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Genetic Control of DNA Replication in Chromosomes of Eukaryotes*

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Summary. It has been found that the formation of multinuclear polyploid cells in plants due to colchicine action results in the "lysis" of some nuclei formed.

The nuclei lacking the chromosome possessing the nuclear organizer (N. O.) are subject to lysis.

In *Haplopappus gracilis*, for example, whose genome consists only of two pairs of chromosomes (2n = 4), tetraploid nuclei having four chromosomes without the nuclear organizer are lyzed. At the same time in the extra nucleus of the cell, *with* one pair of chromosomes possessing the nuclear organizer, these chromosomes undergo the next reproduction.

In experiments with H^3-labelled thymidine, DNA synthesis is blocked in the nuclei having *no* nuclear organizer.

The data obtained suggest a hypothesis concerning the control mechanism of DNA synthesis and chromosome replication in eukaryotes.

1. Introduction

Investigations of the genetic systems regulating chromosome reproduction and DNA synthesis in eukaryotes are still at an early stage. A much larger body of information has been accumulated for the genetics of bacteria and phages; particularly interesting evidence has been obtained in the last few years.

Most, if not all, prokaryotes are known to have a single chromosome, and consequently a single linkage group, which constitutes their genome. Experiments with bacterial transformation, transduction and conjugation have indicated that when only a part (fragment) of chromosome gets into the recipient's cell, this fragment, in a number of cases, proves incapable of reduplication. In contrast, certain episomes having a much smaller DNA (for instance, the phage genome or the F sex factor of *E. coli*) show different behaviour, being capable of autonomic replication.

The rate of episomal replication differs from that of bacterial chromosome replication. It is also known that a bacterial chromosomal fragment unable to replicate acquires such capacity once it is incorporated into the episome. These facts suggest that different prokaryote genomes carry some determinant (or determinants) which regulates their replication. Generally, a replication of prokaryote chromosome, which has started at a certain point, is completed. Jacob, Brenner and Cuzin (1963) have proposed the term "replicon" to denote the replication unit for the prokaryote chromosome. They advanced a hypothesis explaining the regulation of chromosome replication in prokaryotes (Fig. 1). Prokaryote (bacterial, phage) chromosomes or episomes contain one replicon. Each of them has two genetic loci regulating replication. These are the "initiator" gene and the "replicator" gene. The former synthesizes a specific substance which so acts on the replicator locus as to unwind the double DNA strand which thus becomes the template for a new DNA. Certain chromosomal fragments may be unable to replicate because they lack regions having a locus of the "replicator" or "initiator" gene.

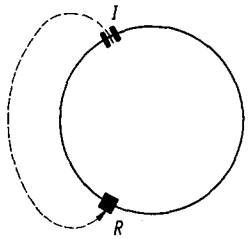

Fig. 1. Scheme illustrating the regulation of chromosome replication in prokaryotes (Jacob, Brenner and Cuzin, 1963). (See text for explanation.) Designations: I — initiator gene; R — replicator gene

DNA synthesis continues until the entire genome (replicon) is doubled. To repeat this process, a new act on the part of the initiator gene is necessary. Although this hypothesis has not yet been adequately validated, there is some evidence in the literature to support it.

2. Replicon in Eukaryotes

Numerous studies have shown that mutagenic factors, both physical and chemical, lead to chromosomal breakages with the formation of individual, sometimes small, fragments. This finding provides an experimental approach to the question of whether the ability of a fragment to replicate is a function of its size and location in the chromosome. It has

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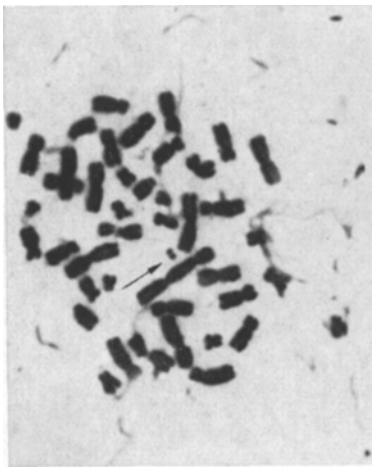

Fig. 2. Micrograph of human lymphocyte culture. Arrow indicates paired chromosome fragment

turned out that any chromosomal fragment can be retained during one nuclear cycle.

One example to show that any fragment invariably undergoes the first replication is provided by studying ordinary chromosomal rearrangement. A fragment arising in the G I stage of the cell cycle in the first metaphase always appears as a pared fragment, i.e. as a replicated fragment (Fig. 2). Not all chromosomal fragments, however, are capable of further replication in a series of cell generations. The ability of fragments to replicate in a series of subsequent replications has been demonstrated by Sidorov and Sokolov (1963 a), studying nuclear cycles in C-mitosis in *Crepis capillaris*. Fig. 3 shows the facts of three chromatid isodeletions (painted black) that have passed three consecutive replications. Note that each of the three fragments and dicentrics has undergone three consecutive replications in strict synchrony with the entire genome so that the preparations each show 8 homologous fragments and dicentrics. The same study has demonstrated that the ability to replicate and actual replication are exhibited even by each microfragment which is equal to a chromatid in diameter and which can be seen in the light microscope. Such a fragment may have neither a centromere nor telomer. These findings suggest the presence of many small replication units or replicons in eukaryote chromosomes.

The above picture, however, is not universal. Sometimes the fragments prove unable to replicate in a series of cell generations. One example of this is the behaviour of so-called "acentric" fragments in normal mitosis. The usual explanation, that they are lost during mitosis because they lack a centromere, is not quite tenable. In fact, no fragment is lost. Because of the absence of centromere, it fails to enter any of the daughter nuclei and forms, in telophase, a third, extra nucleus of small size. Such "micronuclei" are very often pycnotized and die without being involved in the replication.

Cytogenetic evidence thus strongly suggests that a eukaryotic chromosome consists of a multitude of small replicons potentially capable of replication. At the same time, it also shows that certain conditions exist in a living cell under which fragments do, or do not, replicate. A multicentred mode of replication of eucaryotic chromosomes has been further suggested by autoradiographic studies in which tritium-labelled thymidine was injected into a living cell and the isolated DNA, carefully freed of proteins, was subjected to electron microscopy (Cairns, 1966; Huberman and Riggs, 1968; Callan, 1972). All the authors arrived at similar conclusions, that the DNA of eukaryote chromosomes consists of small regions (replicons) not exceeding 30 in length. In each of them, DNA replication occurs independently,

Fig. 3. Meristematic cell of *Crepis capillaris* that has passed several consecutive C-mitoses. Isodeletions — fragments and dicentrics — are painted black

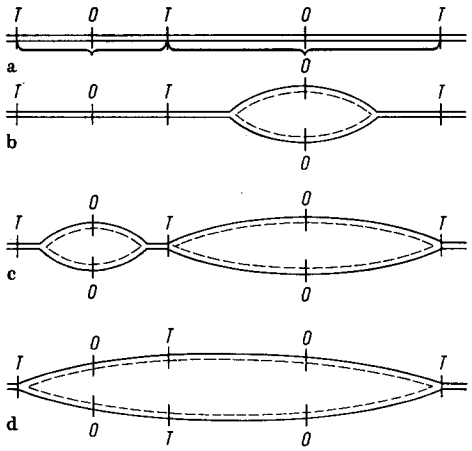

Fig. 4. Scheme of DNA replication in eukaryote chromosomes (Huberman and Riggs, 1968). Designations: O — beginning of synthesis; T — end of synthesis; braces indicate the limits of replicons

starting with the replicon centre and proceeding towards its opposite ends. A schematic picture of DNA replication in eukaryote chromosomes is given in Fig. 4.

According to the above authors, chromosomes of higher organisms consist of thousands of small replicons and exhibit a replication rate lower by one order than prokaryote chromosomes.

However, according to cytogenetic data, chromosomal fragments do not always replicate. What conditions are essential for this process? In the fifties, some investigators attempted to answer this question. McLeish (1954), La Cour (1952) and others reported that not all the acentric fragments were pycnotized and died in extra micronuclei. Some of them were shown to pass normally through the next mitosis, concurrently with the main nucleus. These fragments were invariably found to contain a chromosomal region with a nucleolar organizer. Das (1962) showed, in autoradiographic studies, that both DNA and RNA synthesis occurred in such fragments. Researchers rightly implicated the nucleolar organizer (N. O.) in DNA replication and RNA synthesis by chromosomal fragments.

In 1964 a similar autoradiographic study by Scott and Evans was published, concerning micronuclei formed by fragments. These authors reported similar results but came to quite a different conclusion, suggesting that while all fragments were potentially capable of replication, those fragments which had no N.O. died early and did not survive till the S-stage of the cell cycle and so did not incorporate the label. Those fragments which did contain N.O. lived longer and thus had enough time to synthesize both DNA and RNA. N.O. *per se* had no connection with DNA synthesis. This hypothesis was simple and convincing enough on first sight, because fragments were obtained in most cases under the effect of radiation or chemical mutagens and could be injured by either.

3. Chromosome Lysis in Plants with Blocked Cell Spindle

Following the finding by Blakeslee and Avery (1937) that the alkaloid, colchicine, blocked the cell spindle, numerous studies appeared in which this agent was used (see Eigsti and Dustin, 1955, for a review of the literature).

Colchicine acts on the cell to block the cell spindle and cell division. Chromosomal replication is not, however, disturbed and polyploid cells are formed. Multinuclear cells emerge, with normal ones, in nearly all plant and animal specimens.

Sidorov and Sokolov (1963b, c) have established the occurrence of aneuploid cells in a number of plants. A tetraploid population of *Haplopappus gracilis* has been found to contain 6% of aneuploid cells, that of *Crepis capillaris* 23%, that of *Crepis tectorum* 20%, and that of *Pyrhetrum* 50% of such

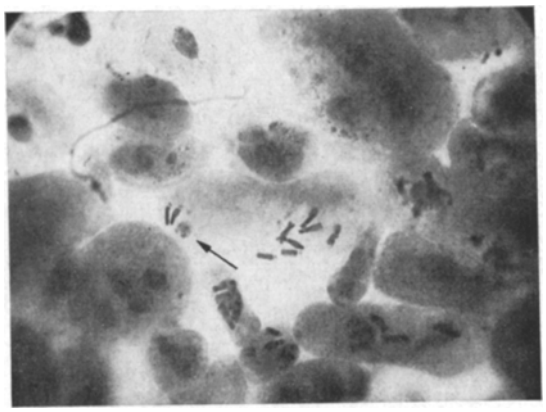

Fig. 5. Micrograph of an aneuploid cell of *Crepis capillaris*. "Shadow" is indicated by arrow

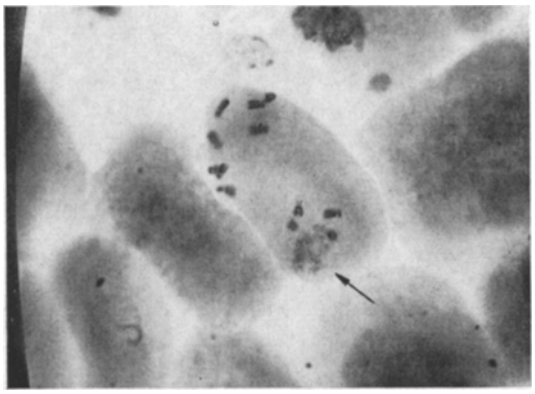

Fig. 6. Micrograph of an aneuploid cell of *Crepis tectorum*. "Shadow" is indicated by arrow

cells. All these aneuploids have to be hypopolyploids. At the same time, in place of the missing chromosomes, 99% of such cells displayed amorphous structures resembling the pycnotic "micronuclei" which arise after the death of acentric fragments. The authors termed these structures "shadows". These shadows became completely resorbed in the cytoplasm in the subsequent nuclear cycle. This process was called "lysis". The photographs in Fig. 5 and 6 depict *Crepis capillaris* and *C. tectorum* cells with an aneuploid chromosomal set and a single shadow.

The number of shadows in a cell was found never to exceed that of the missing chromosomes. This finding led the authors to conclude that the shadows were precisely the missing chromosomes in hypopolyploid cells and that lysis was just the process of disintegration of such chromosomes. This hypothesis seems all the more plausible because the cell does not divide in C-mitosis and its envelope is not destroyed. Therefore, chromosomes died within the cell. The frequency of aneuploid cells in *Crepis capillaris* was reported to be 23%, and it was proposed that the process of lysis might be associated with the formation of multinuclear cells.

These studies of Sidorov and Sokolov have also shown that, of 5699 tetraploid meristematic cells of *Crepis capillaris*, 1297 were aneuploid. Cells with "lyzed" chromosomes occurred in 22.6% of cases. Among the 1551 cells with interphase nuclei, 516, or 33.3%, were multinuclear.

If there is a relationship between multinuclearity and lysis, then a relationship should also exist between the number of nuclei in the cell and the number of shadows. As Table 1 shows, such a correlation does hold.

Table 1. *Variation of nuclear and shadow numbers in tetraploid cells of Crepis capillaris (in %)*

No. of extra nuclei or shadows in the cell		1	2	3	4	No. of cells studied
Percentage of cells with:	extra nuclei	73.8	21.9	3.5	0.2	516
	shadows	84.6	14.3	1.1	—	1214

During C-mitosis, when the spindle is blocked and all the chromosomes are uncontrolled and have lost their ability to organize the equatorial plate and proceed further towards the poles, the organization of two identical daughter cells becomes complicated. Because of a random chromosome distribution in the cell, several nuclei may form, each with a nuclear membrane of its own. Centrifugation was used to affect the process of development of multinuclearity in the cells. During centrifugation, chromosomes show unidirectional, centrifugal movement in colchicine mitosis. It was thought that centrifugation would decrease the frequency of the appearance of multinuclear cells and, consequently, the percentage of shadows, if these two events were correlated. And indeed, the percentage of lysis was found to decrease from 23 to 10% or less, depending on centrifugation time.

The previously advanced hypothesis was thus confirmed experimentally. A proportion of nuclei in a multinuclear cell dies, being lyzed, or, as was said previously, "pycnotized". Since the bulk of lyzed nuclei (about 85%) contain 1 or 2 chromosomes, we may generally refer to them as "extra" nuclei. Because of the small number of chromosomes in *Crepis capillaris* (2n = 6) and the strongly marked individuality of chromosomes of each pair, it is possible to find out (from the number of missing chromosomes) which chromosomes have formed the extra nucleus, considering that chromosomal number in the 4n cell is 12 (4A + 4D + 4C). Because there were cells with 2, 3 or more extra nuclei among multinuclear cells, it was necessary, in order to ensure a precise analysis, to confine oneself to cells with one extra nucleus or, in other words, to metaphase of tetraploid cell with a single shadow.

Table 2. *Number and percentage of lyzed chromosomes in a single extra nucleus*

Number of lyzed chromosomes	1	2	3	4	5	No. of cells viewed
Number of cases	719	731	132	57	8	1647
Percentage	43.6	44.4	8.0	3.4	0.6	100

It follows from the experimental data of Table 2 that the most frequently lyzed in an extra nucleus are 2 and 1 chromosomes, less frequently 3, 4 or 5 chromosomes (4n = 12).

Analysis of chromosomal composition revealed that the extra nuclei containing 3 or more chromosomes (i.e. in 12% of cases) showed practically no (0.2%) lysis in the combination of the three chromosomas (ADC) which constitutes the haploid set of the *Crepis capillaris* genome.

Analysis of lysis frequency for each chromosome of the *Crepis* set indicated (Table 3) that among the 2276 cases of chromosomal lysis observed, the most frequently lyzed was the C chromosome, less often the A chromosome, and very rarely the D chromosome with its satellite carrying the N.O. region.

Table 3. *Lysis of different chromosomes of the Crepis capillaris set in tetraploid cells*

Type of lyzed chromosomes	Number of lyzed chromosomes				Total number of lyzed chromosomes	
	1	2	3	4	No.	%
A	306	247	9	4	843	37.0
D	87	16	0	0	119	5.3
C	504	366	26	0	1314	57.7
Sum	897	629	35	4	2276	100.0

Thus, when individual chromosomes or their groups (5 of 12) are in the "extra" nucleus, they are often incapable of reproduction and undergo "lysis" or "pycnosis".

The fate of lyzed single chromosomes and their groups is therefore similar to that of acentric fragments in normal mitosis. And this is not accidental, since all chromosomes behave in C-mitosis as if "acentric" and come out of control of the cell spindle.

The regularities referred to above for 4n cells, also hold in the subsequent nuclear cycles in continuing C-mitosis, i.e. in 8n and 16n cells. This clears up the role of the chromosome carrying the N.O. region which is very rarely lyzed itself and which protects other chromosomes in the same nucleus from lysis. The chromosomal region containing N.O. appears to code the synthesis of some substance or substances responsible for the normal reproduction and functioning of the chromosome.

These processes also show the special role of the integrity of the nucleus and the nuclear membrane. Both individual chromosomal fragments and whole chromosomes and even chromosome groups lose, once they get into the extra nucleus, the ability to replicate and undergo spontaneous enzymatic break-down — "lysis" or "pycnosis" — despite the fact that another nucleus of the same cell contains chromosomal regions with N.O. These observations suggest that nuclear membranes appear to be impermeable or poorly permeable for the respective substances.

4. DNA Synthesis in "Extra" Nuclei of *Crepis* with Different Chromosome Sets

To find out whether the N.O. actually assures normal replication of the DNA of chromosomes invested by a common nuclear envelope, experiments were carried out using tritium-labelled thymidine. Label incorporation into extra nuclei with different chromosome complements was studied in the second C-mitosis, using only those nuclei in which all chromosomes of the main nucleus were intensively labelled. For a more precise determination of chromosome composition in the "extra" nucleus, only cells with a single "shadow" in metaphase II were viewed. Fig. 7 shows a cell whose "extra" nucleus contains 2 A chromosomes; Fig. 8 shows 5 chromosomes, 2 A plus 3 C. Nevertheless, the label was absent in both cases.

In view of the strong variation of chromosome numbers in extra nuclei, all cases were classified into two groups: group 1 in which the chromosome carrying the N.O. region was present with different sets of other chromosomes; and group 2 in which the chromosome with N.O. was absent.

The data of Table 4 point to a distinct correlation between the presence of N.O. in the extra nucleus and DNA synthesis.

It is true, though, that in 14.5% of cases the label failed to be incorporated into the extra nucleus despite the presence of N.O.-containing chromosomes. Possibly, it is necessary to have such a chromosome in the diploid number. On the other hand, this deviation from the general rule may be caused by the presence in the population of "defective" D-chromosomes resulting from an unequal exchange between sister chromatids in the region of repetition of ribosomal cistrones.

The 12% of labelled shadows without D chromosomes is accounted for by the contamination of chromosomes with the "substance" of the former nucleolus which partly makes up for the activity of N.O.

Table 4. *Label incorporation into the extra nucleus as related to the presence of N. O.*

No. of nuclei	Chromosome composition in the extra nucleus	With label	Without label
374	all combinations +D(N.O.)	85.5%	14.5%
	all combinations −D(N.O.)	11.9%	88.1%

It follows from this data that the genes responsible for normal DNA replication in *Crepis capillaris* are sited in the D chromosome. To ascertain the location of this region, use was made of two translocations between A and D and C and D chromosomes. In both cases, only a small proximal region with a secondary constriction remained from the D chromosome, while the remaining (main) part of the chromosomes was replaced by chromatin from the A or C chromosomes (see Fig. 9). It was found that only those chromosomes which contain the part of the D chromosome with the secondary constriction do not undergo lysis.

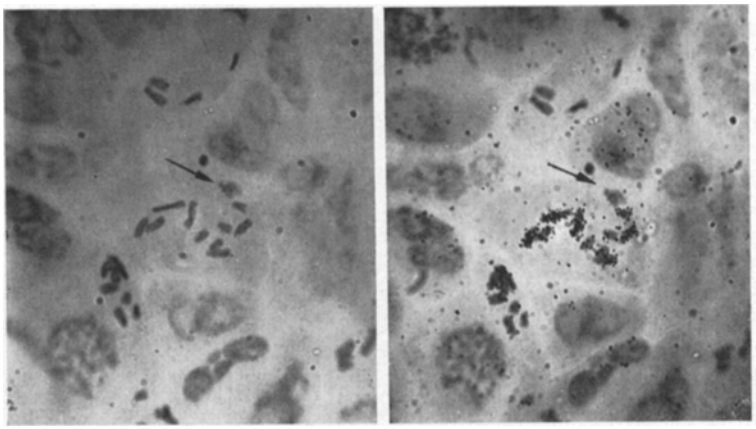

Fig. 7. Micrograph: a — Radioautograph; b — Feulgen stain. Tetraploid cell of *Crepis capillaris* with extra nucleus containing 2 A chromosomes. The extra nucleus is indicated by arrows. The label is seen not to have been incoporated the extra nucleus

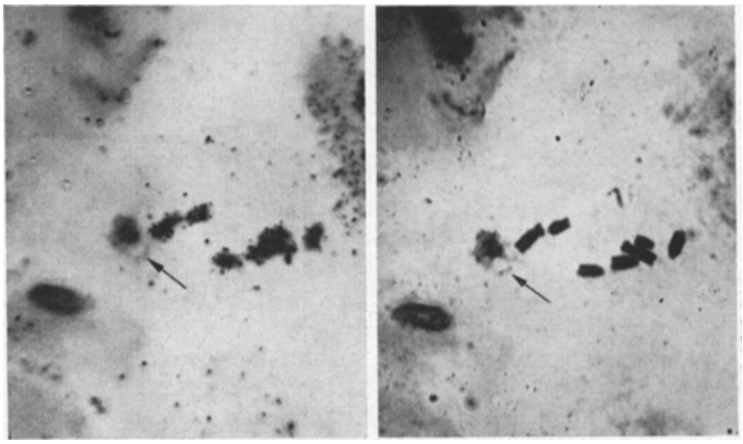

Fig. 8. Micrograph. a — Autoradiograph. b — Feulgen stain. Tetraploid cell of *Crepis capillaris* with extra nucleus containing 2 A and 3 D chromosomes. The extra nucleus is indicated by arrows. The label is seen not to have been incorporated into the extra nucleus

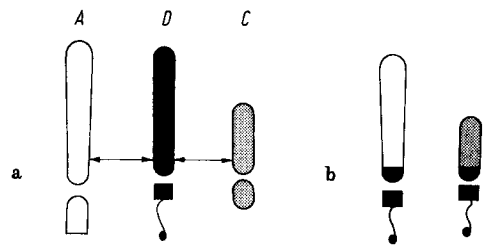

Fig. 9. Scheme to show rearrangements of chromosomes in translocations. Designations: a — haploid chromosome set in *Crepis capillaris*. Arrows indicate breakage sites. b — translocations between chromosomes: on the left, A and D; on the right, C and D

5. Genetic System Regulating DNA Synthesis and Chromosome Replication

The foregoing evidence, although indicative of N.O. involvement in chromosome replication, could be interpreted in terms of Scott and Evans' concept (see above). Evidence contrary to this concept was reported by Crosby (1957), who showed that in wheat the micronuclei which are formed as a result of nondisjunction of chromosomes in the first meiotic division of monosomic forms, and which contain only one chromosome (any of the set), survive to the second division of maturation. It was found that all of them, both those 4 which had N.O. and the remaining 17 chromosomes without N.O., quietly lived and passed a normal prophase and metaphase cycle of the second division. In other words, all the 17 types of extra nuclei (micronuclei) did not undergo "lysis" or "pycnosis" in the absence of N.O., so it might be concluded that the lack of N.O. does not necessarily at once lead to lysis in the extra nuclei. To produce lysis it is necessary that the cell pass through the DNA synthesis phase. Micronuclei do not perish in the case mentioned because no DNA synthesis occurs in the second division of maturation. It could be concluded that chromosome lysis occurs only in proliferating tissues. Such a conclusion seems quite natural because chromosomes often do not replicate in differentiated tissues.

Although this conclusion seems somewhat unexpected, similar phenomena are known in the genetics of prokaryotes, viz.: the so-called "thymineless death" of certain *E. coli* strains. S. Cohen (Russian edition, 1960, p. 546) wrote that "this organism loses its ability to reproduce, or "dies", in the absence of thymine on media which are complete in all other respects."

It is possible that an analogous phenomenon occurs in the case of bacterial lysis under the effect of phages when DNA synthesis is blocked in the host bacterium.

We may thus arrive at a general conclusion that, in multiplying cells, with programmed DNA synthesis and chromosome replication, chromosomes undergo spontaneous enzymatic breakdown, lysis, if the genetic system regulating the replication is blocked.

In eukaryotes, both individual fragments and whole chromosomes of chromosome groups, which are potentially capable of replication, lose or retain this capacity depending on the presence of N.O. in the micronucleus. This suggests the existence of at least two determinants regulating replication. One of them is present even in a small chromosome fragment, and the other, as experiments with *Crepis* have shown, is associated with the only genome region, nucleolar organizer.

The foregoing discussion enables us to formulate a hypothesis about the genetic system controlling DNA replication in chromosomes of higher organisms. We propose as the basis the hypothesis of Jacob, Brenner and Cuzin modified as follows.

In contrast to prokaryotes, the genome of higher organisms contains many replication units, replicons, whose numbers appear to run into thousands, even in a single chromosome. Each of them carries at least one locus of the replicator gene (R), while the initiator gene (or genes) (In) is not associated with an individual replicon but is located in a certain region of one or several N.O.-carrying chromosomes. In many organisms the position of the initiator gene (In) is actually already mapped, since it is associated with the presence of so-called "secondary chromosome constructions", i.e. the site of nucleolus formation.

The product of the initiator gene interacts (chemically or physico-chemically) with the locus of the replicator gene (R) to lead to the replication of that chromosomal region (replicon). An important role is played here by the nuclear membrane which must be involved in the interaction between the product of the initiator genes and the replicator genes (R).

As the foregoing evidence shows, the main nucleus of a binucleate cell of *Crepis capillaris* contains a N.O., but the product of the initiator gene is incapable of passing the nuclear membrane and no chromosome replication occurs in the extra nucleus. So this nucleus must have its own initiator gene or genes, i.e. a N.O., in order that DNA may replicate in it. Clearly, neither an individual fragment, nor a whole chromosome, nor a chromosome group, are capable of replication in the absence of the initiator gene, i.e. N.O.

One should add a highly important observation, viz.: that any damage to this genetic system will result in spontaneous enzymatic breakdown of chromosomes and fragments, i.e. in lysis or pycnosis.

This hypothesis may be illustrated by the following scheme (Fig. 10), in which the consecutive phases of a single cell's cycle are shown:

(a) metaphase stage of the first C-mitosis; (b) stage S of the cell cycle; and (c) stage of the second metaphase.

In metaphase 1 (a), two chromosome pairs can be seen, one of which (with a secondary constriction)

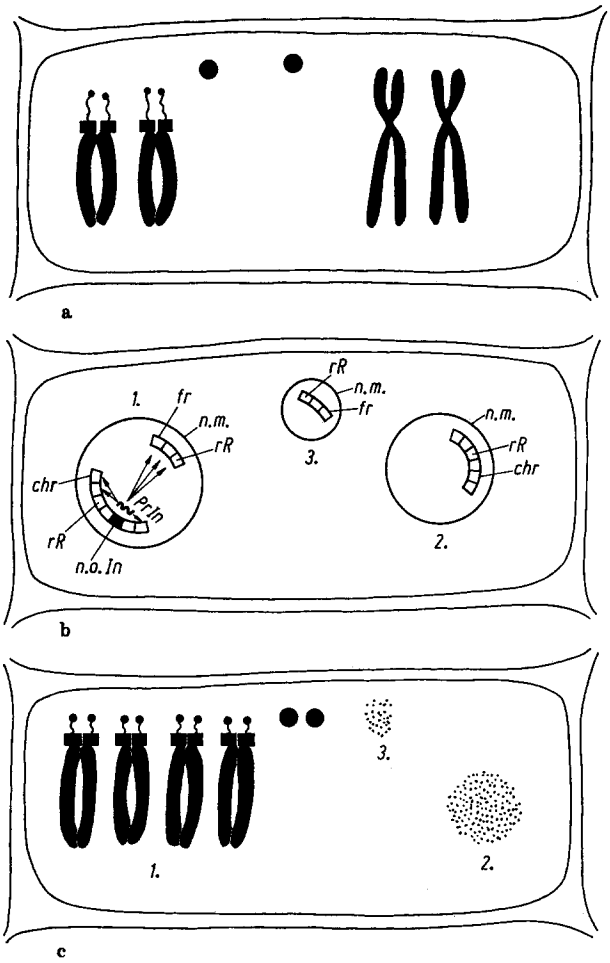

Fig. 10. Scheme illustrating the regulation of genome replication in eukaryotes. (See text for explanation.) Designations: a — metaphase I; b — interphase S phase; c — metaphase II; n.m. — nuclear membrane; chr. — chromosome; fr. — fragment; n.o. In — chromosomal region containing initiator genes; rR — replicon with replicator genes; Pr In — product of initiator genes

contains initiator genes and two fragments. Upon passage to the interphase, three nuclei are formed in this cell (b). One of them (I) contains 2 chromosomes carrying a N.O. region and one fragment; the second contains only 2 chromosomes without N.O.; and the third contains only fragments (only one chromosome is shown for each nucleus in the scheme).

The first nucleus contains a chromosome (chr) and a fragment (fr) within the area circumscribed by the nuclear membrane. The initiator genes (In) located in this chromosome code the synthesis of the product (wavy line) (PrIn) which interacts with the replicator genes (R) to provide normal replication of the DNA contained both in the chromosome and in the fragment. Arrows indicate the action site of the product of the initiator gene.

Since both the determinants essential for normal DNA replication are present in the first nucleus, both the chromosome and the fragment of this nucleus are reproduced. The second and third nuclei have no initiator genes of their own and the product of their initiator gene in the 1st nucleus fails to pass through the nuclear membrane. As a result, no chromosome reproduction occurs either in the 2nd or the 3rd nucleus.

In the next metaphase (c), there are 4 chromosomes, one paired fragment and two "shadows", a large one (lyzing chromosomes of the 2nd nucleus) and a small one (lyzing fragment of the 3rd nucleus).

The presence of a large number of replicons in the genome suggests that the initiator gene (N.O.) is unlikely to be represented by only one locus, because, like any other gene, this gene can mutate and such a system would be too unreliable. The available experimental evidence also shows that the N.O. region, like many other "housekeeping genes", is complex and consists of several identical subunits. As far back as 1934, McClintock succeeded in separating this region into two parts, each part being capable of forming a nucleus. Moreover, many specimens are known to contain several N.O.-carrying chromosomes in their genome.

Studies of inter-species hybrids of *Chironomus* (Beermann, 1960; Pelling and Beermann, 1966) have shown that the three different N.O. found in the initial species were fully interchangeable. Furthermore, one of the N.O. regions was divided into two parts by radiation, and each part replaced the other and provided for normal viability of the hybrid. *Chironomus* thus has at least 6 functionally identical regions. The N.O. region may be considered as a region of "repetitions" of the initiator gene, and it cannot be ruled out that it is not accidental that selection has coupled this region with the repetition system of ribosomal cistrons.

The facts indicate that the N.O.-region-carrying chromosomes have both the genetic determinators of replication, an initiator gene and replicator genes, and, consequently, such a chromosome may assure its own replication even if it is the only chromosome of the micronucleus. To explore this possibility,

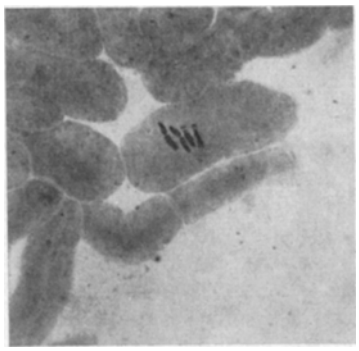

Fig. 11. Micrograph. Karyotype of *Haplopappus gracilis*

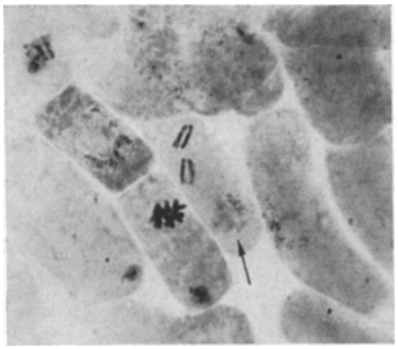

Fig. 12. Metaphase II of C-mitosis in a meristematic cell of *Haplopappus gracilis*, showing 4 B chromosomes and a "shadow". The shadow is indicated by arrow

lysis was studied in the plant *Haplopappus gracilis* (2n = 4) whose haploid set consists of two chromosomes, A and B. The two-shoulder (submetacentric) chromosome A contains about 60% of chromatin, while the acrocentric chromosome B with a secondary constriction which carries the N.O. region contains about 40% of chromatin (Fig. 11). In these circumstances, chromosomes A and B may be expected to be present in different nuclei. If this proposal is valid, then only those nuclei should survive in which chromosome B was retained. And indeed, among the 164 cells with chromosomal lysis in this plant, 7 binucleate cells were found in which the surviving nucleus contained only 4 B chromosomes, while the second nucleus containing chromosome A was lyzed (Fig. 12). The photographs of Figs. 11 and 12 show that B chromosomes have undergone replication, their number having increased from 2 to 4. Consequently, chromosome B, which was alone in the extra nucleus, has proved, as was expected, to be able to replicate although it contained only 40% of the genome. It is unlikely that a cell carrying a homozygotic deficiency for 60% of the genome could survive for long. However, owing to the store of substances of the maternal cell, the nucleus succeeded in passing one cycle and its sole chromosome B replicated.

Thus, in higher organisms, the chromosome bearing the N.O. region and some of the replicons coupled with it represents a self-regulating replication system and resembles, to a degree, the prokaryote episome.

Earlier experiments with *Crepis* (Sidorov and Sokolov, 1963) revealed one cell in whose metaphase there were 8 D chromosomes.

In the light of the evidence discussed here, it is of interest to recall the recently discovered phenomenon of selective replication (or amplification) of individual chromosomal regions in the oocytes of amphibians and some other specimens. The chromosomal regions containing N.O. were found to be freely floating in such oocytes. These "nucleoli" numbered hundreds (e.g. in *Xenopus* they ran into a thousand). These facts make it imperative to recognize that chromosomal regions bearing N.O. can reproduce independently. This phenomenon has not of course been adequately studied, but things are as they stand. We are concerned in oocytes with amplified chromosomal regions which appear to contain both the replication determinants. And if, as we have seen in *Haplopappus* and *Crepis*, single N.O.-forming chromosomes were capable, in mitosis, of passing through one replication, then such chromosomes (in effect fragments, according to Das and McLeish) could undergo up to 10 replications in such a rich cell as the oocyte. Such a coincidence would not appear to be accidental.

The above facts suggest a unity of the mechanisms assuring DNA replication in the chromosomes of pro- and eukaryotes. One may hope that further studies will shed light on a number of complicated problems arising in the modern genetics of higher organisms and will bridge the gap between the molecular and "classic" trends in genetics.

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