Satellite DNA

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Repetitive and Non-repetitive DNA

In recent years it has been discovered that the greater proportion of the DNA of higher organisms (eukaryotes) is composed of repetitive nucleotide sequences (reviewed by Walker, 1971). This finding has revolutionized ideas concerning the organization of genetic material and stimulated much research and discussion on the functions of repetitive DNA. In general its function is not yet understood although DNA of known function has been shown to be repetitive in a number of instances. These mainly involve genes whose terminal products are RNA rather than proteins. Examples are the genes for 28S, 18S, and 5S ribosomal RNA (reviewed in Attardi and Amaldi, 1970), and the transfer RNA genes (Ritossa *et al.*, 1966; Brown and Weber, 1968; Quincey and Wilson, 1969). The genes for histones are also reiterated (Kedes and Birnstiel, 1971). The few examples of genes controlling proteins synthesized by differentiated cells which have so far been examined indicate that they are represented once, or at most a few times per diploid genome (Bishop, Pemberton, and Baglioni, 1972; Harrison *et al.*, 1972; Bishop and Rosbash, 1973).

Satellite DNA forms a distinct class of repetitive DNA. It is the most highly repeated DNA in the genome and it exhibits the property of separation from the rest of the DNA in dense salt gradients in the ultracentrifuge.

Satellite DNA

When the solutions of dense salts such as caesium chloride are subjected to very high gravitational forces in an ultracentrifuge, the molecules migrate centrifugally. This force is opposed by diffusion which tends to equalize the concentration of the salt

throughout the fluid space. On prolonged centrifugation an equilibrium density gradient eventually forms. Macromolecules such as DNA migrate in such a gradient until they reach a zone within it where their buoyant density is the same as that of the surrounding salt solution and is expressed in terms of the salt concentration per ml (ie, g/cm³) at that point. Such a gradient is termed an isopycnic gradient. The buoyant density of native DNA reflects its average base composition and increases in proportion to the percentage of G + C pairs present. The zone or band of DNA therefore represents a distribution of molecules according to base composition. The average base composition for mammalian DNA is about 40% G + C but departures of as much as 10% may occur from this value. Any DNA molecules which differ appreciably from the modal distribution of the bulk of the DNA will broaden and distort the symmetrical shape of the band and if they depart very significantly from the mean they will appear as a separate band. DNA which separates thus is called satellite DNA.

Sequences of highly repetitive DNA remaining within the main band in caesium chloride gradients can sometimes be revealed as satellite DNA in gradients of caesium sulphate containing metal ions, silver, or mercury (Nandi, Wang, and Davidson, 1965; Corneo, Ginelli, and Polli, 1968a; Corneo *et al.*, 1968b). DNAs of particular A + T/G + C ratios bind disproportionate amounts of these ions and change in buoyant density relative to the main band. The reasons for such disproportionate binding are not fully understood but it affords a useful preparative method for such otherwise occult satellite DNAs, among them the satellite DNAs of man and related primates.

DNA satellites purified in this manner have been shown to comprise from 0.2% to more than 50% of the genome; the proportion being roughly

characteristic of a given satellite in a particular species. They are the most highly repetitive naturally occurring DNAs, consisting of short nucleotide sequences reiterated in tandem thousands to millions of times per nucleus (reviewed by Walker, 1971). Of the numerous species that have been surveyed (reviewed by Arrighi *et al*, 1970; Coudray, Quetier, and Guille, 1970; Ingle, Pearson, and Sinclair, 1973) about half showed satellite DNA. The first satellite DNA to be described was that of the mouse (Kit, 1961 and 1962), which has a buoyant density of 1.691 g/cm³ compared with the mean buoyant density of 1.700 g/cm³ for mouse mainband DNA, and comprises about 10% of the total DNA. It consists of as many as several million copies (3.3×10^3) of a sequence 8–13 base pairs in length (Southern, 1970). Twenty to thirty per cent of these copies are identical while the rest show only one or two base substitutions. This homogeneity results in very sharp banding in caesium chloride gradients. The availability of good methods of preparation (detailed by Flamm, Birnstiel, and Walker, 1968), has made satellite DNA an attractive subject for research into the question of the functions of repetitive sequences.

The Organization of Satellite DNA in the Cell Nucleus. The discovery of satellite DNA and its properties necessarily led to questions concerning its function and its organization at the chromosomal level. The first question is still unanswered although many ideas concerning potential functions have been advanced (for example, see Walker, 1971; Flamm, 1972; Mazrimas and Hatch, 1972; Walker, 1972).

Whether satellite DNA sequences are transcribed has been examined in a few instances with different results. In the mouse, failure to find RNA complementary to isolated single strands of satellite DNA led Flamm to conclude that it is not transcribed (see Flamm, 1972). However, Harel *et al* (1968) reported some transcription of mouse satellite and Graham (1971) presented evidence for the transcription of a heavy satellite in *Geocarcinus*. The known nucleotide sequences of guinea pig satellite DNA (Southern, 1970) would not lead to protein synthesis, and the heterochromatic localization of those satellites so far investigated suggests transcriptional inertness.

The gross aspects of nuclear and chromosomal organization of satellite DNA have been determined in a number of cases. First attempts to attack this question were made by Bond *et al* (1967) who showed that most satellite sequences were present in nucleolar fractions in the same proportion as in

the non-nucleolar fractions of mouse nuclei. Schildkraut and Maio (1968) subsequently reported that the DNA obtained from nucleoli was 30% enriched in satellite DNA. Maio and Schildkraut (1969) also reported that different chromosome size classes contained equivalent proportions of satellite DNA, thus implying the presence of satellite sequences on most, if not all, of the chromosomes. Isolated dense chromatin from interphase mouse nuclei was shown by Yasmineh and Yunis (1969 and 1970) to contain 70% of the satellite DNA and in guinea pig the same fractions contained a four-fold enrichment of satellite DNA. The most direct proof of the chromosomal distribution, however, came from the technique of *in-situ* hybridization (Gall and Pardue, 1969; John, Birnstiel, and Jones, 1969).

In-Situ Hybridization and Evidence for Location of Satellite DNA in Centromeric Heterochromatin.

When the complementary strands of DNA are caused to separate by heating or other appropriate means (denaturation) they can be induced to reform duplex molecules (renature) given a favourable concentration of salt at an appropriate temperature of incubation, usually 15–20° C below the mean denaturation temperature (T_m) (for a review see McCarthy and Church, 1970). The process of renaturation involves the collision of complementary base sequences and the reformation of hydrogen bonds. It takes place at a rate which reflects the concentrations of the complementary nucleotide sequences. Thus at a given concentration, a satellite DNA will renature faster than any other naturally occurring DNA, since its basic sequences are very much more highly repeated. The realization of the repetitive nature of a large proportion of DNA in eukaryotes, principally by Britten and his colleagues (Britten and Kohne, 1968a), in fact followed upon the finding that a certain fraction of DNA renatured much faster than expected if each DNA sequence was unique. The renaturation of DNA thus allows a measure of its base sequence complexity as well as of the relatedness of one sample of DNA to another. One of the strands of DNA

FIG. 1. Satellite DNA III in human chromosomes revealed by the *in-situ* hybridization of complementary RNA synthesized from radioactive precursors on a template of pure satellite DNA III *in vitro*. The silver grains on the autoradiograph reveal the sites which contain major amounts of this sequence. Chromosome 9, identified by a pericentric inversion, is particularly heavily labelled. The other chromosomes, arranged roughly in conventional groups, show minor labelling. Some of this is attributable to 'background', but there was consistent labelling of members of the D, F, and G groups. (Adapted from Jones *et al*, 1973a.)

FIG. 1

TABLE 1
CONDITIONS FOR ISOLATION AND THE PHYSICAL

Name of Fraction	% of Genome	Tissue Extracted From	Buoyant Density				Buoyant Density of Separated Strands	T _m
			Main Band	Satellite				
				Native	Denaturated	Renaturated		
Satellite I	1	Bone marrow; lymph node; leucocytes; HeLa	1.700	1.687	1.704	1.694	1.707 1.738	80° C in SSC
Light satellite	0.2	HeLa; cultured diploid; placenta; spleen; liver	1.698	1.686	—	—	1.731 1.764 plus double stranded material at 1.750	—
Satellite II	2	Placenta	1.700	1.693	1.704	1.696	1.740 1.750	85° C in SSC
Satellite III	1.5	Placenta	1.700	1.696	1.715	1.703	1.740 1.754	84° C in SSC
Satellite IV	2	Placenta	1.700	1.700	1.716	1.705	1.730 1.742	—
Homogeneous main band	15	Placenta	1.700	1.696	1.715	1.709	—	87° C (from graph) in SSC
Heavy satellite (a)	—	HeLa	1.698	1.712	1.774	1.774	—	—
Heavy satellite (b)	—	Leucocytes (chronic lymphocytic leukaemia)	1.697	1.703	—	—	—	—

T_m = melting point. (Mid point of the absorbancy rise on heating in solution.)

SSC = standard saline citrate. (0.15M NaCl 0.015M trisodium citrate: pH 7.2.)

R_F = molar ratio of metal ion to DNA-P.

Cot = renaturation conditions expressed as moles of nucleotides times litres per second. (See Britten and Kohne 1968a.)

can be experimentally replaced by a complementary strand of RNA in which case the resulting duplex molecule is termed a hybrid. DNA/DNA renaturation and DNA/RNA hybridization (eg, see Gillespie and Spiegelman, 1965) therefore form two closely related and powerful tools with which to investigate DNA structure. Usually such reactions are performed using purified nucleic acids in solution or bound to a substrate such as filter paper. However, used in this way the methods do not readily lend themselves to analysis of the organization of the DNA in structures within the intact cell. This has led to the development of an approach in which only one of the reacting nucleic acids is purified while the other remains in the cell. Incubation of the radioactively-labelled pure fraction of nucleic acid (complementary RNA or DNA) with a cytological preparation (target) suitably pretreated to denature its DNA allows the input nucleic acid which becomes hybridized to the target to be sub-

sequently detected autoradiographically, thus locating the sequence of interest. This technique is called *in-situ* hybridization (reviewed in Gall and Pardue, 1971; Jones, 1973). For technical reasons, related to rate of reaction and amount of complementary DNA in the individual cell nucleus, *in-situ* hybridization is most suited to the detection of repetitive DNA which forms a significant proportion of the genome: it was obvious that mouse satellite DNA offered a suitable case for investigation by these means. Radioactive complement to this DNA was shown to hybridize *in situ* principally with the centromeric heterochromatin of all of the chromosomes except the Y chromosome and with interphase heterochromatin and nucleoli (Jones, 1970; Pardue and Gall, 1970). These results directly confirmed those obtained biochemically by previous workers and showed that centromeric and chromocentral heterochromatin in mammals represent the same chromosomal regions. Nucleoli

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PROPERTIES OF HUMAN SATELLITE DNA FRACTIONS

Enriched in Heterochromatin	Enriched in Nucleolar	Renaturation Data	Method of Extraction	Reference
Yes	—	—	(1) 25 µg/ml in CsCl (2) Hg ⁺ + Cs ₂ SO ₄ , 50 µg/ml, R _F = 0.1 (3) Ag ⁺ + Cs ₂ SO ₄ , 70 µg/ml, R _F = 0.2 (4) MAK elution, Ag ⁺ + Cs ₂ SO ₄ , 60 µg/ml, R _F = 0.23	Corneo <i>et al.</i> , 1967 Corneo <i>et al.</i> , 1968a Corneo <i>et al.</i> , 1971 Corneo <i>et al.</i> , 1972
—	Yes	—	(1) 15 µg/ml in CsCl (2) Nucleolar preparations	Schildkraut and Maio, 1969
Yes	—	10 µg/ml for 5 hr at 65° C in 2 × SSC. Cot of 5.6 × 10 ⁻¹ yields 73% renaturation	(1) Ag ⁺ + Cs ₂ SO ₄ , 50 µg/ml, R _F = 0.27 (2) Ag ⁺ + Cs ₂ SO ₄ , 70 µg/ml, R _F = 0.20 (3) MAK elution, Ag ⁺ + Cs ₂ SO ₄ , 60 µg/ml, R _F = 0.23	Corneo <i>et al.</i> , 1970 Corneo <i>et al.</i> , 1971 Corneo <i>et al.</i> , 1972
Yes	—	10 µg/ml at 60° C in 2 × SSC. Cot 5.6 × 10 ⁻¹ yields 63% renaturation	(1) Ag ⁺ + Cs ₂ SO ₄ , 70 µg/ml, R _F = 0.20 (2) MAK elution, Ag ⁺ + Cs ₂ SO ₄ , 60 µg/ml, R _F = 0.23	Corneo <i>et al.</i> , 1971 Corneo <i>et al.</i> , 1972
—	—	5 µg/ml for 2 hr 60° C in 2 × SSC. Cot 1.1 × 10 ⁻¹ yields 63% renaturation	(1) MAK elution, Ag ⁺ + Cs ₂ SO ₄ , 60 µg/ml, R _F = 0.23	Corneo <i>et al.</i> , 1972
—	—	10 µg/ml for 5 hr at 65° C in 2 × SSC. Cot 5.6 × 10 ⁻¹ yields 58% renaturation	(1) Hg ⁺ + Cs ₂ SO ₄ , 50 µg/ml, R _F = 0.1 (2) Ag ⁺ + Cs ₂ SO ₄ , 70 µg/ml, R _F = 0.20	Corneo <i>et al.</i> , 1970 Corneo <i>et al.</i> , 1971
—	Yes	—	(1) Nucleolar preparations from HeLa CsCl, 1 µg/ml	Schildkraut and Maio, 1969
—	—	72.9° C in 0.025 M Tris HCl pH 8.0	Ag ⁺ + Cs ₂ SO ₄ , R _F = 0.27	Saunders <i>et al.</i> , 1972

which form in association with chromocentres are therefore closely associated with centromeric heterochromatin in the interphase nucleus. The circumscribed physical nature of satellite DNA as preparatively defined is therefore matched by a discrete cytological localization. These pioneering studies, using mouse satellite DNA, have been repeatedly confirmed in later work with a wide variety of satellite DNAs from different species (reviewed by Hennig, 1973) including man and higher primates (Jones and Corneo, 1971; Jones *et al.*, 1973a and b). As a consequence, constitutive heterochromatin, previously a murky concept, is now better defined, although its function remains mysterious.

Satellite DNA in Man. The case of satellite DNA in mouse is not by any means typical for all other genomes. Human DNA has been shown to contain not one but several distinct DNA satellites

(Table I), each of which constitutes different amounts varying from 0.5–2.0% of the total DNA (Corneo, Ginelli, and Polli, 1967, 1970, and 1971; Corneo, Zardi, and Polli, 1972). Those most intensively studied have been designated by Roman numerals from I to IV and they differ from each other in important respects. Two of these satellite DNAs, II and III, have been assigned genomic locations by *in-situ* hybridization (Jones and Corneo, 1971; Jones *et al.*, 1973a and b). A third, possibly different, satellite DNA has also been described by Saunders *et al.* (1972). Satellite II is present in major amounts on chromosomes 1 and 16 and in lesser amounts on chromosome 9 and on the acrocentrics of the D, F, and G chromosomes. The chromosomal regions bearing this satellite DNA show heteromorphism which affects the amount of the sequence present. Satellite III on the other hand is located predominantly on the No. 9 chromosome as is Saunders' satellite (Saunders *et al.*,

1972), and on the smaller median and acrocentric chromosomes of the groups D and G (Fig. 1). There is therefore partial overlap in the distribution of these sequences and cytological distribution *in situ* is roughly commensurate with their genomic quantity as shown by analytical ultracentrifugation. They are confined to centromeric heterochromatin but, unlike the mouse autosomes, some chromosomes do not appear by this technique to possess either sequence. Therefore among the potential functions imaginable for these sequences, those requiring their major presence on all chromosomes would appear to be excluded. The fact that heterochromatin at a given location is composed of more than one sequence family, is of potential interest regarding the origin of such sequences (Walker, 1971). The genomic distribution of the other human satellite DNAs have yet to be determined. Satellite I has been annealed on two separate occasions without success. The reasons for this lack of success in locating it are presently unclear but may have to do with its very small amount in the genome and the possibility it is on more than a few chromosomal pairs.

Origin and Evolution of Satellite DNA. Satellite DNA is presumed to originate in a replicative event termed 'saltation' (Britten and Kohne, 1968b) which results in the tandem repetition of an original simple nucleotide sequence. Nothing is known about the events instrumental in accomplishing this process. It is possible that in some cases it occurred as a relatively discrete event. However, data on the sequencing of one satellite in the guinea pig have been interpreted (Southern, 1970) to suggest that a series of such saltatory events occurred, separated by appreciable intervals of time.

Satellite DNAs have been widely observed to be species-restricted and different species even within taxonomically related groups have been shown to possess their own characteristic spectra of these highly repetitive sequences (see Walker, 1971; Flamm, 1972). However, exceptions to this generalization are known in *Drosophila* (Fansler *et al.*, 1970; Skinner and Kerr, 1970), in Crustacea (Graham and Skinner, 1973), and in species of *Mus* (Sutton and McCallum, 1972). Satellite DNAs are for the most part relatively young in evolution as seen from their high rate of evolutionary turnover. That some balance has been struck in the rate of appearance and disappearance of satellite DNA is reflected in the fact that the size of the genome in most mammals appears to be fairly constant (Ohta and Kimura, 1971). Mechanisms for loss as well as for generation of satellite sequences

must therefore exist in evolution but we presently have no evidence of their nature.

The situation in humans and related primates, also departs from the general picture of species restriction of satellite DNA. In *in-situ* hybridization studies on human satellite III DNA it has been found that cross homology exists between it and centromeric heterochromatin of the chimpanzee and the orang-outang (Jones *et al.*, 1973b). The homologous satellite DNA of the chimpanzee, which was implied by these findings, has been isolated, transcribed and shown to cross hybridize with human DNA (Prosser *et al.*, 1973). Studies are in progress to isolate the implied satellite DNA of the orang-outang. These data show that the original satellite sequence existed in a common primate ancestor of man, chimpanzee, and orang-outang. It appears that satellite DNA may be of particular value in researching evolutionary events in primates since it transcends species, can be isolated as a pure fraction and remains cytologically discrete despite its evident great age.

Chromosome Staining and Satellite DNA. The various heterochromatic regions of human chromosomes have been shown in many studies (reviewed by Pearson, 1972) to exhibit characteristically different responses to staining methods, such as the variations on Giemsa staining and fluorescent staining. Chromosomes 1, 9, and 16 are particularly interesting in this respect. All show staining of centromeric heterochromatin after the Giemsa C-banding technique (Pardue and Gall, 1970; Arrighi and Hsu, 1971), but only chromosomes 1 and 16 respond positively to the ASG variation of this method (Evans, Buckton, and Sumner, 1971), and, when used at pH 11, Giemsa stains only the heterochromatin of No. 9 (Bobrow, Madan, and Pearson, 1972; Gagné and Laberge, 1972). Staining variations thus correspond to the molecular differences and similarities which characterize the satellite DNAs of these three chromosomes. This correlation was also pointed out in the recent work of Bobrow and Madan (1973) who showed that Giemsa-11 staining of the chromosomes of the chimpanzee gave an intense colour in those heterochromatic regions which cross react with the human satellite III c-RNA (Jones *et al.*, 1973a). On the reasonable assumption that Giemsa is not specific for DNA sequences, these findings suggest that these regions in man and chimpanzee retain features in common other than related satellite DNAs. Pearson (1972) has suggested that acidic chromosomal proteins may be primarily involved in

FIG. 2. Chromosomal location of human satellite DNA II in an individual showing heteromorphism in centric heterochromatin on the No. 1 chromosome pair. There is clearly a difference in the extent of hybridization to the homologues as revealed by the size of the masses of silver grains over the affected regions in this autoradiograph which was obtained by annealing RNA complementary to satellite DNA II. In contrast the No. 16 chromosome pair, which also contains satellite DNA II in centric heterochromatin, has hybridized uniformly. (From Jones and Corneo, 1971, *Nature New Biology*, 27 October 1971, by permission of the Editor.)

such staining differences, but direct evidence for this suggestion is lacking.

The ability to differentially stain satellite-rich constitutive heterochromatin has led in many cases to the observation that it is heteromorphic in normal human populations (for examples see Starkman and Shaw, 1967; Craig-Holmes and Shaw, 1971; Lubs

and Ruddle, 1971). The heteromorphic features are heritable (Craig-Holmes, Moore and Shaw, 1973). Analysis of the relative amounts of satellite II sequences in an individual exhibiting heteromorphic No. 1 chromosomes (Fig. 2) showed a positive correlation between the size of the stainable centromeric heterochromatin and the intensity of

hybridization with RNA complementary to the satellite (Jones and Corneo, 1971). This suggested that heteromorphism is based in some instances on inheritance of different amounts of satellite DNA. Such a suggestion is supported by comparison between the chimpanzee No. 1 chromosome and the human homologue (Turleau and de Grouchy, 1972; Bobrow and Madan, 1973). The chimpanzee chromosome lacks the centromeric heterochromatin which is a prominent feature of the human No. 1 chromosome, and its DNA appears to be devoid of any fraction homologous with satellite II of humans. We might therefore suppose that the evolution of satellite DNA II has accompanied the appearance of the heterochromatin on chromosome No. 1.

Concluding Remarks

The discovery and subsequent study of satellite DNA have done much to advance ideas about the molecular structure of chromosomes but so far little to explain the significance of this DNA itself. The strongest correlates are between satellite DNA, speciation, and constitutive heterochromatin; particularly that near the centromere. It is therefore very probable that some causal connection will eventually be shown to exist between them. Already there are observations which demonstrate a reciprocal relationship between degree of evolutionary specialization and amount of satellite DNA (Mazrimas and Hatch, 1972) and attention has been drawn to possible evolutionary advantages connected with the acquisition of centromeric heterochromatin (Walker, 1972). Documentation of the phenomenology of constitutive heterochromatin is voluminous (for references see Schultz, 1965; Brown, 1966; Baker, 1968) and testifies convincingly regarding its role(s) in controlling a broad range of cellular and developmental activities. The involvement of satellite DNA is probably structural and changes in satellite sequences presumably bear witness to prolific alterations in constitutive heterochromatin in evolution. Such changes have stabilized within species such that each individual of a given species, regardless of global habitat, as far as is known exhibits the same spectrum of satellite DNA components. The conclusion from this must be that alterations in satellite DNA are not permissible except in the context of evolution and speciation. This conclusion suggests that satellite DNA is involved in mechanisms, resident in constitutive heterochromatin, which are essential to the balance of gene expression which we recognize as a species. Further studies of satellite DNA should reveal the nature of the processes involved.

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