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EDITED BY

G. H. BOURNE J. F. DANIELLI

ASSISTANT EDITOR
K. W. JEON

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Part B: General Topics

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Part B: General Topics

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Plant Chromosomes: A Perspective

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At the simplest level of classification chromosomes of organisms with a wall-bound protoplast fall into three groups.

1. Neither nucleosomal nor linear nor membrane limited (bacteria and blue green algae).
2. Neither nucleosomal nor linear but membrane limited (dinoflagellates).
3. Nucleosomal, linear, and membrane limited (eukaryotes).

Beyond this, subdivision into "primitive" and "advanced" is possible and forms the subject of stimulating reviews by, for example, Cavalier-Smith (1981) and Picket-Heaps (1974). For perspective here, however, a probable sequence of the major evolutionary events involving the nucleus could be the following.

1. A prokaryote ancestor had a closed ring of DNA where the terminus of replication (T.O.R.) associated with the cell membrane.
2. Invagination of the cell membrane gave a persistent nuclear envelope with which the T.O.R. continued to associate.
3. Elaboration of the chromosome involved evolution of plural replicons, linearization together with a mechanism for perpetuating "ends" (telomeres) and evolution of nucleosomes.
4. Fenestration of the nuclear envelope gave the kinetochore a role that linked the original (?) T.O.R. with cytoplasmic elements.
5. Diversification of the chromosome by duplication and rearrangement prompted the elaboration of both homology and nonhomology.
6. Elaboration of function gave split genes, noncoding sequences, gene amplification, partition of the chromosome into eu- and heterochromatin, and the emergence of a (probably persistent) nucleolus.
7. The kinetochore became integral to the chromosome and thus a "centromere."
8. Fenestration of the nuclear membrane was taken virtually to total disin-

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tegration at metaphase and was linked with a sharply defined condensation cycle and a nonpersistent nucleolus.

9. Centromere activity was in some cases dispersed to several parts of the chromosome.

10. Specialization of chromosomes at particular points in the life cycle occurred, examples being emergence of single chromatid types from interphase nuclei for meiotic pairing, multistrandedness in secretory tissues, and loss of totipotency in some aged cells.

Numerous rearrangements of such a sequence are possible but it seems unlikely for example that a nonpersistent nucleolus originally preceded a persistent one or that dispersed centromere activity preceded a nondispersed one however much subsequent evolution reversed these directions. Conversely the elaboration of homology and the evolution of noncoding sequences may or may not be regarded as independent or unrelated events.

The events outlined from 1 to 10 could lead equally to chromosomes of multicellular plants or animals. All higher plant chromosomes however operate within a wall-bound protoplast and additionally, those plants that are green include not only mitochondrial but chloroplast DNA within their cell environment, the inference being that unlike animal chromosomes, those of plants intimately coevolve not with one but two types of subsidiary organelle.

At two extremes detail is readily available. The optical microscopy of plant chromosomes and the base sequences of numerous examples of plant gene are to hand but between these extremes the organization of the chromosome as an arena for molecular events is but little understood and among the questions that could be asked, perhaps the following presently are the most relevant and thus guided the choice of subject matter in the first part of this volume.

1. In the evolution of linear nucleosomal chromosomes do dinoflagellates provide evidence of an intermediate type or are they examples only of an "alternative chromatin"?

2. How are distinctively "plant" characteristics organized and how does plant and animal chromatin compare?

3. How physically manipulable are plant chromosomes and to what extent can techniques for animal chromosomes be applied to them?

4. Lest conclusions be based on too few or unrepresentative samples, how varied is plant chromatin ultrastructurally?

5. How do the centromere and the telomeres, the major "suborganelles" vary?

6. At the applied level what scope is there for *directed* alteration likely to be useful in plant breeding?

As the answers, partial at first, to these questions begin to accumulate, so the study of plant chromosomes can contribute to resolving the enigma of chromosome phylogeny set out earlier.

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The Chromosomes of Dinoflagellates

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I. Introduction

The chromosomes of dinoflagellates have long been suspected of being unusual. The nucleus is generally big and conspicuous (Fig. 1) and, in the larger species at least, chromosomes can be seen even without any specal staining. The nucleus has been described as a dinocaryon. Early work on nuclear division, such as that of Hall (1925), revealed that the chromosomes exist in a permanently condensed state. In the late 1950s electron microscopy revealed a quite unique ultrastructure to the chromosomes and cytochemical studies showed that their major compenent is DNA. It is now known that no true histones are present and histone-like protein represents only a small proportion of the chromosome. Compared to other organisms dinoflagellates appear to contain a disproportionately large amount of DNA which may be as much as 200 pg per cell in *Gonyaulax polyedra* as compared with only about 5 pg in human cells (Allen *et al.*, 1975). Consequently, dinoflagellate chromosomes have been the subject of many investigations and a fair amount of speculation. In this article the historical work will be reviewed and an attempt made to bring together a current view on the structure of the chromosome.

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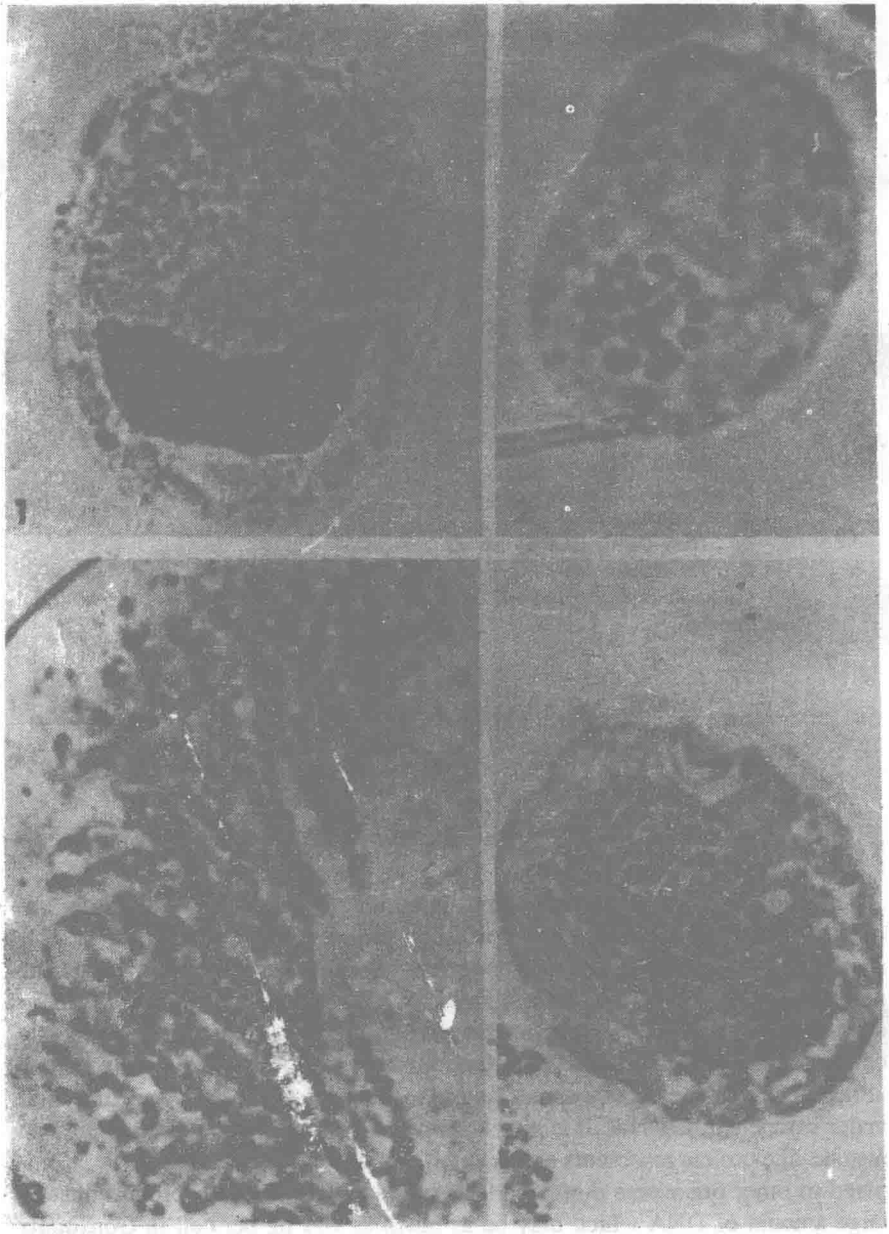

FIGS. 1–4. Light micrographs of stained dinoflagellate chromosomes. Fig. 1. *Amphidinium herdmanae* showing the numerous long chromosomes. Acetocarmine. ×2000. Fig. 2. *A. carterae* with a small number of short chromosomes. Acetocarmine. ×3500. Fig. 3. The long chromosomes of *Prorocentrum micans* which have been treated to reveal evidence of a chromonema which is thinner than the chromosome. Acetocarmine. ×2500. Fig. 4. A section of a plastic-embedded cell of *P. micans* stained with Azur B to show the presence of DNA in the chromosomes. ×2000.

II. Chromosome Numbers

Following the life history studies of von Stosch (1973) it is generally thought that the normal motile dinoflagellate cell has a haploid chromosome number. Nevertheless, the numbers recorded, apart from those of a few parasitic dinoflagellates, are extremely high and because of the difficulty of making squash preparations in general the counts given are only approximate. There is no metaphase plate in the sense that this is found in higher eukaryotes and the counts have generally been made on interphase nuclei where the chromosomes, when long, may be tangled together (Fig. 3). The chromosomes also appear to be rather easily fragmented, perhaps a consequence of the lack of histone in their construction. Recently, special techniques involving cell lysis and enzymic digestion have been devised to assist in spreading and counting these chromosomes (Holt and Pfiester, 1982; Loper *et al.*, 1980) in species where the chromosomes are long and numerous.

The earliest known count (approximation) was that of Borgert (1910) who found that *Ceratium tripos* had about 200 chromosomes. A freshwater species, *C. hirundinella,* was found to have an even higher number, 264–284 (Entz, 1921) but an *Amphidinium* species had only 25 ± 1 (Grassé and Dragesco, 1957). Dodge (1963b) presented interphase counts of acetocarmine-stained nuclei of 11 species. These ranged from 18–22 in *Prorocentrum balticum* to 134–152 in *Gonyaulax tamarensis.* It was of interest that in the genus *Prorocentrum* where five species were counted, there was a clear range of chromosome numbers: *P. balticum,* 20; *P. triestinum,* 24; *P. pusilla,* 24; *P. mariae-lebouriae,* 32; *P. micans,* 68. These could possibly represent different levels of ploidy although the size of the chromosomes was also fairly variable. Among other marine dinoflagellates which have been examined are the much used experimental organism, *Crypthecodinium cohnii,* with 99–100 chromosomes (Allen *et al.*, 1975), *Scripsiella sweeneyae,* with 80–90 (Fine and Loeblich, 1976), and *Heterocapsa pygmaea,* with 61–65 (Loeblich *et al.*, 1981). Chromosome numbers of numerous freshwater dinoflagellates have been counted, particularly for species from India (Sarma and Shyam, 1974; Shyam and Sarma, 1978) and the United States (Holt and Pfiester, 1982). In the case of the Florida red-tide organism, *Gymnodinium breve,* counts of field material and recent isolates were 121 ± 3 but a 25-year-old isolate gave a count of approximately twice as many chromosomes (240 ± 6) and is thought to represent an autodiploid state (Loper *et al.*, 1980).

The shape and size of dinoflagellate chromosomes vary considerably from the small almost oval bodies of *Amphidinium carterae* (Fig. 2) to the long 1-μm-wide threads of *Prorocentrum micans* (Fig. 3). In general, the chromosomes all appear similar within any species and it is arguable that any variations, apart perhaps from those of nucleolar organizing chromosomes, result from the prepa-

ration techniques. However, Gavrila (1977) has attempted a karyotype analysis of the 87 chromosomes of *Peridinium balticum* which appear to range in shape from small spheres to long threads.

III. Chemical Composition of the Chromosomes

The use of standard cytochemical tests such as Feulgen and azur B stains (Fig. 4) clearly shows the presence of substantial amounts of DNA in dinoflagellate chromosomes (Ris, 1962; Dodge, 1964). However, attempts to demonstrate proteins were unsuccessful, leading to the conclusion that the chromosomes lack histone. It should be noted, though, that fast green staining has indicated the presence of substantial amounts of basic protein in the parasitic organism *Syndinium* (Ris and Kubai, 1974) and the rather unusual and possibly primitive dinoflagellate *Oxyrrhis* (Hollande, 1974). Many molecular biological techniques have now been used in investigation of the basic components of the chromosomes.

A. DNA

One of the first discoveries concerning the DNA of dinoflagellate chromosomes was that it has an unusual composition. In analyzing the DNA from *Crypthecodinium cohnii* Rae (1973) found a discrepancy in both the buoyant density in CsCl and in thermal gradient studies. He then discovered that this was caused by the replacement of some of the usual base thymidine by 5-hydroxymethyluracil which is not known from any other eukaryotes. This was subsequently confirmed for a number of other dinoflagellates (Rae, 1976). Recently, it has been shown (Herzog and Soyer, 1982a) for *Prorocentrum micans* that 5-hydroxymethyluridylate represents 13.4% of the total nucleotides replacing 63% of the expected thymidine.

Studies of the renaturation kinetics of dinoflagellate DNA (Allen *et al.*, 1975; Roberts *et al.*, 1974; Loeblich, 1976) have shown that there is a clear difference to the DNA of bacteria. It is also suggested that, on the basis of the curve obtained, there is no evidence that the chromosomes are highly polytene for the portion of repeated DNA (of 500–600 base pairs) is interspersed with unique DNA representing approximately 40% of the whole, as happens in higher eukaryotes. The question of polyteny has been disputed by other workers who suggest that in *Prorocentrum micans* the degree of polyteny is about 700 (Haapala and Soyer, 1974). More recent studies of the DNA sequence in *Crypthecodinium* have shown that roughly half the genome is made up of unique sequences which are interspersed with repeated sequences representing about

600 nucleotides (Hinnebusch *et al.*, 1980). Whereas in most respects the arrangement is as in higher plants and animals an unusual class of heteroduplexes was detected by electron microscopy. These are thought to represent the reassociation of repeated sequences from different families and may indicate that there is an unusual organization within the dinoflagellate chromosome.

B. Protein

The first study on isolated dinoflagellate chromatin (Rizzo and Nooden, 1972) showed that although histones were not major constituents, there was a small amount of acid-insoluble protein. Subsequent studies have shown that this protein gives an acrylamide gel banding pattern quite different from that of typical histones (Rizzo and Nooden, 1974) and it has a molecular weight of about 16,000. It is a basic protein differing from histone in containing both cysteine and aromatic amino acids.

C. Metals

Studies on dinoflagellate chromosomes using X-ray microanalysis have resulted in the somewhat surprising discovery of the presence of fairly high levels of transition metals: iron, nickel, copper, and zinc together with chromatin-associated calcium (Kearns and Sigee, 1980; Sigee and Kearns, 1981b, 1982). When similar analyses were carried out on *Glenodinium foliaceum*, a dinoflagellate which contains a eukaryotic endosymbiont, it was discovered that whereas the dinoflagellate nucleus contained the transition elements the nucleus of the symbiont lacked iron and nickel (Sigee and Kearns, 1981a,c).

In an effort to discover the function of the transition metals autoradiographic experiments have been carried out on the uptake of nickel into the chromosomes (Sigee, 1982). Cells labeled for 2 hours showed active uptake throughout the population and 83% of the label was over the dinoflagellate nucleus. The function of transition metals in these particular nuclei is now thought to be related to the stabilization of the chromosome structure in which they may act to form ionic bridges between nucleic acid and the protein matrix (Kearns and Sigee, 1980). It may also be that they form an important structural linking agent within the chromatin which is necessary because of the absence of histones (Sigee, 1983). This suggestion is perhaps at variance with a recent study (Herzog and Soyer, 1983) which suggests that divalent cations (Ca^{2+}, Mg^{2+}) are mainly responsible for the stabilization of the permanently condensed dinoflagellate chromosome architecture. When isolated chromosomes were incubated in EDTA the structure of the chromosomes collapsed and the fibrils (nucleofilaments) separated.