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EDITED BY

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ASSISTANT EDITOR
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Functional Aspects of Satellite DNA and Heterochromatin

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"We know so much about the structure, variability and location of satellite DNA, that it is surprising and increasingly significant that we know nothing about the origin and function of these special DNA sequences" (P. M. B. Walker, 1972).

I. Introduction

Although classic genetics considered the eukaryote chromosome simply a linear sequence of linked gene loci, biochemical work has made it clear that many eukaryotes carry far more DNA than appears to be required in terms of this simple model. It is now generally agreed that there is considerably more DNA in the nucleus than is needed to code for all the proteins made by a plant or an animal (see Addendum, note 1). While this is usually regarded as a distinctive feature of eukaryotes, it is worth drawing attention to the fact that bacterial

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genomes, consisting almost entirely of unique DNA, may vary over a 10-fold range (Kingsbury, 1969). In eukaryotes, however, the variation applies both to the unique DNA fraction, not all of which appears to function in a conventional coding sense, and to the repetitive DNA fraction which characterizes most eukaryotes (see Addendum, note 2). It is especially true of the simple-sequence DNA which is highly repeated within a genome. This DNA is sometimes identifiable as a satellite in buoyant density gradients, but it can be cryptic and require the presence of metal ions or antibiotics for its visualization and isolation.

Figure 1 illustrates some of the striking differences which obtain between related species in terms of their satellite components. Three important facts are immediately obvious:

1. In some cases (e.g., the antelope squirrel, *Ammospermophilus harrisi*, and the mouse, *Mus musculus*) each species has its own distinctive satellite or satellites.

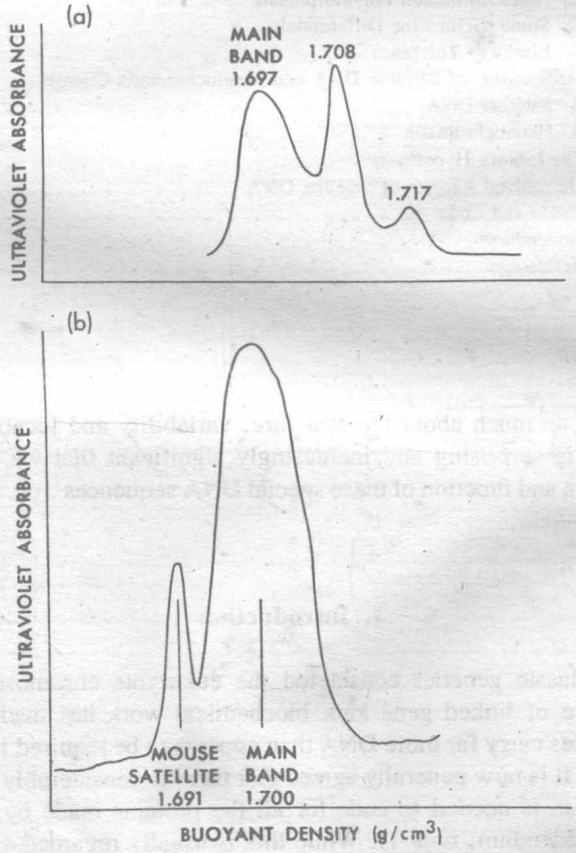


FIG. 1a and b.

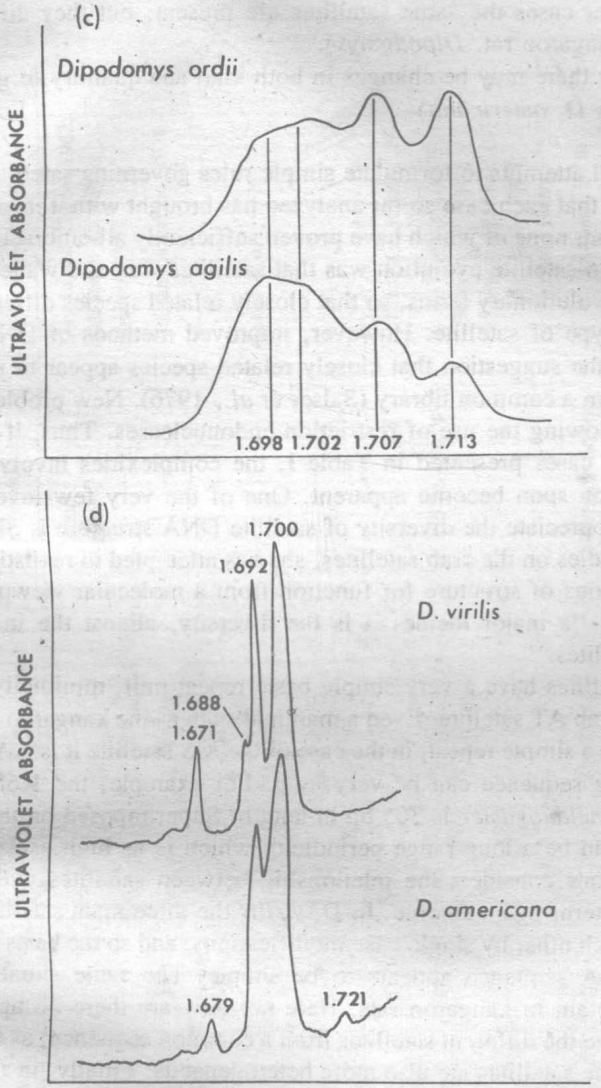


FIG. 1. Buoyant density patterns of DNA preparations centrifuged to equilibrium in neutral cesium chloride. (a) *Ammospermophilus harrisi* (after Mascarello and Mazrimas, 1977); (b) *Mus musculus* (after Walker, 1968); (c) *Dipodomys ordii* and *D. agilis* (after Mazrimas and Hatch, 1972); and (d) *Drosophila virilis* and *D. americana*. (After Gall and Atherton, 1974.)

2. In other cases the same satellites are present, but they differ in amount (e.g., the kangaroo rat, *Dipodomys*).

3. Finally there may be changes in both kind and quantity (e.g., *Drosophila virilis* versus *D. americana*).

Despite all attempts to formulate simple rules governing satellite evolution, it is now clear that each case so far analyzed has brought with it its own claims for generalization, none of which have proven sufficiently all-embracing. One initial hypothesis on satellite evolution was that satellites wax and wane with amazing rapidity in evolutionary terms, so that closely related species differ drastically in amount or type of satellite. However, improved methods of DNA sequencing have led to the suggestion that closely related species appear to modulate their satellites from a common library (Salser *et al.*, 1976). New problems have been revealed following the use of restriction endonucleases. Thus, if one examines some of the cases presented in Table I, the complexities involved in satellite DNA function soon become apparent. One of the very few investigators who appears to appreciate the diversity of satellite DNA structure is Skinner (1977). From her studies on the crab satellites, she has attempted to realistically evaluate the implications of structure for function from a molecular viewpoint. She has stressed that "a major theme . . . is the diversity, almost the individuality of various satellites."

Some satellites have a very simple basic repeat unit, minimally 2 base pairs (bp) in the crab AT satellite. Even a mammal such as the kangaroo rat can have a satellite with a simple repeat; in the case of the MS satellite it is AAG. However, the repeating sequence can be very long. For example, the 1.688 satellite of *Drosophila melanogaster* is 365 bp in length. Superimposed on the short repeat sequences can be a long-range periodicity which is as high as 1408 bp in calf satellite. If one considers the relationship between satellites within a species, different patterns again emerge. In *D. virilis* the three main satellites are clearly related to each other by single base modifications, and so the basis for changes in satellite DNA sequence appears to be simple. The same situation does not, however, obtain in kangaroo rats. Here not only are there no apparent simple rules to derive the different satellites from a common sequence, as there are in *D. virilis*, but the satellites are also more heterogeneous. Finally, in *D. melanogaster*, even though most satellites appear to be related simply to each other, the 1.688 satellite is complex.

In spite of the large amount of information which now exists on the structure of satellite DNA, it is clear that the central issue, namely, *function*, has not been directly tackled. Probably the most important reason for this unsatisfactory state of affairs has been the signal failure to approach the problem of function experimentally, despite the considerable effort that has gone toward elucidating structural properties. In part this refractory state of affairs stems from the assumption

TABLE I

STRUCTURAL PROPERTIES OF SATELLITE DNA

Animal	Satellite	Sequence	Periodicity	Reference
Crab	—	AT	None	Sueoka and Cheng, 1962;
	—	ATCC	None	Skinner *et al.*, 1974
	—	AGTGCAG(CTG)n	—	Skinner, 1977
Fruit fly, *D. virilis*	I	ACAAACT	—	Gall and Atherton, 1974
	II	ATAAACT	—	
	III	ACAAATT	—	
Kangaroo rat	MS	AA G GC A	—	Salser *et al.*, 1976
	HS-α	GG GTTA AAT G	—	
	HS-β	ACACAGC GGG AG G_4 or G_5	[a]	Fry *et al.*, 1973; Marx and Hearst, 1975
Mouse	Mouse satellite	GAAAAATGA and variants	235 bp, 245 bp	E. M. Southern, 1975; Biro *et al.*, 1975; Horz and Zachau, 1977; Maio *et al.*, 1977; Marx and Hearst, 1975
Guinea pig	α = I	GGGTTA and variants	Heterogeneous	E. M. Southern, 1970
	III	—	215 bp	Altenburger *et al.*, 1977; Horz *et al.*, 1974

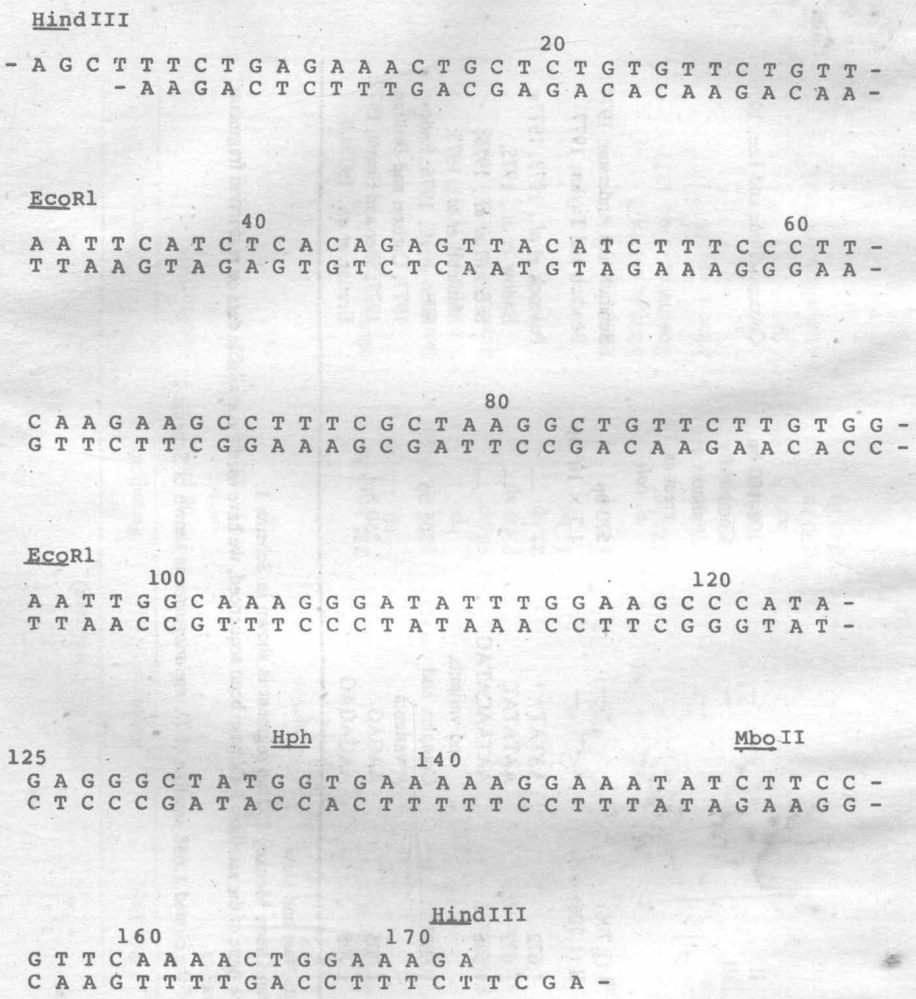
(*continued*)

TABLE I (*continued*)

Animal	Satellite	Sequence	Periodicity	Reference
Field mouse, *Apodemus sylvaticus*, *A. flavicollis*, *A. microps*, *A. agrarius*, *A. mystacinus*			370 bp plus variants, 430, 1850 bp	Cooke, 1975
Sheep	Satellite II		235 bp, 176 bp, 125 bp	Maio *et al.*, 1977
Calf	Satellite I		1400 bp, 1408 bp	Botchan, 1974; Maio *et al.*, 1977
	Satellite II		45 bp	Streeck and Zachau, 1978
	Satellite III		2350 bp	
			22 bp	
			11 bp	
African green monkey	α	—	176 bp	Fittler, 1977; Maio *et al.*, 1977
			172 bp[b]	Rosenberg *et al.*, 1978
Human[c]	A	—	176 bp	Maio *et al.*, 1977
	B	—	352 bp and variants of both	
			170 bp	Manuelidis, 1978a,b
			340 bp	

Drosophila nasuloides	II	—	100–120 bp	Cordeiro-Stone and Lee, 1976
	III	—	Complexity almost as great as *E. coli*	
Muskmelon, *Cucumis melo*	I (1.706)	—	570 bp	Bendich and Anderson, 1974
	II (1.706)	—	1.7×10^6 bp[d]	Bendich and Taylor, 1977
D. melanogaster	1.672	AATAT	—	Peacock *et al.*, 1973, 1977a;
	1.672	AATATAT	—	Endow *et al.*, 1975;
	1.686	AATAACATAG and variants	—	Sederoff *et al.*, 1975; Manteuil *et al.*, 1975;
	1.688[e]	Complex and variants	365 bp	Shen *et al.*, 1976; Endow, 1977; Carlson and Brutlag,
	1.705	AAGAG	250 bp[f]	1977; Shen and Hearst, 1977;
	1.705	AAGAGAG		Brutlag *et al.*, 1977a,b

[a] Two renaturing components, fast and slow.
[b] The sequence of the African Green Monkey 172 bp segment is shown in Scheme 1.
[c] In the case of humans, since none of the satellites I–IV have been sequenced, we have used the available data on restriction fragments.
[d] Based on reassociation kinetics.
[e] The sequence variations of the cloned 1.688 satellite of *D. melanogaster* is shown in Scheme 2.
[f] Based on trioxalen binding.



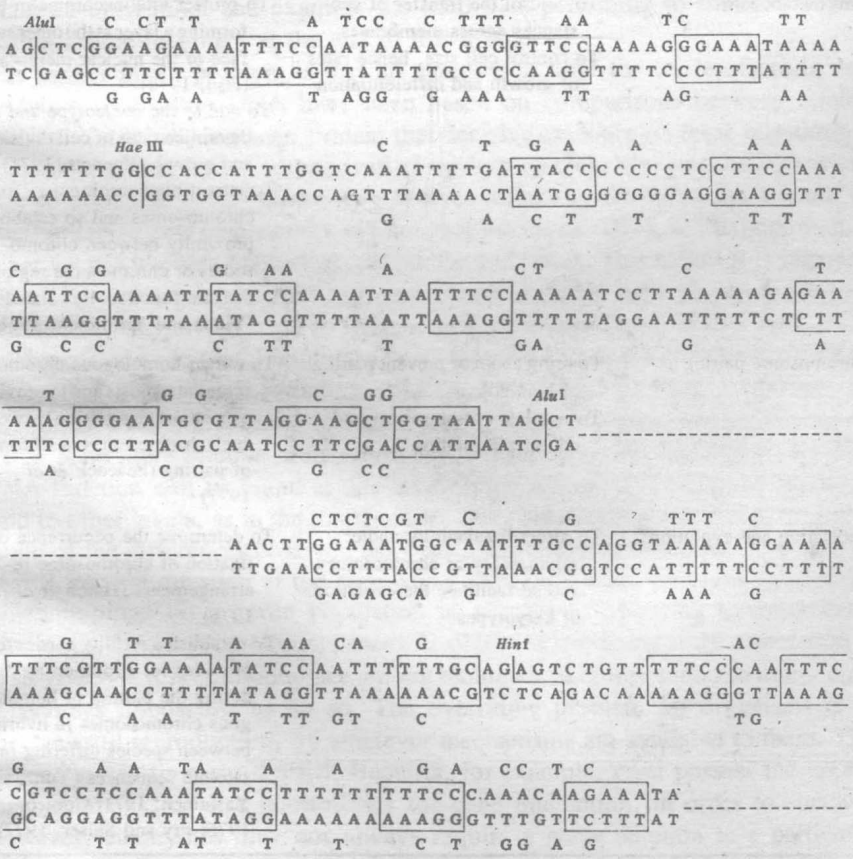
SCHEME 1. The nucleotide sequence of a population of uncloned 172 bp *Hind* III segments of the African Green Monkey (*Cercopithecus aethiops*). (From Rosenberg *et al.*, 1978.)

that a knowledge of function necessarily follows from a knowledge of structure. In part too it is explained by the fact that the properties of satellite DNA have been evaluated within the framework of prokaryotic dogma without sufficient consideration of the higher-order phenomena which characterize the biology of eukaryotes.

It appears very obvious that we have now reached a stage in satellite DNA research where additional structural analyses are not revealing the nature of its

function—and indeed there is a very good reason for this. The initial success of the prokaryotic approach to genetic function was due to its manipulative aspects. This approach, involving perturbation of a system by mutation, deletion, substitution and translocation, proved critical. Only recently has a similar approach been applied specifically in investigating satellite DNA function, although an enormous literature exists on experimental and natural modifications of heterochromatin, which bear directly on this issue.

In the absence of experimental evidence the problem has in general been discussed in terms largely modified from earlier theoretical considerations relating to the functions of heterochromatin. A summary of the comparisons of heterochromatin and satellite DNA functions is presented in Table II. As can be seen from this table, the assumption has generally been made that there is at least one positive function. However, since similar organisms have widely different



SCHEME 2. Sequence variations in cloned 1.688 satellite DNA. (From Brutlag, 1977b.)

TABLE II

A COMPARISON OF THE SUGGESTED FUNCTIONS OF HETEROCHROMATIN AND SATELLITE DNA

Type of function	Heterochromatin[a]	Satellite DNA
Chromosome organization	To stabilize centromeres or telomeres	To protect vital chromosome organelles such as centromeres and nucleolus organizers (Yunis and Yasmineh, 1971) To alter the properties of the centromere or to stabilize chromosome ends (Walker, 1972) To specify folding patterns of chromosomes (Walker, 1972)
Cell metabolism	To control the transfer of substances across membranes To control cell size, hence rates of growth and differentiation	To protect vital euchromatin by forming a layer at the outer surface of the nuclear membrane (Hsu, 1975) To add to the nucleotype and so determine rates of cell division and growth (Bennett, 1971) To attract nonhomologous chromosomes and so establish proximity between chromosomes or chromosome regions that are functionally related (Yunis and Yasmineh, 1971)
Chromosome pairing	To bring about or prevent pairing of homologs To regulate crossing-over and chiasma formation	To attract homologous chromosomes at meiosis and to provide a means of recognition between such chromosomes in all forms of pairing (Peacock *et al.*, 1977)
Speciation and evolution	To affect breakability and/or rejoinability of chromosomes and so facilitate the evolution of karyotypes	To determine the occurrence or fixation of chromosome rearrangements (Hatch *et al.*, 1976) To establish a fertility barrier that provides for evolution by hindering the pairing of homologous chromosomes in hybrids between species differing in satellite sequences (Yunis and Yasmineh, 1971; Corneo, 1976; Fry and Salser, 1977)

[a] Summarized by Cooper (1959).