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THE ORGANIZATION OF GENETIC UNITS IN CHROMOSOMES

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SUMMARY

In the genomes of chromosomal organisms, cytological evidence from disparate sources suggests that each unit of information encoded as a DNA base sequence is serially repeated. Further cytological and genetical evidence suggests that among such serially repeated sequences a terminal unit serves as the 'master' sequence, within which recombinational events can occur, followed by 'slave' sequences which are not directly involved in recombination but which are made congruent to the master sequence once per life-cycle. The formation of lateral 'lampbrush' loops in meiotic prophase, after synapsis, is claimed to represent the outcome of the master/slave matching process.

Whitehouse (1965) has divided living organisms into two major categories: a chromonemal kingdom (bacteria and blue-green algae) in which the DNA is not associated with basic protein, and no nuclear membrane encloses the DNA; and a chromosomal kingdom (all the rest) in which the DNA is associated with basic protein, and a nuclear membrane encloses the DNP except during cell division. It is widely recognized that the chromonemal and chromosomal kingdoms represent different levels of complexity of organization of both primary genetic and somatic material. Whereas the organization of genetic material in some chromonemal organisms has already been largely explored, that of chromosomal organisms is still very much the subject of debate and speculation. In the present paper certain observations which have a bearing on this debate will be considered.

Several authors, following the pioneer observations of Mirsky & Ris (1951), have drawn attention to the astonishing range in DNA content of the gamete nuclei of diploid chromosomal organisms. Within the class Amphibia *Necturus* has a 'DNA value' more than 10 times that of *Rana pipiens* (J. G. Gall, personal communication). Within the order Leguminosae *Vicia faba* has a DNA value more than 10 times that of *Lupinus albus* (Sunderland & McLeish, 1961).

Even within a genus there may be grossly different DNA values in different diploid species. Dr H.-G. Keyl has informed me that spermatids of the amphipod crustacean *Gammarus pulex* ($n = 26$ or 27) contain about 3 times as much DNA as those of *G. chevreuxi* ($n = 26$); and even more striking, spermatids of the rhabdocoel planarian *Mesostoma ehrenbergi* contain about 11 times as much DNA as those of *M. lingua*, yet both species have the haploid chromosome number of 4.

There are many references in the older cytogenetic literature to related diploid organisms whose otherwise similar chromosome complements differ from one

another markedly in volume. While such differences do not necessarily imply that the organisms being compared have markedly dissimilar DNA values, this at least seems likely in the case of two species of *Crepis*; the complement of *C. neglecta* is about 4 times larger than that of *C. fuliginosa*, and in F_1 hybrids the size difference is maintained (Tobgy, 1943).

No doubt supernumerary chromosomes, where they occur, and variation in the amount of 'heterochromatin', if such is present, make their contributions to contrasting DNA values; but these can hardly account for gross differences. Cytologists who consider chromosomes to be multistranded, i.e. as containing several DNA fibres in parallel, find no particular difficulty in accounting for such differences; other things being equal, the higher the DNA value the greater the number of strands assumed to be present. But the evidence against multistrandedness of chromosomes other than those which are demonstrably polytenic (e.g. in some somatic cells of Diptera), and in particular of germ-line chromosomes generally, is compelling.

The semi-conservative mechanism of DNA replication in plant and animal chromosomes, even in species such as *Vicia faba*, the first studied, with exceptionally large chromosomes (Taylor, Woods & Hughes, 1957), was demonstrable by virtue of the very simplicity of the pattern of labelled chromatid segregation. The pattern of segregation requires that a chromatid should contain one DNA duplex. If more than one is present, subsidiary hypotheses have to be invoked to explain the simplicity of the pattern. Furthermore the relative frequency of 'single' and 'twin' exchanges of labelled segments between sister chromatids, as observed by Taylor (1958), is compatible with chromatids each containing two different subunits, reunion between subunits being restricted to those which are alike. This requirement is in telling accord with the opposite polarities of the polynucleotide chains of a DNA duplex; if more than one duplex is assumed to be present, more subsidiary hypotheses have to be erected to explain the observations.

In the meiotic lampbrush chromosomes of female newts the most recent determinations (Miller, 1964) of the thickness of the fibre between chromomeres (about 50 Å, two chromatids) and of the axial fibre in lateral loops (about 30 Å, one chromatid), exclude the possibility that the DNA component of these chromosomes is multistranded. Yet newts have particularly large DNA values. Furthermore Gall (1963) has shown that the kinetics of breakage of newt lampbrush chromosomes by DNase is substantially consonant with the supposition that the chromatid contains two separately breakable subunits; when DNase degrades DNA in chemically defined conditions, the subunit polynucleotide chains are cleaved independently and the kinetics of duplex breakage is similarly second order (Thomas, 1956).

When genetical evidence is also taken into consideration—the phenomena of mutation and recombination in a wide range of organisms are assuredly attributes of chromatids, not subunits of chromatids—the case against multistrandedness of DNA in chromatids is overwhelming. How then are gross differences in DNA values between related species to be explained? Recent observations of Keyl (1964, 1965*a*, *b*) provide an acceptable explanation in terms of the serial duplication of units of replication of DNA within the lengths of chromatids.

The essence of Keyl's discovery is that when the DNA contents of homologous (though evidently heterozygous) bands of *Chironomus* salivary gland chromosomes are compared, the ratios of DNA quantities conform to a geometric doubling series 1:2, 4, 8 or 16. At first sight such a relationship might seem to depend on one or more extra cycles of DNA replication having occurred in particular bands but not in their partners; indeed, during their replication, bands containing more DNA incorporate [^{3}H]thymidine for longer periods than homologous bands containing less (Keyl & Pelling, 1963). But this explanation is not acceptable. Keyl (1965*b*) has compared the integrated salivary gland chromosome and gametic DNA values of *Chironomus thummi thummi* with those of its near relative *Ch. th. piger*; *thummi* has 27% more DNA than *piger* in its gametes as well as in its salivary gland chromosomes. The larger DNA value for the salivary gland chromosomes of *thummi* evidently reflects the sum total contributions of the higher DNA contents of many of *thummi's* individual bands, but as it is equally a feature of germ line nuclei the excess DNA must be an intrinsic feature of the genome.

Keyl provides evidence that *thummi* has evolved from *piger*, or at least that *piger* is nearer to the ancestral *Chironomus* stock. Keyl explains the geometric increases in DNA contents of units characteristic of the *thummi* genome as having resulted from errors in the replication process. The replicons are assumed to be loops of DNA which normally separate from one another after replication. Occasionally, however, irregular union at the base of a replicating loop results in a tandem duplication of the loop on one chromatid, and a loop deficiency on its sister. If tandem duplication arising in this manner is indeed responsible for DNA differences between homologous but heterozygous replicons in *Chironomus*, it is open to speculation whether homologous and homozygous replicons with comparable DNA quantities have similarly evolved from shorter, more primitive units.

Keyl dismisses the possibility that the duplications may have arisen as a result of unequal crossing-over during meiosis, for if this were the mode of origin, DNA ratios of 1:3, 1:5, etc., would be expected as well as the observed 1:2, 1:4, 1:8 and 1:16. From Keyl's data a further inference can be drawn. Not only is unequal crossing-over ruled out as a prime cause of these DNA duplications; unequal crossing-over evidently does not occur between already established duplications and their ancestral homologues, for if it did occur disturbance of the geometric ratios would again be anticipated. That there is no such disturbance indicates that a restriction on recombination operates at meiosis. When synapsis occurs between two replicons, one of which is a 4-fold serial repeat of its homologue, either recombination between the two is totally inhibited or recombination is restricted to the 'first' (alternatively the 'last') member of the series. I wish now to give further attention to the second possibility, for its implications are considerable.

The organization of the lampbrush chromosomes in the oocytes of urodele Amphibia is such that the DNA fibre which forms the axis of a lateral loop should be considered part of a very long DNA fibre running throughout the length of a chromatid (Gall & Callan, 1962). This conclusion follows from the observation that at certain sites where 'double loop bridges' occur naturally, notably where the giant fusing loops arise

on chromosome XII of *Triturus cristatus cristatus* (Callan & Lloyd, 1960), the only physical continuity across the site is provided by the loops themselves. However, despite this structural relationship between lateral loops and the 'major' chromosome axis of chromomeres and DNA-containing fibres between chromomeres, chiasmata never involve the lateral loops. In other words the restriction on sites of recombination to be inferred from Keyl's data on *Chironomus* is matched by an otherwise puzzling feature of the lampbrush chromosomes of *Triturus*.

The asymmetrical distribution of RNP matrix on the lateral loops of lampbrush chromosomes, which for particular loops shows constant polarity with respect to the chromosome as a whole, prompted the speculation (Callan & Lloyd, 1960) that lateral loop axes progressively extend from chromomeres and retract back into chromomeres in a polarized manner, engaging in RNA synthesis while they are extended. Movement of the kind postulated, in the direction postulated, and taking about 10 days to complete the transit from the 'left' side to the 'right' side of the chromomere, has been demonstrated for the RNP of the giant granular loop of chromosome XII of *T. cristatus cristatus* (Gall & Callan, 1962), though whether the loop axis moves along with the matrix is debatable. On morphological grounds it is easier to envisage both moving together than to envisage movement of matrix relative to a stationary loop axis, and I am going to assume that loop axes do in fact move in the manner proposed. If this be granted, then the DNA axis of a given loop involved in RNA synthesis at a certain time will be a different stretch of DNA from that which was similarly engaged, say, 1 month earlier. Yet particular textural qualities of loop-RNP matrix enable loops at certain sites to be identified in oocytes over a considerable developmental range. In other words, although the DNA extended as a loop axis may consist of different material at different stages during the development of an oocyte, the characteristic qualities of the RNP whose synthesis it supports may remain unaltered.

Since lateral loops of characteristic morphology show classical Mendelian inheritance in F_1 and backcross hybrids between subspecies of *T. cristatus*, and since as an outcome of meiosis in F_1 female hybrids intrachromosomal recombination between loops has been demonstrated (H. G. Callan & L. Lloyd, unpublished) it is evident that characteristic lateral loop morphologies are determined genetically. Taken in conjunction, these attributes of lampbrush chromosomes require that there should be a serial repetition of genetic information encoded in the DNA at each lateral loop locus (Callan & Lloyd, 1960).

Two independent lines of investigation thus lead to a similar conclusion, yet this conclusion seems at first sight entirely at variance with a well-established fact of genetics: in diploid organisms heterozygosity is normally restricted to pairs, and not more than pairs, of allelic alternatives. If units of genetic function, each capable of mutation, were many times repeated along a chromosome, one would expect in heterozygotes to observe families of related products determined by different segments of a repeated code, not merely two alternatives.

Confronted by this incompatibility of evidence, Callan & Lloyd (1960) proposed that each unit of genetic function along a chromosome consists of one master copy of the coded information followed by a series of slave repeats which in some obscure

manner are further specified by the master copy. I wish now to extend this argument.

If the master copy of the code for any particular genetic function consists of a particular nucleotide sequence, matching of a slave to the master sequence might be achieved by separation of the polynucleotide chains followed by base pairing between complementary segments of master and slave, followed in turn by correction of base sequence in the slave wherever incongruities happen to be present. A formal diagram of the scheme proposed is shown in Fig. 1 A, B. In order to correct the code in the

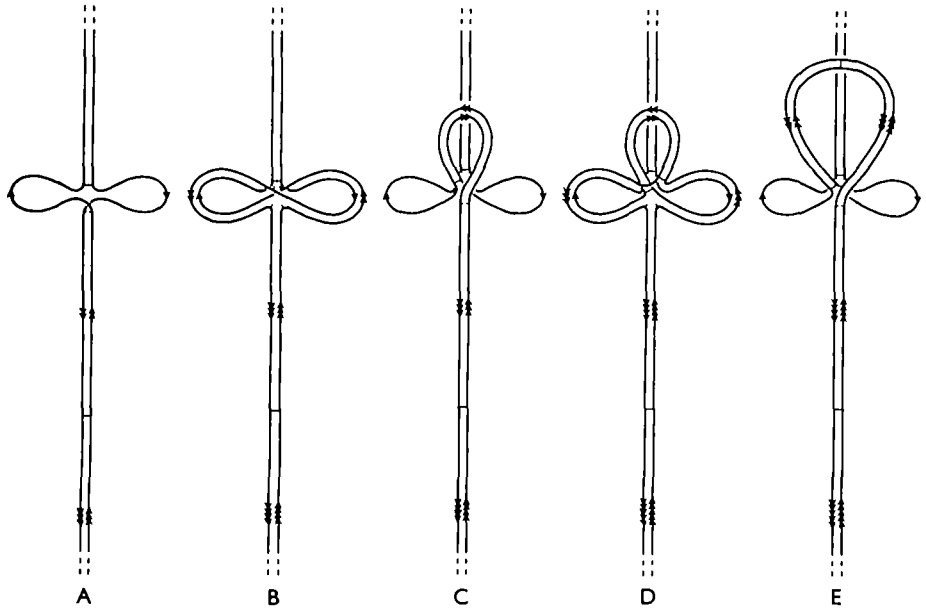

Fig. 1. Diagrams to illustrate the postulated matching of slave sequences to the master sequence, and the polarized extension of a lateral loop formed by slave sequences after they have been matched, in a portion of a single chromatid. The opposite polarity of complementary polynucleotide chains is indicated by arrows pointing in opposite directions. The limits of repeated coding sequences are indicated by short lines at right angles to the polynucleotide chains. The first (master) sequence is marked with single arrows, the second (first slave) is marked by two, the third (second slave) is marked by three, and so forth. Further explanation is given in the text.

next slave sequence, the complementary sequences already corrected detach from the master sequences, base pair with one another to form a loop, and the next slave proceeds to match bases with the master (Fig. 1 C, D). Matching having occurred, these slave sequences detach from the master sequences, base pair with one another, and extend the already initiated loop from one side (Fig. 1 E). The process is assumed to continue until all slaves have been matched to the master sequence.

The scheme outlined above would explain 3 characteristic features of lampbrush chromosomes: the lateral loops; the polarized extension of lateral loops; and the formation of loop bridges when the chromosomes are stretched (by the breakdown of base pairing between master and slave sequences).

In the diagram, for the sake of clarity, the matching of slaves to the master sequence has been shown as an intermittent process, but it could just as well be continuous. The series of slaves not yet matched to the master are shown extended, but in a lampbrush chromosome they are presumed to be packed closely together in the chromomere at the base of a loop. Equally, after engaging in RNA synthesis, the already matched slaves are presumed to pack down in the chromomere. To be compatible with genetic data, the process of slave correction should occur once per life-cycle, and immediately after meiotic recombination. The lampbrush phase precisely fits this requirement.

An evident defect of the scheme proposed is that relational coiling between polynucleotide chains of the DNA duplex is neither indicated in the diagram nor accommodated in the theory. Dr H. L. K. Whitehouse takes up this and further questions in a following paper (see this Journal, p. 9).

The concept of an organization of chromosomal DNA as master gene sequences, in which recombination may occur, followed by slave gene sequences which only reflect recombination in master genes, helps to explain two other well-recognized peculiarities of meiosis: the chromomeral pattern of leptotene, and the synaptinemal complex of zygotene/pachytene. At first sight it seems odd that chromosomes which are about to synapse should form a series of densely packed aggregates of DNA when the very specificity of synapsis and of the genetic recombination which may ensue demands that homologous extended base sequences should recognize one another. But the aggregation to form chromomeres may be a mechanism which ensures that slave gene sequences are excluded from the essential preliminary to recombination, precise pairing of homologous base sequences. It is furthermore a characteristic feature of synapsed chromosomes that most of the homologues' DNA is spaced well apart and lateral to the cores of the synaptinemal complex (Coleman & Moses, 1964). Proportionately very little of the DNA lies in the pairing space between cores. The cores may represent lines of demarcation which separate master gene sequences within the pairing space from slave genes which lie without.

In this paper the term 'master gene sequence' has been used to designate the first member of a repeating series: the term is not intended to imply any attribute other than particular place in an order. This notion takes account of the fact that the nucleolar organizer locus in *Zea* (McClintock, 1934) and also in *Chironomus* (Beermann, 1960) can be broken by X-irradiation into fragments which after passage through meiosis retain their nucleolar-organizing capacities.

It will be apparent that the concept of a master/slave gene organization of the chromosomes of higher organisms is essentially an extension of Weismann's theory (1885) of the continuity of the germ plasm and its segregation from somatic material. It is envisaged that master genes in the germ line may not at any time engage directly in RNA synthesis, this operation being left to the slaves. The master genes are thus supposedly sequestered from the remainder of the genetic material, and less exposed to degradative processes than otherwise they would be.

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