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Mechanisms of DNA Sequence Amplification and their Evolutionary Consequences

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Mechanisms of DNA sequence amplification and their evolutionary consequences

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[Plate 1]

DNA sequence amplification is a phenomenon that occurs predictably at defined stages during normal development in some organisms and has been shown to occur spontaneously, but sporadically, in a variety of cells, including mammalian cells, selected for overproduction of a gene product. Developmentally programmed gene amplification includes rDNA amplification during oögenesis in amphibia, chorion protein gene amplification in *Drosophila* and the chromosomal changes accompanying macronuclear formation in ciliates. Selected gene amplification is illustrated by mutant mammalian cells which have been selected *in vitro* or *in vivo* for the overproduction of a gene product. In these cells the unit of DNA that is amplified is much larger than the gene under selection, and appears to be formed by multiple recombination events, which bring together sequences not normally adjacent to each other. Often the product of amplification can be seen microscopically as aberrant chromosome forms. The vast majority of DNA amplification events occur in somatic nuclei, and thus would not have any direct effect on the evolution of a genome. However, the ability to amplify DNA in somatic cells does have consequences for the composition of the genomes of the organisms in which it can occur, and should DNA amplification occur, even sporadically, in germ-line cells the potential effect on evolution would be great.

The term amplification can be applied to any event that increases the number of copies of a gene or DNA sequence per haploid genome over and above that which is characteristic for an organism. To detect amplification it is necessary to know what the 'normal' copy number for the organism is, which in turn implies that the process of amplification is infrequent and, depending on which time scale is under consideration, is either restricted to a characteristic stage of organismal development or has occurred only rarely during the evolution of a species. It is a term that covers a wide range of different phenomena. I am going to consider only those amplification events that occur within the time scale of the life span of an organism or that can be selected in cells growing in culture. These events involve the amplification of identifiable genes, although in many cases large stretches of apparently non-genic DNA also become amplified.

Gene amplification as part of normal development

Ribosomal RNA genes in amphibia

Most structural genes are present as a single copy in a genome, but those that code for the structural RNAs (for example, ribosomal, transfer and small nuclear) and some proteins (for example, histones) are, almost without exception, repeated many times within the haploid

genome. This evolutionarily established repetition reflects the constant requirement of all cells for the products of these genes. However, in the case of the ribosomal RNA (rRNA) genes the multiplicity is sufficient to meet the demands of somatic cells, but not those of gametogenesis.

Ribosomal DNA (rDNA) is amplified during gametogenesis in many organisms (see reviews by Bird 1980; Macgregor 1982). In amphibians amplification proceeds in two phases; an initial low-level amplification of up to 40-fold in the primordial germ cells of both sexes (Gall & Pardue 1969) and a subsequent massive 2500-fold amplification which occurs only in oöcytes (Gall 1969). Amplified rDNA in both premeiotic and meiotic cells is found in an extrachromosomal form (figure 1).

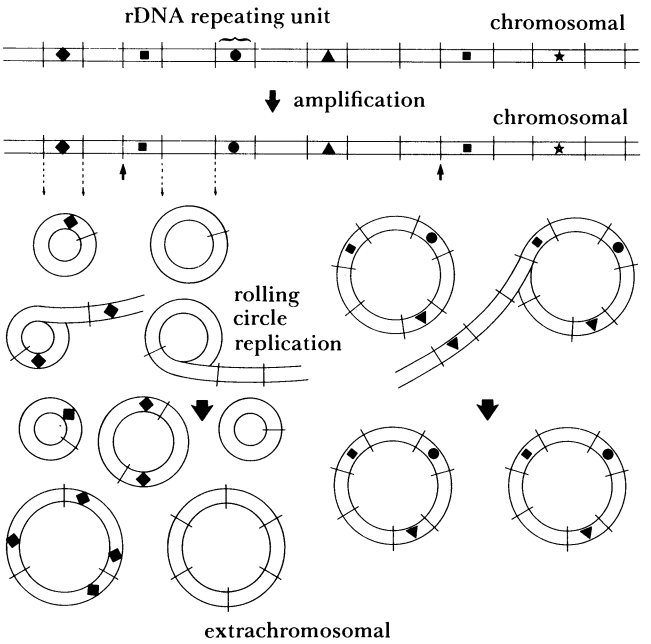

FIGURE 1. Amplification of rDNA in amphibian oöcytes. The chromosomal rDNA is arranged in tandem arrays of a repeating unit which is composed of a segment of non-transcribed spacer and a segment which is transcribed and includes the rRNA genes. Heterogeneity in the length of rDNA units results from length variation in the non-transcribed spacer segment. Such variation allows the identification of particular rDNA units (represented here by different symbols) which can then be identified after amplification. Amplification involves the formation of an extrachromosomal circular copy, by an as yet unknown mechanism, of a small subset of rDNA units, either singly or in short segments, which then amplify by multiple rounds of rolling circle replication to form the extrachromosomal nucleoli of the oöcytes.

Some insight into the mechanism of rDNA amplification can be gained by comparing the structure of chromosomal and amplified rDNAs. The multiple copies of chromosomal rDNA are arranged in tandem arrays, although many individual copies of the rDNA repeat unit can be distinguished on the basis of the variation in the length of their non-transcribed spacer region. Wellauer *et al.* (1976) showed that the heterogeneity of length in rDNA repeat units is much less in the extrachromosomal copies than in the chromosomal copies of the same individual. Although different oöcytes amplify a different subset of length variants (Bird 1978) only a very small number of possible length variants are represented in the extrachromosomal rDNA of any one oöcyte.

Amplification of rDNA must therefore involve initially the apparently random selection of a small number of all the possible repeats available, and their transfer into extrachromosomal

circular forms (Bird 1978; Miller 1964) which are either a single unit of rDNA or are simple multiples of the rDNA repeat length (Hourcade *et al.* 1973). It is not known whether the forms that are multiples of the rDNA unit length are excised directly from the chromosome, and thus represent tandem copies as they are in the chromosome, or whether they are formed extrachromosomally from replicating forms. The relative homogeneity of neighbouring unit lengths in amplified rDNA suggests the latter.

Once in an extrachromosomal form the rDNA is capable of autonomous replication by the rolling circle mechanism (Gilbert & Dressler 1968; Bird 1978) so further amplification does not require continuous copying or excision from the chromosome. Nothing is known about how the first extrachromosomal copies are formed, although several mechanisms have been proposed (Bird 1978). These are based either on localized cycles of extra replication within the rDNA arrays, followed by excision of the extra DNA strand or strands, which has similarities to the 'onion skin' model for amplification (see later section), or on the involvement of reverse transcriptase to copy an rRNA intermediate.

Ribosomal RNA genes in Tetrahymena

I mentioned above that 'almost without exception' the genes for structural RNAs are repeated many times within the haploid genome, but there are exceptions and they are found in the ciliated protozoa. Ciliates have two types of nuclei: a vegetative, transcriptionally active, macronucleus and a 'germ-line', transcriptionally inactive, micronucleus. A well studied example is *Tetrahymena*.

From the point of view of the evolutionary implications of amplification it is significant that the micronucleus, which, as far as it is known, is not transcribed, has only a single copy of rDNA integrated within a chromosome (Yao & Gall 1977). After conjugation, when the macronucleus is reformed from the syncaryon (itself derived from the meiotic products of micronuclear divisions) the single rDNA copy becomes excised from the chromosome and amplified several hundred-fold (figure 2). In the early stages of amplification single copies of the extrachromosomal rDNA unit become joined together in a head to head configuration to form a giant palindrome and the single units are lost (Engberg *et al.* 1976; Karrer & Gall 1976). About 3 kilobases of DNA, which flank the rDNA at its 3′ chromosomal site in the micronucleus, are lost from the developing macronucleus (Yao *et al.* 1979) and multiple tandem copies of a simple repeating sequence C_4A_2 become added to the telomeres of what is now a small linear 'chromosome'.

The micronucleus contains only one copy of the C_4A_2 repeat at the 3′ end of the chromosomal DNA, so the amplification of the rDNA unit must also involve the amplification of the telomeric C_4A_2 sequence. The addition of multiple copies of the C_4A_2 repeat would seem to have mechanistic parallels in the growth of the tandemly repeated CCCTAA sequence at the ends of trypanosome minichromosomes (Blackburn & Challoner 1984; Van der Ploeg *et al.* 1984) and the growth of yeast (Walmsley *et al.* 1984; Shampay *et al.* 1984) and perhaps *Ascaris* (Streek *et al.* 1982; Roth & Moritz *et al.* 1981) telomeric sequences. In the evolutionary context of this meeting it is interesting that these telomeric sequences are very similar to tandemly repeated satellite DNA sequences; in fact, the sequence of the trypanosome telomere is the same as the consensus sequence of the guinea pig α and the kangaroo rat HS α satellites. This is not to say that the mammalian satellites have evolved from those of the trypanosome, but the observed growth of the telomeric simple sequences suggests a possible mechanism by which satellite sequences could have become amplified over evolutionary time.

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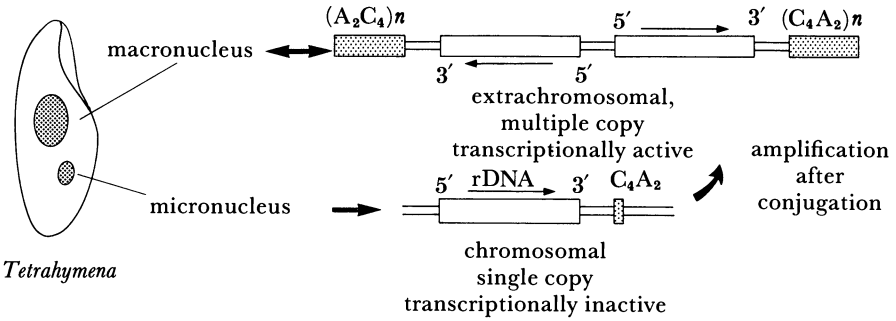

FIGURE 2. Amplification of rDNA in *Tetrahymena*. The vegetative macronucleus contains multiple copies of an extrachromosomal linear DNA element which contains two copies of the rDNA unit forming a large inverted repeat structure with ends that are composed of multiple tandem repeats of the sequence C_4A_2. The 'germ-line' micronucleus contains only a single copy of the rDNA unit integrated into chromosomal DNA with a single copy of the C_4A_2 repeat close to one end of the rDNA unit. Amplification of rDNA occurs during the formation of the macronucleus from the micronucleus following conjugation. Amplification first involves the excision of the rDNA unit from its chromosomal environment, with the loss of about 3 kilobases of DNA downstream from the rDNA unit. In some way the rDNA unit must form an inverted dimer and the C_4A_2 sequence serve as a 'nucleation' site for the addition of more copies to form a functional telomere structure.

Amplification of rDNA shows that mechanisms have evolved that allow organisms to maintain a number of rRNA genes compatible with somatic growth while being free of the burden of maintaining a vast excess of somatically redundant rDNA necessary for oögenesis. Since this cellular machinery exists, one wonders why the same evolutionary solution has not been arrived at for 5 *S* RNA genes. *Xenopus laevis* carries permanently in its genome 25000 copies of the 5 *S* RNA genes, most of which are oöcyte-specific and not expressed in somatic cells (Ford & Southern 1973).

Gene amplification in the macronucleus of hypotrichous ciliates

The hypotrichous ciliates provide clear examples of large scale gene amplification during macronuclear development which has been studied in detail (see reviews by Prescott *et al.* 1973; Swanton *et al.* 1980).

The overall process of amplification occurs in three distinguishable phases. The chromosomes first undergo multiple rounds of replication and become polytenized, when non-satellite DNA sequences become over-represented. This is analogous to the virtual absence of satellite DNA sequences in dipteran polytene chromosomes (Endow & Gall 1975). Polytenization is followed by a process that transects the chromosomes such that individual segments or bands become enclosed in vesicles. Within the vesicles selective degradation of the majority of the DNA sequences occurs so that only 5% of the single-copy sequences remain as short DNA fragments. During the third phase these fragments are replicated many times resulting in a macronucleus that contains some 24000 different, originally single-copy, DNA sequences, each of which is repeated about 1000 times. Like the *Tetrahymena* rDNA minichromosome, these chromosomal fragments are found to contain blocks of several tandem repeats of the sequence C_4A_4 at both ends. Thus in these organisms amplification involves several mechanisms; differential replication, selective cleavage and degradation and finally replication of the remaining DNA fragments.

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Differential polytenization leads to amplification

The under-replication of satellite DNA sequences in the formation of polytene chromosomes of *Drosophila* can be considered as amplification of the non-satellite sequences. This view becomes clearer in the DNA puffs of polytene chromosomes of sciarid flies. Here a few specific bands go through additional rounds of DNA replication (Rudkin & Corlette 1957; Crouse & Keyl 1968) resulting in the further amplification of the DNA sequences they contain. In the related fly *Rhynchosciara* these amplified DNA sequences contain genes that code for proteins expressed during the late larval stage (Glover *et al.* 1982).

Selective amplification of the chorion protein genes of *Drosophila* in follicle cells is achieved by a similar mechanism. In this case four to six additional rounds of replication proceed from a defined origin and extend over at least 100 kilobases of chromosomal DNA sequences. The DNA sequences near the replication origin are amplified to a higher degree than those further away suggesting that subsequent replication forks do not travel as far as their predecessors (Spradling 1981; Spradling & Mahowald 1980). The observation that the degree of amplification is dependent on the distance between the DNA sequence and the origin of replication is also found in the amplification of defective thymidine kinase genes in mammalian cells (see below). Developmentally regulated amplification is not the only solution to the problem of how an insect meets the high demand for chorion proteins during egg maturation, since the silkmoth permanently carries within its genome multiple copies of the genes (Jones & Kafatos 1980). The ability to amplify genes provides an evolutionary alternative to the problem of how best to meet a short, sharp but heavy demand for a stage-specific protein, and one way of avoiding the problem of maintenance of homogeneity in a multigene set.

Multiple rounds of initiation of DNA replication as a mechanism of DNA amplification

Polytene chromosomes illustrate, and provide good evidence for, one aspect of a proposed mechanism of gene amplification popularly known as the 'onion skin model' (see figure 3). During polytenization most non-heterochromatic regions are thought to undergo multiple complete rounds of replication, preserving the linear integrity of each chromatid. The available evidence suggests that amplification of the chorion protein genes, or the genes in the DNA puffs, reflects *additional* local multiple rounds of DNA synthesis, resulting in a hierarchy of branch points as each new replication fork proceeds away from the origin. In a non-dividing 'dead-end' polytenized cell of an insect there would be no requirement for the structure to resolve itself into something capable of segregating at mitosis. However, similar models have been proposed for gene amplification in mammalian cells that do divide. In this situation it would be necessary for the branched complex to be resolved into the structures seen in these cells. Presumably this would involve multiple recombination events between sequences within the complex.

Gene amplification as an experimentally selected phenomenon

There are now numerous examples in which the amplification of a gene has been demonstrated in a variety of prokaryotic and eukaryotic cells following selection for increased amounts of the gene product. This field has been comprehensively reviewed recently (Cowell 1982; Schimke 1982; Stark & Wahl 1984) and I will summarize here only some essential features of amplification in mammalian cells.

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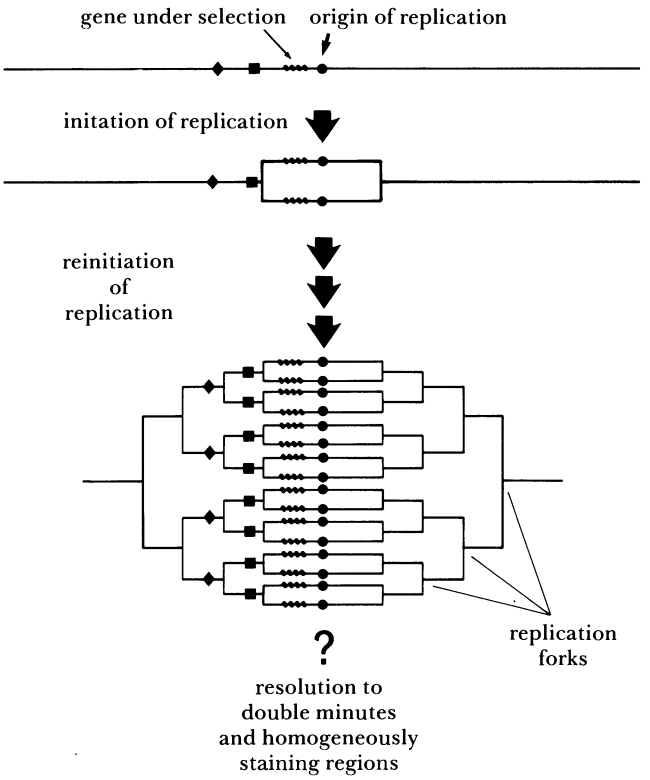

FIGURE 3. The 'onion-skin' model of DNA sequence amplification. This model proposes that amplification is brought about by the differential replication of particular sequences through multiple initiation events at a single origin of replication. Multiple replication forks travelling away from the origin produce a complex branched chromosome structure in which sequences close to the origin will be amplified to a greater extent that those more distant from the origin. A structure such as this is probably the end-product of amplification in dipteran polytene chromosomes, but would require resolution into mitotically stable forms if it serves as a mechanism for amplification in mammalian cells. It has been proposed that recombination between homologous sequences might play a role in this, although there is no direct evidence. Notice, however, that the integrity of the original chromatid could be preserved if all the hypothetical newly replicated DNA is removed from the complex, and the single DNA strands of the original chromatid are allowed to 're-anneal'. It is therefore theoretically possible to resolve this type of structure into either extrachromosomal forms (for example, d.m.) or integrated forms (for example, h.s.r.) depending on how the proposed recombination events are envisaged.

Gene amplification as the basis for drug resistance

The basis for resistance to the inhibitory effects of many drugs is the overproduction of the drug's target enzyme or binding protein (see tables 1 and 2 of Stark & Wahl 1984). In some of our mouse cell lines, which are highly resistant to methotrexate (MTX), the target enzyme (dihydrofolate reductase, DHFR) can account for as much as 20% of the soluble protein, whereas normally it represents less than 0.002% (C. Tyler-Smith, unpublished results; Bostock *et al.* 1979). Alt *et al.* (1978) were the first to show that amplification of DHFR genes formed the basis for the elevated levels of DHFR. The degree of gene amplification depends on the degree of resistance sought, but can be built up to several thousand-fold through multiple rounds of selection at increasing concentrations of the drug.

Large amounts of DNA are coamplified with the selected gene

One property that appears to be common to different mammalian gene amplification events is the coamplification of large stretches of DNA which are not part of the gene under selection or part of its normal immediate chromosomal environment. For example, the mouse DHFR gene is some 32 kilobases in size, but in MTX-resistant cells the average size of the unit of DNA which is amplified per extra DHFR gene copy is commonly between 500 and 1000 kilobases, but can be as much as 3000 kilobases (see Cowell 1982).

There are two ways in which these additional sequences can be made visible. Ethidium-bromide-stained agarose gels of restriction enzyme digested DNA (see figure 4; Bostock & Tyler-Smith 1981; Heintz & Hamlin 1982) show complex patterns of bands in addition to those characteristic of the gene. Alternatively, abnormal chromosomal structures called 'double minutes' (d.m.) or 'homogeneously staining regions' (h.s.r.) can be seen in mitotically dividing cells (figure 5). One general requirement of gene amplification in mammals, which is true also for *in vivo* selected oncogene amplification (see later section), would therefore seem to be 'bulk' in the unit that can be amplified.

Gene amplification occurs in the absence of selection

Although metabolic inhibitors are used to select for cells in which gene amplification has occurred, it is clear that the initial amplification event occurs spontaneously at a frequency of around 10^{-3} to 10^{-4} events per cell generation in the absence of the selective agent (Kempe *et al.* 1976; Johnston *et al.* 1983). This spontaneous rate for DHFR gene amplification can be enhanced by various treatments, some of which interfere with DNA replication or are mutagenic (see, for example, Varshavsky 1981; Mariani *et al.* 1984), but the CAD gene system does not respond in this way (Kempe *et al.* 1976).

Assuming that the gene systems that have been studied are not especially prone to amplification, that amplification events occur at random and that a particular selective condition identifies only those cells that have amplified the gene under selection, we can estimate that at any one time up to one in every three cells in a population must be undergoing some form of amplification event. (Say 1 in 10000 cells amplifies a unit of DNA which is on average 1000 kilobases in size and happens to include the gene under selection. The genome size of mammalian cells is approximately 3×10^6 kilobases, so a unit of 1000 kilobases represents 1/3000 of the total genome. Thus there must be 3000 amplification events of a unit of DNA about 1000 kilobases in every 10000 cells in the population.) It remains to be shown whether these high rates of gene amplification are only found in transformed cells.

DNA sequence amplification involves DNA rearrangement

Since the initial event of amplification happens spontaneously in a single cell that cannot be identified until after the event it is difficult to think of ways of analysing it as it happens! It is really only possible to study the structure of the product of amplification many cell generations later. Following a single low level of selection for resistance to *N*-phosphonacetyl-L-aspartate (PALA) clones of Chinese hamster ovary (CHO) cells grow out which are capable of growing at much higher concentrations of PALA than that used for their initial selection (Zieg *et al.* 1983). Cells contain between 10 and 20 amplified copies of the gene that codes for the multifunctional CAD protein, suggesting that in this system the initial event involves the

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saltatory production of several copies rather than involving a process, for example, unequal recombination between sister chromatids, by which the copy number increases gradually according to demand.

There is no doubt that multiple recombination events of some kind are involved in amplification. By using the DHFR gene again as an example, rearrangements can be detected which occur within the gene as well as at either the 5′-, 3′- or at both ends of some copies of the gene (for example, Federspiel *et al.* 1984; Tyler-Smith & Alderson 1981; see figure 4*b*). The rearrangements are characteristic of one particular clone, or its subclones, and so represent unique events in the amplification process in a single cell. The molar ratios of the rearranged copies to normal DHFR gene copies can be different for different rearrangements, but once established they can be maintained through subsequent rounds of amplification, a change from double minutes (d.m.) to homogeneously staining regions (h.s.r.) and transfection of amplified DNA into a different host cell type (Tyler-Smith & Bostock 1981; Bostock & Clark 1983). On the other hand Federspiel *et al.* (1984) identified some variation in the composition of amplification-specific rearrangements over extended periods of maintenance in culture.

The same kind of rearrangements have been identified in non-DHFR gene sequences by 'walking' away from the gene along overlapping cloned fragments (Federspiel *et al.* 1984) or by using cloned amplified non-DHFR gene sequences as probes to the DNAs of different MTX-resistant cell lines (Caizzi & Bostock 1983). In addition to DNA fragments containing novel rearrangements, several DNA sequences were found to be amplified only in the cell line from which they were cloned, suggesting that the recombination events bring together at random DNA sequences from distant parts of the genome. Similar conclusions have been reached by Ardeshir *et al.* (1983) who found 'novel joints' in a series of PALA-resistant clones of Syrian hamster cells.

The overall effect of all these rearrangements can be made visible in ethidium-bromide-stained gels of DNAs from highly MTX-resistant cells (figure 4*a*, plate 1). The DNA of each cell line

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Description of plate 1

Figure 4. Amplified DNA in MTX-resistant mouse cells. (*a*) Ethidium-bromide-stained gel of *Eco*RI restriction enzyme digested DNAs isolated from wild-type mouse EL4 lymphoma cells (track A), four highly MTX-resistant EL4–lymphoma cell lines: EL4/3 (track B), EL4/8 (track C), EL4/11 (track D) and EL4/12 (track E) and one highly MTX-resistant PG19 mouse melanoma cell line (track F). Notice that each of the MTX-resistant DNAs contain multiple brightly fluorescing bands not present in the wild-type DNA. The overall patterns of these bands is different in each DNA, although several bands are common to two or more of the DNAs. The track labelled λ contains fragments of λ DNA digested with both *Eco*R1 and *Hind*III, which served as size markers. (*b*) Probing a Southern transfer of the DNAs shown in (*a*) with DHFR gene sequences shows the degree of DHFR gene amplification and the presence of fragments (arrowed) which represent rearrangements close to the DHFR gene in three of the five MTX-resistant lines. The radioautograph of the wild-type DNA in track A′ has been exposed for 50 h compared to 1 h for the radioautographs of tracks A–F. This gives an indication of the 1000-fold amplification of DHFR gene sequences. The wild-type DHFR gene is contained in four *Eco*RI restriction fragments, all of which are amplified. However EL4/8 and EL4/11 each contain an additional amplified DHFR gene-containing fragment, which results from a rearrangement at the 3′ end of the gene. EL4/3 contains two rearrangements, one at the 5′ end of some copies and the other at the 3′ end of some copies. Only one of these rearrangements can be seen in *Eco*RI digested DNA.

Figure 5. Examples of aberrant chromosome forms that carry amplified sequences in MTX-resistant cells. (*a*) Double minutes and ring chromosomes in L cells originally transfected with EL4/8 chromosomes, (*b*) h.s.r. in PG19 cells and (*c*) double minutes in EL/8 cells. In these examples d.m. are clearly heterogeneous, varying in size up to structures which appear as ring chromosomes. The h.s.r. of the PG19 cells (*b*) contain large amounts of mouse satellite DNA which was amplified along with the DHFR gene.

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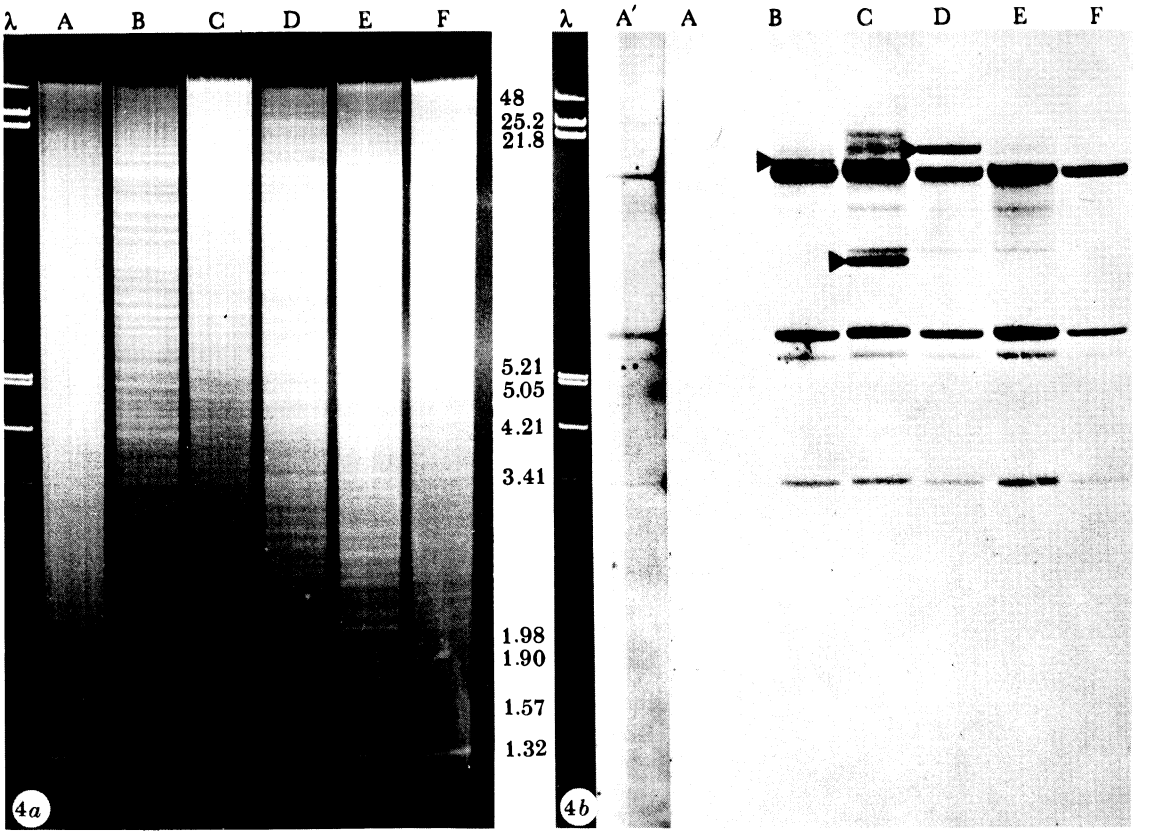

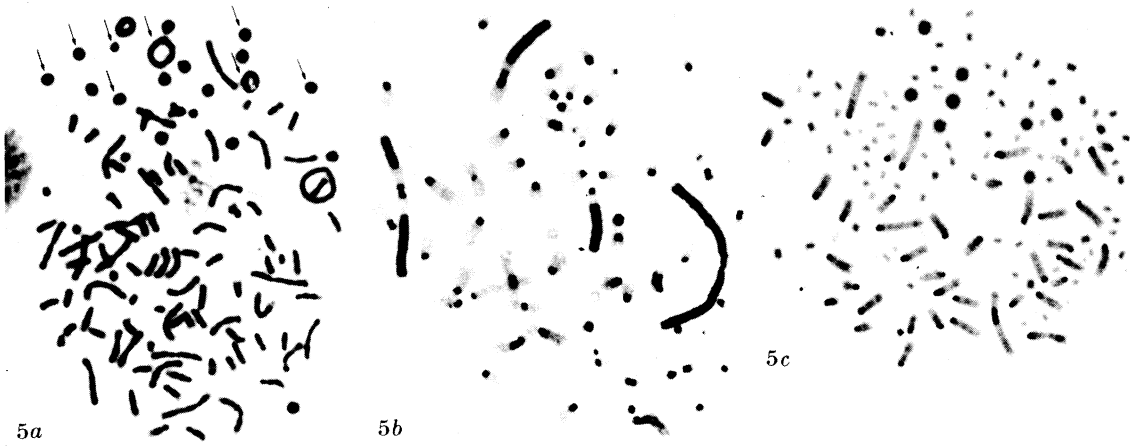

FIGURES 4 AND 5. For description see opposite.

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produces a complex pattern of distinct bands (fragments containing amplified sequences) against a background smear (characteristic of non-amplified DNA). The fact that the overall pattern is quite different in each independently selected cell line shows that a unique set of rearrangements has produced a unique amplified structure in each cell line.

The important involvement of chromosomal rearrangements in the amplification process is also indicated by cytogenetic observations. The very nature of d.m. and their fluidity in size and form (see figure 5) suggests constant rearrangement. More specifically, the amplification of DHFR (Worton *et al.* 1981; Flintoff *et al.* 1982) and asparagine synthetase (Andrulis *et al.* 1983) genes in CHO cells has been associated with an initial requirement for a chromosome translocation. In a set of several independently selected MTX-resistant Chinese hamster cells Biedler *et al.* (1980) found an h.s.r. frequently located on chromosome 2. However, the position within chromosome 2 is variable, again indicating that rearrangement may be an integral part of the amplification process.

Amplification of experimentally introduced genes

Most studies on gene amplification have started with an endogenous 'target' gene and attempted to deduce events that may have happened upon selection. An alternative approach has been to introduce marked selectable genes into cells in such a way that their behaviour can be followed upon selection for amplification. In this way one can attempt to distinguish between host cell effects, gene-specific effects and those effects that are mediated via flanking chromosomal sequences. Roberts *et al.* (1983) used a partly defective thymidine kinase (TK) gene which could be amplified by selection after transfection into a TK-deficient recipient cell. They found that the structure of the amplified unit was different in each independent cell line, indicating that each amplification was accompanied by 'unique' rearrangements. The molar ratios of different cotransfected sequences varied depending on their position relative to the TK gene in the original transformant. Those sequences close to the TK gene were amplified three- to fourfold more than those at the periphery of the transfected structure. This is reminiscent of what happens when the chorion protein genes are amplified, and has been interpreted to suggest that amplification proceeds via the 'onion skin' model.

It is difficult to assess to what extent this result is caused by the integration of multiple copies of homologous plasmid DNA sequences in the immediate vicinity of the TK gene. Similar experiments with cloned CAD (Wahl *et al.* 1984) and DHFR (Kauffman *et al.* 1983) genes usually resulted in amplification of the transfected structure intact, so that any amplification-specific rearrangements must have occurred in host chromosomal sequences which lay outside the integrated DNA. Host chromosomal effects could be detected by comparing the frequency with which different initial transformants could be selected for subsequent amplification, suggesting that different chromosomal domains or sequences differ in their ability to promote amplification.

Gene amplification *in vivo* as a sporadic natural phenomenon

DNA amplification in tumours

Chromosomal changes, which are suspected of having some involvement in the development or progression of the tumour phenotype, occur in the lineage of many tumour cells. Often abnormal chromosome entities such as d.m. and h.s.r. are found, which, because of their

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association with amplified DNA in the experimentally induced systems, suggests that they carry 'spontaneously' amplified DNA in the tumour. DNA sequence amplification has now been directly demonstrated to have occurred in several tumours (see Stark & Wahl 1984; Bishop 1983).

Up to 40-fold amplification of the N-*myc* oncogene has been found in different neuroblastomas or derived cell lines, many of which also contained an h.s.r. (Brodeur *et al.* 1984; Schwab *et al.* 1983). In one case, at least, amplification was confined to the tumour cells of the patient only (Schwab *et al.* 1984). Although most of these neuroblastomas carry the amplified DNA in the form of an h.s.r., its position is not limited to a specific chromosomal location (Schwab *et al.* 1983). This suggests that, since there are amplified sequences in common to the different tumours (for example, the N-*myc* oncogene), tumour-specific translocation events must have moved the h.s.r. to different locations in each tumour. Kanda *et al.* (1983) isolated by fluorescence activated sorting the h.s.r.-containing human chromosome 1 from the neuroblastoma cell line IMR32. The sequences contained in the h.s.r. were found to be derived by rearrangement and amplification of sequences normally present on human chromosome 2 (Kohl *et al.* 1983).

The observations that the size of an h.s.r., and thus the degree of DNA amplification, can be correlated with the degree of tumorigenicity (Gilbert *et al.* 1983), and that DNA amplification is found more frequently in advanced stages of neuroblastoma (Brodeur *et al.* 1984) suggest that amplification may enhance the severity of the malignant phenotype rather than cause the initial transformation event. The demonstration that DNA amplification, with many of the properties previously described for drug selection systems, has occurred in some tumours confirms the spontaneous nature of the amplification event. Since it is not yet known whether DNA amplification actually causes the change from normal to tumour cell it is not possible to say whether it occurs in normal diploid cells. If it did occur in diploid germ-line cells it would clearly have a significant cumulative effect on the evolution of genotypes.

rDNA amplification in Drosophila

Direct evidence for the inheritance of amplified DNA is sparse, but the clearest demonstration that it can, and does, occur is the phenomenon of rDNA magnification in *Drosophila*. Wild-type *Drosophila* have tandemly repeated blocks of rDNA on both X and Y chromosomes, but mutants, called *bobbed*, arise which carry major deletions of the rDNA on one or other of the sex chromosomes (Ritossa *et al.* 1966). Normally chromosomes carrying such deletions can revert at a low rate to wild-type chromosomes, presumably by a process of unequal crossing-over within the rDNA cluster during meiosis, but against certain genetic backgrounds in males the *bobbed* genotype can revert to wild types much more rapidly (Boncinelli *et al.* 1972). The mechanism by which rDNA magnification occurs is not known, but it is sensitive to mutations which affect meiotic recombination and replicative repair processes (Hawley & Tartoff 1983). Also implicated in the magnification process are the chromosome-specific insertion sequences found in rDNA in *Drosophila* (Hawley & Tartoff 1983; de Cicco & Glover 1983). These are also found in tandem arrays adjacent to, but independent of, the rDNA clusters and have characteristics in common with transposable elements. Interestingly, the independent copies of insertion sequences also amplify during the early stages of magnification, but subsequently their number decreases.

Transposable elements have already been discussed in detail in this volume, and I do not have space to elaborate further here on their properties with respect to DNA sequence

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amplification. Nevertheless, it is clear that they could mediate amplification – replicative transposition is by definition an amplification – and models based on their involvement in amplification have been proposed by Stark & Wahl (1984).

Conclusions

We have seen that there are a number of situations in which amplification of DNA sequences occurs either as an ordered and predictable event in the development of an organism or as a spontaneous but random event during growth and division of cells. They all have effects on the survival of cells or organisms, and as such must have the potential to affect the genome composition over evolutionary time. This potential can be seen in a rather direct way, for example, in the re-establishment of rDNA copy number by rDNA magnification, the requirement for only a single rDNA unit in micronuclear DNA of *Tetrahymena*, or the 'growth' of simple tandem repeating sequences at telomeres. The existence of amplification events of the kind observed in mammalian tumour cells, or cells selected *in vitro*, indicates that large scale rearrangements and low level amplification can occur, but it remains to be shown whether they also occur in germ-line cells. Their random, spontaneous and sporadic nature would make their individual detection difficult, especially if the frequency of their occurrence was low. Nevertheless, it would not require many events of this type to alter substantially the genomes of organisms whose ancestry has included them.

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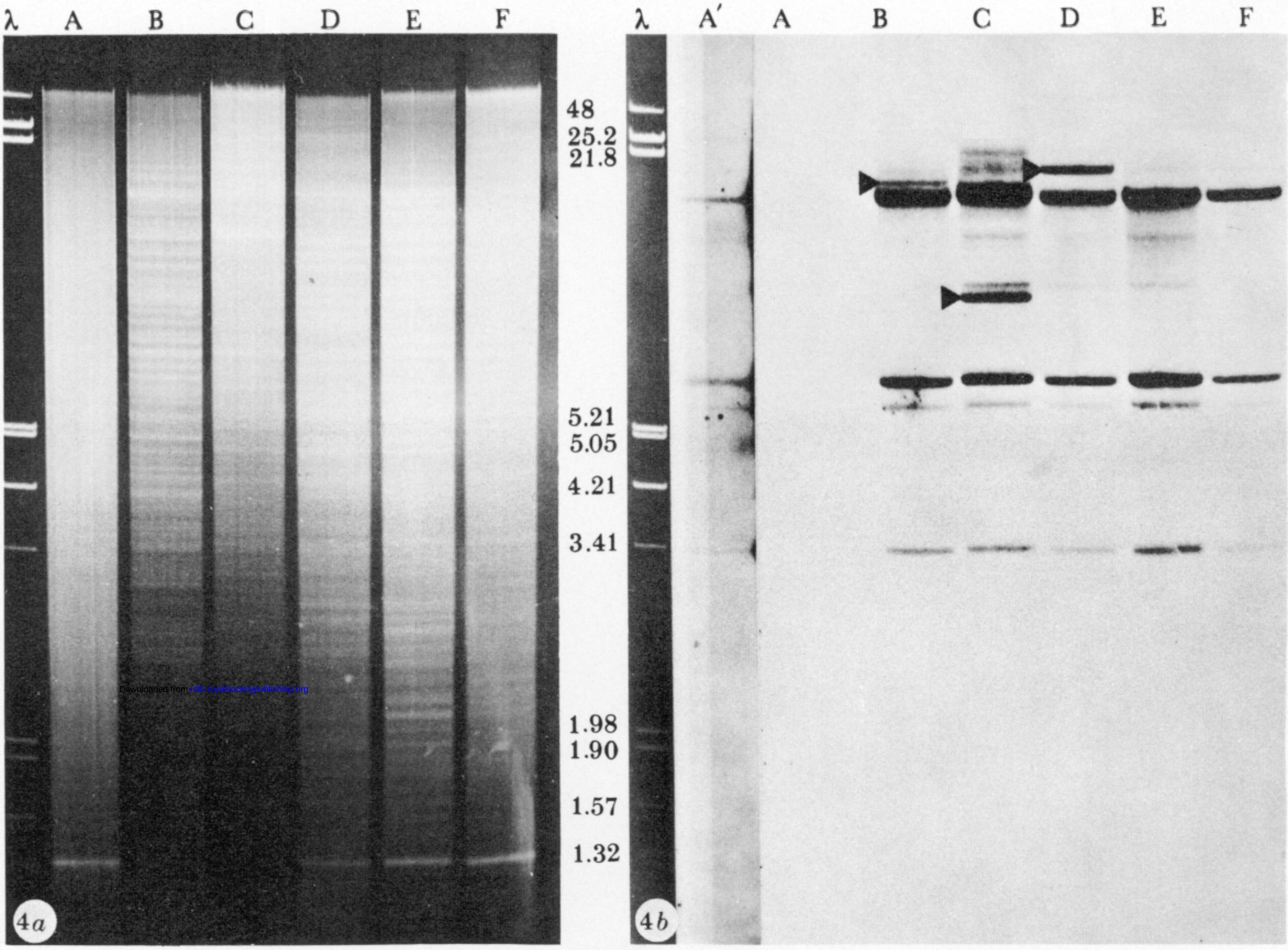

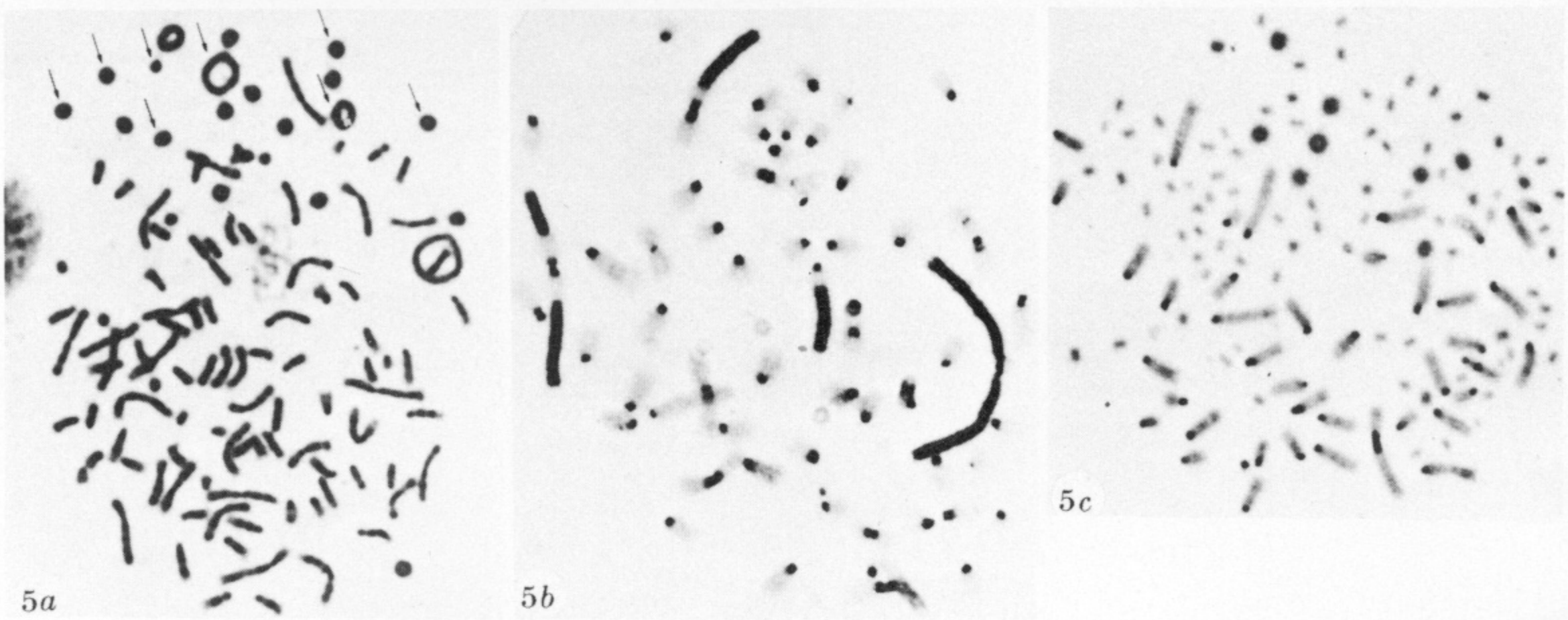

FIGURES 4 AND 5. For description see opposite.