes a number of their "ts" alleles). They found that all temperature-sensitive alleles, with the exception of one (fa), localize to the right of spl. The significance of the

clustering of both recessive lethal and ts alleles (not mutually exclusive classes) to the right of $\underline{fa^g}$ remains speculative.

Notch: Organization and Function

Two major schools of thought have arisen in attempts to elucidate the structure-function relationship of the Drosophila chromomere. Paradigms proposed by members of each group generally reflect the acceptance of different premises. First, and currently the more popular view, the chromomere is thought to contain a single structural gene sequence. Primary support for this contention comes from the oft-cited studies of Judd et al. (1972; see Introduction). Various of the chromosome models previously discussed include allowances for this interpretation (e.g. those proposed by Britten and Davidson (1969) and Crick (1971)). In such models, the bulk of chromomeric DNA is thought to perform regulatory and/or recognition functions.

The second group includes investigators who consider some chromomeres (not necessarily all) to be comprised of multiple structural elements, whose products function in sequential reactions, comparable to similar systems recognized in lower organisms. Functionally related cistrons, grouped in a chromomeric complex, might then be coordinately controlled as required. Lewis (1964, 1967), it will be

recalled, early suggested that the bithorax complex typified an operon-like form of organization. More recently, Fristrom and his colleagues (Fristrom and Yund, 1973; Rawls and Fristrom, 1975) drew similar conclusions from their biochemical studies of complementation groups assigned to the rudimentary locus. Primarily, this type of argument has been invoked to explain the irregular patterns of complementation exhibited by spatially distinct groups within a particular complex locus (e.g. at white; Green, 1959).

How, then, do these interpretations apply to the cytogenetics of Notch? Or conversely, do the cytogenetics of Notch fit any of the models suggested for chromosome organization? To these ends the remainder of this discussion will be directed.

Notch: One cistron or many?

Welshons (1965) early concluded that Notch could best be described as a single cistron assigned to salivary band 3C7. His conclusion was based, primarily, on a comparison of Notch with other complex loci, including bithorax and dumpy, when criteria such as polarization effect, allele-type distribution, and cytological ubiety were considered.

However, the complex interallelic complementation patterns, the diverse pleiotropic effects (both temporally and morphologically), and the apparent clustering of related alleles have hauntingly been a basis for objections to such

an interpretation. For example, in the absence of a definitive gene product(s), the trans-complementation test has classically been employed to distinguish independent coding units.

Shellenbarger and Mohler (1975) have challenged this objection. The results of their temperature-sensitivity and temperature-shift experiments involving several Notch alleles have led them to conclude that Notch is a single cistron. They argue that interallelic complementation results from the spatially and temporally distinct functions performed by complementary alleles. For instance, the heterozygote, $\underline{fa^g}/\underline{nd}$, is phenotypically normal, they say, because the product of the $\underline{fa^g}$ allele is able to compensate for nd (and reciprocally) under developmental conditions requiring $\underline{nd^+}$ function in which the mutant effect of $\underline{fa^g}$ is not manifested.

On the other hand, the Notch series might as easily, with about the same number of postulates, be viewed as an operon-like complex, comprised of both structural sequences and one or more regulatory elements. The cluster of recessive lethal and temperature-sensitive Notches to the right of $\underline{fa^g}$ may demarcate two distinctive structural sequences. The left-most segment, including the facet-like recessive visible alleles, may represent a second unit which functions in interdependent activities with the first.

One potentially serious flaw in this interpretation

might be exposed when the cytogenetics of $\underline{N^{62b1}}$ are considered. If Notch activities are localized to the 3C7-3C8 interband as $\underline{N^{62b1}}$ data suggests, one might question whether enough DNA comprises this interval to constitute more than one cistron. However, if recombination distances do reflect the content of DNA in the interband, Notch would seem to possess considerably more DNA than other loci thought to be monocistronic. For instance, Gelbart *et al.* (1974) have concluded that the rosy locus represents a single cistron. Its total map length based on recombination tests, however, is considerably less than that of Notch (i.e. rosy is approximately .009 map units in length compared to .13 units at Notch). Difficulties with interband DNA content might be conveniently circumvented, however, by assigning regulatory roles to each of the postulated independent units; Foster (1975) has recently suggested that the entire Notch locus performs regulatory functions, but both possibilities remain speculative.

With regards to either interpretation (i.e. mono- or multicistronic), it is tempting to hypothesize the involvement of the 3C7 band material and a short segment to its immediate left in a regulatory capacity. If such were true, the left-side deficiency mutants described in this report (and possibly $\underline{N^{55e11}}$, as well) might then be considered as *cis*-dominant regulatory mutants which delete all or part of

the regulatory element(s) associated with $\underline{N^+}$. Inactivation of the regulatory sequences would predictably, in turn, render the structural element (or elements) partially or totally inactive. Unfortunately, the lethality resulting from these deletions prevents the determination of whether one, or more than one, structural element is inactivated. That is, were the hemizygous males viable under these conditions, one might expect them to express the abnormal phenotypes associated with both the recessive facet series and the Notch or notchoid series of alleles.

Notch and models of organization

The Notch data reported here neither unequivocally substantiate nor refute any of the interpretations of chromomere organization and function. The Notch system seems uniquely complex: it shares many characteristics with other complex loci but would seem to singularly possess others. Proponents of various interpretations might conceiveably use any or all available Notch data to support their respective positions.

However, certain features of operon-type models (without regard to the number of cistrons present) proposed by Britten and Davidson (1969), Crick (1971), and others (see Introduction) are attractive. For example, Crick has postulated that the chromomere's informational sequences reside in the adjacent interband. The cytogenetics of the

right-side deficiency, N^{62b1}, would point to a similar interpretation. But, the cytogenetics of the left-side deficiencies reported here and elsewhere (Welshons, 1974; Welshons and Keppy, 1975) would further suggest the additional involvement of the 3C6-3C7 interband in N^{+} function.

Operon-type models generally also contain provisions for the functional activities of the bulk DNA comprising the chromomere. Various types of regulatory sequences, both for replication and transcription, are thought to occupy the banded regions of the Drosophila chromosome.

Whether the chromomere in general, and Notch in particular, represents a single cistron in association with a single salivary band remains undetermined. In the absence of the specific gene product (or products) coded by Notch, many questions remain unanswered. The ultimate resolution of Notch structure-function will require this determination.

SUMMARY AND CONCLUSIONS

The eucaryotic chromosome, in particular that of Drosophila melanogaster, exhibits a level of complexity that precludes the comprehensive analysis of its properties. Instead, biologists have focused on individual structural characteristics and functional activities in efforts to describe its collective genetic role.

The chromomere observed in polytene chromosomes has provided a suitable system for such studies. Its structural independence, though necessarily a part of the chromosomal whole, is readily recognized cytologically; so too, its relative autonomy in replication and transcription has lately been appreciated. These properties, then, have led many geneticists to view the chromomere as a rather independent unit of the chromosome, both functionally and genetically. However, the characterization of the chromomere is not without controversy.

Particularly, the structure-function relationship of the abundant quantities of DNA per chromomere has stirred much debate. That the chromomere is apparently comprised primarily of unique sequence DNA (see Laird et al., 1973), linearly ordered in a single fiber (Kavenoff and Zimm, 1973) requires the elucidation of its structural organization.

Fine structure analyses of complex loci in Drosophila, including Notch, have been employed to this end. In the investigation reported here, the cytogenetic analyses of recessive lethal and visible mutations, which, on the basis of trans-complementation tests and pseudodominant effects, localize to Notch, have been conducted.

Cytological examinations of the salivary gland chromosomes of the left-side deficiencies, N^{68f19}, $N^{66h26,12}$, $N^{66h26,31}$, and $N^{66h10,1}$, reveals that each is deficient for bands 3C2 to 3C6, inclusive. Genetically, each is phenotypically Notch and noncomplementary with any of the recessive visible alleles. Recombination data gathered on the left-side deficiencies indicate that N^{68f19}, $N^{66h26,12}$, and $N^{66h26,31}$ are separable from N^{62b1} and all tester sites to its right; $N^{66h10,1}$ has yet to be separated from N^{62b1} although it recombines freely with all other sites to the right that have been tested. None, including N^{66i25} (Welshons, 1974), recombined with the left-most tester site, N^{55e11}, in tests involving nearly one half million chromosomes. The positive recombination values obtained in these tests, especially with N^{62b1}, are inexplicably lower than expected, assuming the cytological interpretations are valid. Possible explanations for this observation have been discussed.

Thus, the genetic data presented here and previously

(Welshons, 1974; Welshons and Keppy, 1975) would seem to indicate that Notch, defined by its recombinational sites, occupies the 3C7-3C8 interband. However, conclusions drawn from cytological and phenotypic observations would include the involvement of the 3C6-3C7 interval, as well, in $\underline{N^+}$ function. The structural and functional roles of the bulk, banded DNA remain undetermined.

Also, a number of EMS-induced recessive lethal Notches have been recovered and studied. Recombination tests involving six EMS-$\underline{N}$'s and the coupled alleles, $\underline{fa^g}$ and $\underline{spl}$, have been conducted. Each mapped in the right-most half of the genic map, defined as proximal to the position of $\underline{fa^g}$. In view of these results, and others, which show a clustering of phenotypically similar alleles on the map, it is perhaps instructive to consider the possibility that Notch is comprised of two or more structural elements which function in a common metabolic process. Of additional value, future endeavors might be directed toward discerning the probable participating functions of the banded DNA in the regulation of replication, recombination, and transcription of the chromomere.

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