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DNA Sequences in Man

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I. Introduction 35
II. Analysis of the Human Genome by Density Gradient Centrifugation 36
A. The Experimental Approach 37
B. Results 39
C. Analysis of Human DNA Degraded by Restriction Enzymes. . 42
III. Human Satellite DNA's 45
A. Satellite I 48
B. Satellite II 49
C. Satellite III 49
D. Satellite IV 50
E. Satellite A 50
F. Satellite B 50
G. Satellites C and D 51
IV. *In Situ* Hybridization 52
A. Satellite DNA 52
B. Repetitive DNA 53
V. Renaturation Properties of Human DNA 53
A. Total DNA 53
B. Satellite DNA 55
References 55

I. INTRODUCTION

The organization of the eukaryotic genome is one of the major problems in molecular biology today. While our understanding of some specific facets, such as

the organization of repetitive genes (e.g. the ribosomal genes) or the nucleotide sequences of some satellite* DNA's, can be considered satisfactory, we are still far from having a clear picture of the overall problem. This also applies to the eukaryotic genome of greatest interest to us, the human genome. In fact, surprising as it may seem, we know even less about it than about some other eukaryotic genomes. The literature on the organization of the human genome is scanty and complex, if not contradictory. We have, in this article, attempted to give a detailed presentation of some specific issues, such as human satellites.

II. ANALYSIS OF THE HUMAN GENOME BY DENSITY GRADIENT CENTRIFUGATION

Since the classic work of Meselson *et al.* (1957), it has become well known that when centrifuged in CsCl density gradients, bacterial DNA's show unimodal, symmetrical bands, whereas calf thymus DNA exhibits a multimodal, asymmetrical band. A number of theoretical and experimental investigations using density-gradient equilibrium centrifugation have dealt with uniform populations of DNA molecules such as phage DNA's (Schmid and Hearst, 1969, 1971, 1972). Early attempts to study the much more complex animal DNA's (Sueoka, 1959, 1961, 1962) have not been followed up.

Research in this field was stimulated by the work of Filipski *et al.* (1973) that showed that the CsCl main band of calf DNA, MW = $5–7 \times 10^6$, was not formed by genome fragments having a continuous distribution of their average G + C content, as was commonly believed, but by three distinct families of fragments. These were identified by their buoyant densities in CsCl and were equal to 1.697, 1.704, and 1.709 gm/cm^3, representing about 50, 25, and 10% of the DNA, respectively. These DNA components could be separated from one another by preparative centrifugation in Cs_2SO_4-Ag^+ density gradients. They were characterized by symmetrical bands that exhibited standard deviations close to those of bacterial DNA's of comparable molecular weight. These components were responsible for the asymmetry of the CsCl main band of calf DNA.

Three important questions raised by the existence of the components of main band DNA from calf concerned their evolutionary significance, their relative arrangement in the genome, and their intramolecular heterogeneity. These problems were investigated by Thiery *et al.* (1976) and by Macaya *et al.* (1976). These authors investigated the DNA's from twenty-five eukaryotes by density gradient centrifugation; a smaller number of DNA's were studied after degradation with restriction enzymes. For the sake of clarity, a brief outline of the experimental approach used will be presented first.

*For the sake of convenience, we will call the simple-sequence or highly repetitive DNA's satellite DNA's.

A. The Experimental Approach

The approach involved the following steps:

1. DNA's, characterized by their sedimentation properties and G + C contents, were centrifuged to equilibrium in CsCl, and their modal and mean buoyant densities, ρ_0 and $\langle\rho\rangle$, were determined. The buoyant density, ρ, at any distance r from the rotation axis, can be calculated using Eq. (1),

$$\rho = \rho_\kappa - \frac{\omega^2}{2\beta_0}(r_\kappa^2 - r^2) \quad (1)$$

where the subscript κ refers to a marker DNA, ω is the angular velocity in radians sec^{-1}, and β_0 is taken as equal to 1.19×10^9 $cm^5 gm^{-1} sec^{-2}$ (Ifft *et al.*, 1961). Under such conditions, using phage 2C DNA (ρ = 1.742 gm/cm^3; Szybalski, 1968) as a density marker, a reproducible modal buoyant density, ρ_0 (density at the peak maximum, located at a distance r_0 from the rotation axis), of 1.7103 gm/cm^3 is obtained for *Escherichia coli* DNA.

The mean buoyant density, $\langle\rho\rangle$, is calculated from the first moment of the band profile about the center of rotation [Eq. (2)],

$$\langle r\rangle = \frac{\int_0^\infty cr\,dr}{\int_0^\infty c\,dr} \quad (2)$$

and from Eq. (1), c being the DNA concentration at point of abscissa r.

2. DNA's were fractionated by preparative Cs_2SO_4-Ag^+ density gradient centrifugation. Owing to differential binding of Ag^+ ions, such a procedure can resolve not only a number of apparent and cryptic satellite DNA's (Corneo *et al.*, 1968b), but also the discrete components forming the main band of calf thymus DNA (Filipski *et al.*, 1973).

Since the resolution of DNA components in Cs_2SO_4-Ag^+ density gradient centrifugation depends on pH and r ratio (the molar Ag^+/DNA-P ratio), preliminary experiments were done, usually in the analytical ultracentrifuge, to assess the influence of these two parameters on the band profiles of the DNA's investigated. The ranges explored were 7.8–10.3 for pH and 0.2–0.4 for the r ratio. Figure 1 shows the profiles of human placenta DNA obtained in the analytical ultracentrifuge in Cs_2SO_4-Ag^+ density gradients at two pH's. Since Ag^+ has an overall preferential affinity for GC base pairs at pH <8 and for AT base pairs at pH >8, the skewness of the profiles in Fig. 1 changes between pH 7.8 and pH 9.2. At pH 7.8, the G + C-rich molecules will be heavier than the A + T-rich ones, due to preferential Ag^+ binding (Jensen and Davidson, 1966);

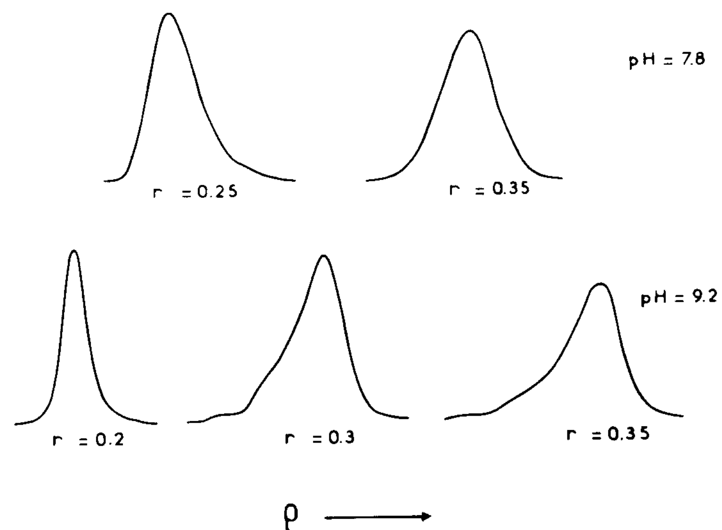

Fig. 1. Human placenta DNA in Cs_2SO_4-Ag^+ analytical density gradients; 0.04 A_{260} units of DNA in 0.005 *M* borate buffer were centrifuged, at the pH and *r* ratio indicated, for 24 hours at 44,000 rpm and 25°C in a Beckman Model E analytical ultracentrifuge, equipped with a photoelectric scanner, multiplexer, and mirror optics. The bands are shown in an arbitrary density scale, only for band profile comparison.

thus the skewness of the peak is toward high buoyant densities. At pH 9.2, the skewness is toward low buoyant densities, due to preferential Ag^+ binding by A + T-rich molecules. At pH 9.2 and r = 0.30 and 0.35 (Fig. 1), two satellites are apparent on the light side of the profile. These satellites will be discussed later (see Fig. 2, fractions 10–13) in preparative Cs_2SO_4-Ag^+ density gradients. Experimental conditions (pH, r, slope of the Cs_2SO_4 density gradient, load) giving the maximum spread and skewness of the main band were chosen; for human placenta DNA, they were pH 9.2 and r = 0.30–0.35.

After equilibrium centrifugation in preparative Cs_2SO_4-Ag^+ density gradients, the 15–25 fractions obtained from total DNA were centrifuged to equilibrium in analytical CsCl density gradients and analyzed. The band profiles were resolved into a number of Gaussian curves and the corresponding DNA components were defined in terms of their buoyant densities and relative amounts in the DNA's analyzed. Resolution of the CsCl profiles was done on a DuPont de Nemours (Wilmington, Delaware) curve resolver model 310. The criteria used to obtain such resolution are given elsewhere (Thiery *et al.*, 1976).

3. On the basis of the experience acquired in step (2), direct analysis of the CsCl band profiles of unfractionated DNA was attempted, and the results satisfactorily agreed with those obtained in the more detailed investigations involving preliminary Cs_2SO_4-Ag^+ centrifugation.

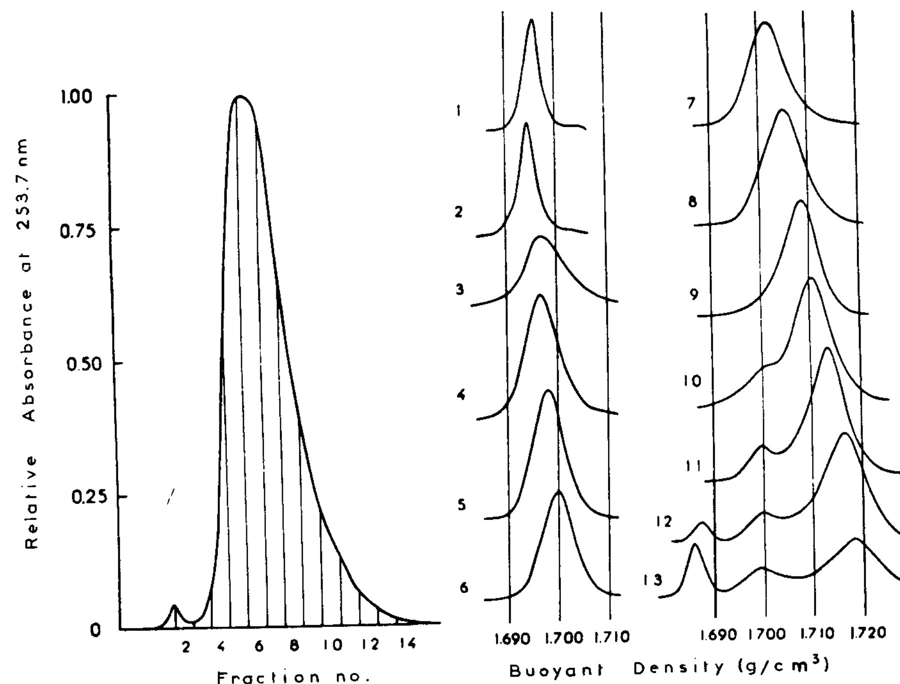

Fig. 2. Analysis of human placenta DNA components in preparative Cs_2SO_4-Ag^+ density gradient. DNA (A_{260} = 0.86) in 0.005 *M* borate buffer pH 9.2, containing $AgNO_3$ (r = 0.32) and Cs_2SO_4 (ρ_i = 1.48 gm/cm^3), was centrifuged in a Beckman type 30 rotor at 25,000 rpm for 90 hours at 25°C. Fractions were analyzed in analytical CsCl gradients.

B. Results

1. Buoyant Density and Symmetry of the CsCl Band

The modal and mean buoyant densities in CsCl, ρ_0 and $\langle\rho\rangle$ for human DNA, G + C = 40.3%, are given in Table I. Table I shows the buoyant density values for Hind II + III degraded DNA (see Section II,C). The large difference between $\langle\rho\rangle$ and ρ_0, 0.002 gm/cm^3, is due to the asymmetry of the peak. Such asymmetry was found in all mammalian DNA's studied by Thiery *et al.* (1976). The buoyant density differences between the DNA preparations from leukocytes and placenta are not significant.

2. Combined Cs_2SO_4-Ag^+ and CsCl Density Gradient Analysis

The greatest amount of information one can obtain from density gradient equilibrium sedimentation is derived from the combined Cs_2SO_4-Ag^+, CsCl density gradient analysis described in Section II,A,2.

TABLE I
Properties of Human DNA's before and after Degradation by the Restriction Endonucleases Hind II + III[a]

Tissue	$s_{20,w}$	ρ_0 [b]	$\langle\rho\rangle$	$\langle\rho\rangle - \rho_0$ [c]
Leukocytes, undegraded	25.0	1.6985	1.7008	2.3
Placenta				
Undegraded	22.3	1.6990	1.7010	2.0
Degraded	13.0	1.6995	1.7011	1.6

[a]Samples were degraded with a mixture of the restriction endonucleases II and III from *Haemophilus influenzae*, Hind II + III, prepared according to Kopecka (1975), at 37°C and at an enzyme concentration of 0.01 unit/50 μg DNA (units as defined by Smith and Wilcox, 1970).

[b]The error on the ρ_0 values is ±0.0005 gm/cm³; that on the $\langle\rho\rangle$ values is ±0.0002 gm/cm³.

[c]Values in mg/cm³.

Human DNA can be resolved (Table II; Fig. 2) into three major components, four minor components and three satellites, the major components each forming more than 10% of the DNA, and the minor components each forming up to 3% of the DNA. The satellites characteristically show hypersharp peaks in CsCl density gradients, a feature due not only to their well-known degree of homogeneity, but also to concatenation phenomena (Macaya *et al.*, 1976).

The three major components of human placenta DNA consist of (1) a main component forming 65% of the DNA; this component is formed by two subcomponents, banding at 1.697 and 1.699 gm/cm³ and forming 16% and 49% of the genome, respectively; (2) two major components banding at 1.704 and 1.709 gm/cm³ and forming 18 and 11% of the DNA, respectively.

The three major components have been detected, by the same procedure, in the DNA's of three more mammals: calf, guinea pig, and mouse. The buoyant

TABLE II
Component Pattern of Human Placenta DNA[a]

Components				Satellites	
Major		Minor			
ρ	%	ρ	%	ρ	%
1.697	16	1.712	0.7	1.6875	0.15
1.699	49	1.7135	1.5	1.6955	1.2
1.704	18	1.716	1.3	1.6995	0.5
1.709	11	1.718	0.3		

[a]Combined Cs_2SO_4-Ag^+ and CsCl analysis.

density and the relative amount of the subcomponents of the main component of mammalian genome showed species- and tissue-specific differences (Thiery *et al.*, 1976), a finding justifying the use of the term subcomponents. In contrast, the 1.704 and 1.709 gm/cm³ components did not show any variation in density and only very slight differences in concentration in other mammalian DNA's (Thiery *et al.*, 1976).

Concerning the minor components of human DNA (1.712, 1.7135, 1.716, 1.718 gm/cm³), it is possible that one of these components corresponds to ribosomal DNA. It is difficult, however, to decide whether rDNA corresponds to the 1.718 gm/cm³ component, as would be suggested by the report (Sinclair and Brown, 1971) that rRNA hybridizes at 1.719 gm/cm³, or to the 1.712 gm/cm³ component, as suggested by the fact that DNA enriched in nucleolar material bands at 1.713 gm/cm³ (Schildkraut and Maio, 1969) or at 1.710 gm/cm³ (Chuang and Saunders, 1974). Nothing that could be identified as mitochondrial DNA (1.707 gm/cm³, Corneo *et al.*, 1968a) could be detected. This could be due to the very small amount of this DNA and to its behavior in the gradient. Neither have we observed the DNA components banding at 1.703 and 1.726 gm/cm³ reported by Saunders *et al.* (1975), although the 1.720 gm/cm³ component of these authors might correspond to our 1.718 gm/cm³ component.

So far as satellites are concerned, only three were found: the 1.6875 gm/cm³ (Fig. 2, fractions 12,13), 1.6955 gm/cm³ (Fig. 2, fractions 1,2), and the 1.6995

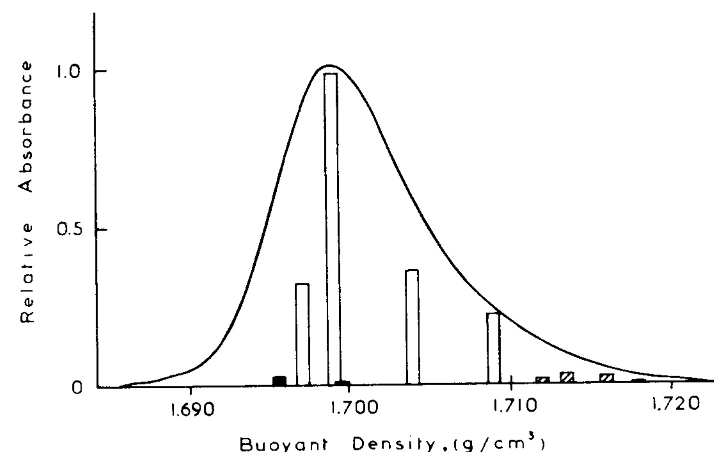

Fig. 3. A histogram of the DNA components of the human genome. The height of the bars is proportional to the percentage of each component, their width is arbitrary; empty bars correspond to main components, hatched and solid bars correspond to minor and "satellite" components, respectively. The CsCl band profile of this DNA is shown superimposed on the histogram.

gm/cm^3 (Fig. 2, fractions 10–13). A detailed discussion of human satellite DNA's will be given in Section III.

A histogram of the DNA components of the human genome, superimposed on the CsCl profile of this DNA is shown in Fig. 3.

3. *CsCl Analysis*

Thiery *et al.* (1976) have shown that direct resolution of the DNA components analyzed above was possible with fairly satisfactory precision, so far as buoyant densities and relative amounts of DNA are concerned, by using the CsCl profiles of unfractionated DNA. This approach is evidently much less laborious than the Cs_2SO_4-Ag^+ density gradient fractionation followed by CsCl band analysis of the fractions.

The results obtained with two different preparations of human DNA, from placenta and leukocytes, are shown in Table III; Fig. 4 shows the CsCl analysis of placenta DNA. The component pattern obtained for placenta DNA is in good agreement with that obtained by the more elaborate analysis presented in Section II,B,2, with the expected exception of minor and satellite components. An interesting finding is that different relative amounts of the two subcomponents were observed in DNA preparations from two different tissues; in contrast, the 1.704 and 1.709 gm/cm^3 are present in equivalent amounts in the DNA from both sources.

C. Analysis of Human DNA Degraded by Restriction Enzymes

Since all observations mentioned so far concern DNA's having molecular weights in the 9–12 × 10^6 range, two important questions raised by the existence of discrete components in the main band of human DNA are (1) Up to what molecular weight could discrete components be seen, or at which DNA fragment size did the components begin to intersperse with one another? (2) The discrete components isolated at the 9–12 × 10^6 molecular weight level exhibited CsCl bands which were at least as symmetrical as bacterial DNA's of comparable molecular weights, yet they evidently had some degree of intramolecular compositional heterogeneity; at what molecular weight could this intramolecular heterogeneity be transformed into an intermolecular heterogeneity? Quite clearly both questions can be seen as a single one, namely: In what molecular size range do the discrete components exist? We have attempted to answer these questions by investigating, in terms of buoyant densities and relative component amounts, human DNA degraded to a molecular weight of 2 × 10^6 by the restriction enzymes from *Haemophilus influenzae* Hind II and III.

TABLE III
Component Patterns of Human DNA's[a]

DNA source	Major components		Minor components and satellites	
	ρ	%	ρ	%
Leukocytes	1.697	27	1.693	3
	1.699	39	1.713	2
	1.704	19		
	1.709	9		
Placenta	1.697	13	1.692	3
	1.699	52	1.716	1
	1.704	19		
	1.709	11		

[a] CsCl analysis.

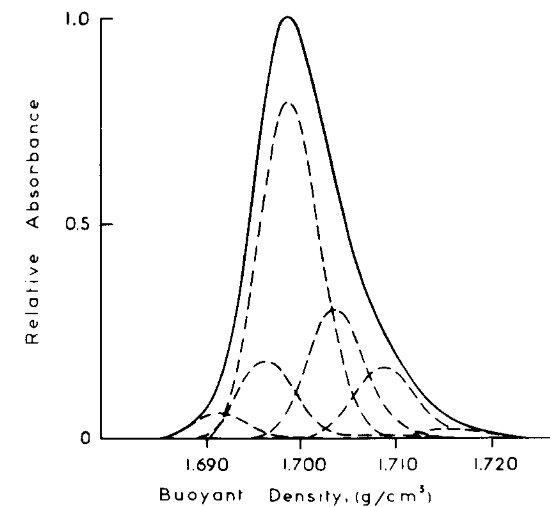

Fig. 4. CsCl analysis of human placenta DNA; 0.05 A_{260} units of DNA in 0.01 *M* Tris, 0.005 *M* NaCl pH 7.6 were centrifuged for 24 hours at 44,000 rpm and 25°C in the analytical ultracentrifuge. The optical density profile so obtained was analyzed in terms of component curves using a DuPont 310 curve resolver.

1. *Buoyant Density and Symmetry of the CsCl Band*

Table I shows the sedimentation coefficient, modal and mean buoyant densities in CsCl, ρ_0 and $\langle\rho\rangle$, and the $\langle\rho\rangle - \rho_0$ values for Hind II + III degraded human placenta DNA. The ρ_0 values of undegraded and degraded samples are exactly the same within accepted experimental error; the $\langle\rho\rangle - \rho_0$ values reflects the error in ρ_0.

2. *Fractionation in Preparative Cs_2SO_4-Ag^+ Density Gradients and CsCl Analysis of the Fractions*

Figure 5 shows the results obtained for human placenta DNA; the component pattern is shown in Table IV. A comparison of the data in this table with those in Table II shows that the major components of the degraded DNA were similar, in both buoyant density and relative amounts, to those of the undegraded samples; slight but significant density shifts were found for the 1.697, 1.704, and 1.709 gm/cm^3 components.

So far as minor components are concerned, the 1.716 gm/cm^3 component does not seem to change in buoyant density and relative amount. The 1.712, 1.7135, and 1.718 gm/cm^3 components cannot be recognized anymore, and a new minor component, 1.695 gm/cm^3, appears after degradation.

As for the satellite DNA's, two of them were not degraded or only very

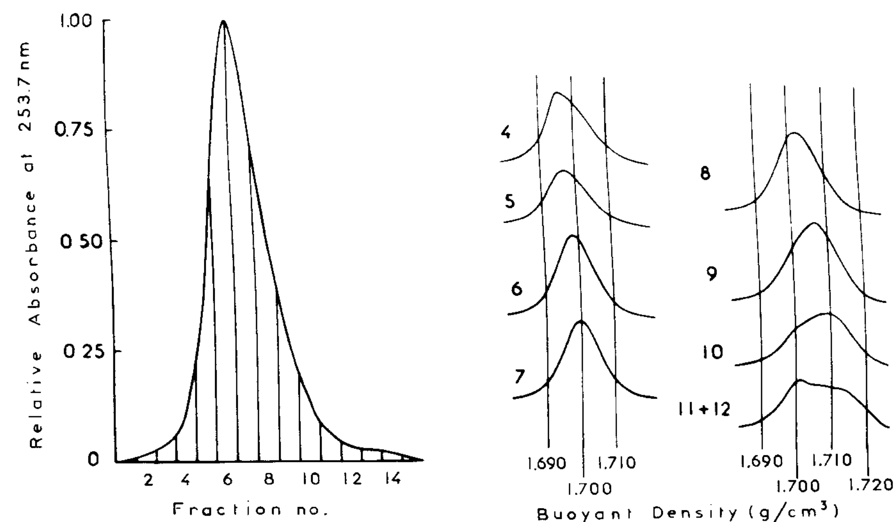

Fig. 5. Analysis of human placenta DNA components in preparative Cs_2SO_4-Ag^+ density gradient after degradation by Hind II + III. DNA (A_{260} = 0.49) in 0.005 *M* borate buffer pH = 9.2, containing $AgNO_3$ (r = 0.32) and Cs_2SO_4 (ρ_i = 1.50 gm/cm^3), was centrifuged in a Beckman type 65 rotor at 35,000 rpm for 64 hours at 25°C.

TABLE IV
Component Pattern of Human Placenta DNA Degraded by the Restriction Enzymes Hind II + III[a]

Components					
Major		Minor		Satellites	
ρ	%	ρ	%	ρ	%
1.6965	16	1.695	2.5	1.695	1.2
1.699	46	1.716	1	1.700	0.5
1.7045	19				
1.710	9				

[a]Combined Cs_2SO_4-Ag^+ and CsCl analysis.

partially degraded (1.695 and 1.700 gm/cm^3, Fig. 5, fractions 4 and 11 + 12) as shown by the appearance of the corresponding bands; the 1.6875 gm/cm^3 satellite was probably degraded and was difficult to recognize as a satellite in the digested DNA (Fig. 5, fractions 11 + 12, compared with Fig. 2, fractions 12 and 13).

In conclusion, the major components of human DNA (as well as those of the other mammalian DNA's investigated by Macaya *et al.*, 1976) did not show major changes in relative amounts or buoyant density when the molecular weight of the DNA preparation under study was lowered from 9×10^6 to 2×10^6. Experiments on higher molecular weight human DNA preparations were not done. In the case of mouse DNA, however, preparations having molecular weights in excess of 200×10^6 showed CsCl band profiles exhibiting maxima corresponding to the buoyant densities of the major components (Macaya *et al.*, 1976).

III. HUMAN SATELLITE DNA's

Eight satellite DNA's have been reported in human DNA: four, named chronologically, I, II, III and IV by Corneo *et al.* (1967, 1970, 1971, 1972) and four named A, B, C and D by Chuang and Saunders (1974) and Saunders *et al.* (1972b, 1975).

Table V shows some of the properties reported for these satellite DNA's. The situation now seems so complex that a detailed discussion on each satellite will be given separately, but some general comments appear to be useful at this point.

Not all of the satellites reported to date were isolated by the same procedure and from the same DNA sample. Usually three different methods were used:

TABLE V
Properties of Human DNA Satellites

Satellite	ρNeut. CsCl	ρAlk. CsCl	ρRean. CsCl	% Total	Separation properties	% G + C	T_m	References[d]
I	1.687	1.707 1.738	1.697	0.5	Heavy side Cs_2SO_4-Hg^{2+}, $r = 0.1$, pH = 9.2	26.1[a] 27.5[b]	80°C in 1× SSC	1,2
					Light side Cs_2SO_4-Ag^+, $r = 0.2$, pH = 9.2, separated at $r < 0.2$			3
					Late eluted from MAK; Light side Cs_2SO_4-Ag^+, $r = 0.23$, pH = 9.2			4
	1.686	1.731 1.764		0.2		26.4[c]		5
	1.687	1.732 1.765						6
II	1.693	1.740 1.750	1.696	2.0	Heavy side Cs_2SO_4-Ag^+, $r = 0.35$, pH = 9.2	43.2[a] 33.7[b]	87°C in 1× SSC	7
					Heavy side Cs_2SO_4-Ag^+, $r = 0.20$, pH = 9.2; separation increases with r, not separated at $r < 0.2$; Early eluted from MAK			3 4
	1.695					34[b]		6
III	1.696	1.740 1.754	1.703		Light side Cs_2SO_4-Ag^+, $r = 0.2$, pH = 9.2; separated at $r < 0.2$.	35.9[a] 36.7[b]	84°C in 1× SSC	3
				1.5	Late eluted from MAK; light side Cs_2SO_4-Ag^+, $r = 0.23$, pH = 9.2			4
				2–3	Light side Cs_2SO_4-Hg^{2+}, $r = 0.1$, pH = 9.2			8
	1.699	1.759 1.772						6
IV	1.700	1.730 1.742	1.706	2	Light side Cs_2SO_4-Ag^+, $r = 0.23$, pH = 9.2; early eluted from MAK	41[b]		4
A	1.710	1.775	1.715	0.5–1	Light side Cs_2SO_4-Ag^+, $r > 0.2$, pH = 9.2; separation increases with r	52[a] 51[b]	88.2°C in 0.18 M Na^+	9
	1.712					53.8[c]		5
B	1.726				Light side Cs_2SO_4-Ag^+, pH = 9.2; lighter than A			9
	1.726	1.792		<0.5	(Ribosomal genes)	67[b]		10
C	1.703	1.760	1.712		Light side Cs_2SO_4-Ag^+, $r = 0.27$, pH = 9.2	44[b]	72.9°C in 0.025 M Tris-HCl	11
D	1.720	1.782				61[b]		6
"1.687"	1.6875			0.15	Light side Cs_2SO_4-Ag^+, $r = 0.32$, pH = 9.2			12
"1.695"	1.6955			1.2	Heavy side Cs_2SO_4-Ag^+, $r = 0.32$, pH = 9.2			12
"1.700"	1.6995			0.5	Light side Cs_2SO_4-Ag^+, $r = 0.32$, pH = 9.2			12

[a] Base composition calculated from the melting temperature (T_m) according to Marmur and Doty (1962).
[b] Base composition calculated from the buoyant density (ρ) according to Schildkraut *et al.* (1962).
[c] Base composition measured by direct chemical analysis.
[d] References:

1. Corneo *et al.* (1967).
2. Corneo *et al.* (1968a).
3. Corneo *et al.* (1971).
4. Corneo *et al.* (1972).
5. Schildkraut and Maio (1969).
6. Saunders *et al.* (1975).
7. Corneo *et al.* (1970).
8. Mitchell (1974).
9. Chuang and Saunders (1974).
10. Chuang (1974).
11. Saunders *et al.* (1972a).
12. Thiery *et al.* (1976).

(1) CsCl density gradient centrifugation (Corneo *et al.*, 1967; Schildkraut and Maio, 1969); (2) Cs_2SO_4-Ag^+ density gradient centrifugation (Corneo *et al.*, 1970, 1971, 1972; Chuang, 1974; Chuang and Saunders, 1974; Gosden and Mitchell, 1975; Saunders *et al.*, 1975); and (3) Cs_2SO_4-Hg^{2+} density gradient centrifugation (Corneo *et al.*, 1967, 1970). These were used on total or on previously fractionated DNA via nucleoli preparations (Chuang, 1974; Schildkraut and Maio, 1969); chromatin fractions (Corneo *et al.*, 1971; Gosden and Mitchell, 1975); MAK column chromatography fractions (Corneo *et al.*, 1972); and "Cot fractions" (Hearst *et al.*, 1973; Saunders *et al.*, 1972b).

The general properties of the different satellites are given in Table V. In the following discussion, we have tried to collate the results and compare them with our own. Basically, a "satellite" DNA is characterized according to its density in neutral CsCl, in alkaline CsCl where strand separation can be analyzed, and in neutral CsCl after denaturation and renaturation under defined conditions. Base composition, when measured analytically, will be useful in this characterization, particularly when compared with values calculated from the melting temperature (T_m) or from the buoyant density. Intramolecular heterogeneity of the isolated satellites can be analyzed by thermal denaturation and reassociation analysis.

A. Satellite I

First isolated by Corneo *et al.* (1967), this satellite has been found by several other authors (Saunders *et al.*, 1975; Schildkraut and Maio, 1969; Thiery *et al.*, 1976). It exhibits a buoyant density of 1.687 gm/cm^3 in neutral CsCl and strand separation in alkaline CsCl; the density difference between the two strands is about 33 mg/cm^3. The absolute ρ values of the strands in alkaline CsCl as obtained by different authors are difficult to compare because of differences in the methods used to calculate the buoyant density in these experiments. This satellite is found on the heavy side of the main band in a Cs_2SO_4-Hg^{2+} density gradient, at pH 9.2 and r = 0.1 (Corneo *et al.*, 1967, 1968a); in Cs_2SO_4-Ag^+ density gradients it is found on the light side of the main band, at pH 9.2, being well separated at $r < 0.2$ (Corneo *et al.*, 1971). In agreement with these results, we found this satellite on the light side of the main band in a Cs_2SO_4-Ag^+ density gradient at pH 9.2 and r = 0.32 (Fig. 2, fractions 12 and 13). According to our data, this satellite accounts for 0.15% of the genome, a value lower than the 0.5% reported by Corneo *et al.* (1967, 1968a, 1971, 1972), but of the same order as that of 0.2% given by Schildkraut and Maio (1969) or 0.15–0.175% calculated from hybridization experiments by Jones *et al.* (1974) and Moar *et al.* (1975). Base compositions calculated from ρ_0 and T_m are in good agreement with the analytical value of 26.4% G + C content found by Schildkraut and Maio (1969).

B. Satellite II

This satellite DNA, first found by Corneo *et al.* (1970), has a density in neutral CsCl of 1.693 gm/cm^3 and shows strand separation in alkaline CsCl; the density difference between the strands is 10 mg/cm^3. This satellite, found on the heavy side of the main band in Cs_2SO_4-Ag^+ density gradients at pH 9.2, is separated at $r > 0.2$, the separation increasing with r (Corneo *et al.*, 1970, 1971). Corneo *et al.* (1971) reported a T_m of 87°C in 1X SSC, but Moar *et al.* (1975) report a value of 84°C in the same solvent. G + C contents of 43.2% and 35.9% can be calculated from T_m values of 87°C and 84°C, respectively, using the relationship of Marmur and Doty (1962); a G + C content of 33.7% can be calculated from ρ = 1.693 gm/cm^3, using the relationship of Schildkraut *et al.* (1962). In the analysis of human placenta DNA using the combined Cs_2SO_4-Ag^+ and CsCl method, we did not find, on either side of the main band, a satellite with a density of 1.693 gm/cm^3. On the heavy side of the main band, at pH = 9.2 and r = 0.32, we found a satellite DNA similar in that respect to satellite II, but with a buoyant density of 1.6955 gm/cm^3 in neutral CsCl. This density value is very close to that reported by Corneo *et al.* (1971) for their satellite III (see Section III,C). Saunders *et al.* (1975) found a density of 1.695 gm/cm^3 for human satellite II. It is thus very difficult with the data available to decide if our 1.6955 gm/cm^3 satellite is equivalent to satellite II. Corneo *et al.* (1970) gave as 2% the amount of this satellite in the genome, but by hybridization experiments Moar *et al.* (1975) found a value of 0.7%; we found 1.2% for our 1.6955 gm/cm^3 satellite in human placenta DNA.

C. Satellite III

First described by Corneo *et al.* (1971), this satellite has a buoyant density in neutral CsCl of 1.696 gm/cm^3; the two strands separate in alkaline CsCl with a buoyant density difference of 14 mg/cm^3 (Corneo *et al.*, 1971). This satellite is found on the light side of the main band in a Cs_2SO_4-Ag^+ density gradient at pH 9.2 and r = 0.2 (Corneo *et al.*, 1971, 1972). Saunders *et al.* (1975) found a buoyant density of 1.699 gm/cm^3 for what they called satellite III, and a difference in buoyant density of the separated strands in alkaline CsCl of 13 mg/cm^3. In this respect, these data are closer to those for satellite IV (see Section III,D). Our 1.6955 gm/cm^3 satellite DNA, even if it is found on the opposite side of the main band in a Cs_2SO_4-Ag^+ density gradient with respect to satellite III, has a buoyant density and amount (1.2%) closer to that reported for satellite III (1.5% Corneo *et al.*, 1972). Moar *et al.* (1975) found that satellite III accounts for 0.85% of the genome using hybridization experiments. Base compo-

sitions calculated from T_m (84°C, Corneo *et al.*, 1971) and ρ are in good agreement, 35.9% and 36.7% G + C, respectively (Marmur and Doty, 1962; Schildkraut *et al.*, 1962).

D. Satellite IV

Found by Corneo *et al.* (1972) after MAK fractionation of human DNA, satellite IV has a buoyant density of 1.700 gm/cm^3 in neutral CsCl and exhibits strand separation in alkaline CsCl, with a density difference of 12 mg/cm^3. It accounts for 2% of the genome (Corneo *et al.*, 1972). Our 1.6995 gm/cm^3 satellite DNA is present in a lower amount, 0.5%, but, as for satellite IV (Corneo *et al.*, 1972), it is found on the light side of the main band in a Cs_2SO_4-Ag^+ density gradient at pH 9.2 and $r = 0.32$ (see Fig. 2, fractions 10–13).

E. Satellite A

By CsCl density centrifugation of HeLa nucleolar DNA, Schildkraut and Maio (1969) fractionated a component having a buoyant density in neutral CsCl of 1.712 gm/cm^3. This component can be seen as a satellite band in CsCl band profiles of total nucleolar DNA preparations. In alkaline CsCl it does not show strand separation; its analytical base composition is 53.8% G + C (Schildkraut and Maio, 1969). Chuang and Saunders (1974) have isolated, by centrifuging human nucleolar DNA in Cs_2SO_4-Ag^+ density gradient at pH 9.2, a satellite with a density in neutral CsCl of 1.710 gm/cm^3; its buoyant density in alkaline CsCl is 1.775 gm/cm^3 and there is no strand separation (Chuang and Saunders, 1974). Renaturation analysis of this satellite (see Section V,B) shows a biphasic renaturation curve with a fast reassociating component accounting for 22% of the total (Chuang and Saunders, 1974). Schildkraut and Maio (1969) reported that, on heating and rapid cooling, their 1.712 gm/cm^3 heavy satellite shows in CsCl two bands of equal area (1.727 and 1.716 gm/cm^3). Each of these bands had the base composition of the double stranded DNA; thus they did not consist of complementary strands separated as a result of heating. On the contrary, after denaturation-renaturation, Chuang and Saunders (1974) found a single band at 1.715 gm/cm^3. If there are close similarities between the two heavy satellites described by Schildkraut and Maio (1969) and Chuang and Saunders (1974), more work should be done to establish their identity.

F. Satellite B

This satellite, described by Chuang and Saunders (1974) is found as a small shoulder on the light side of satellite A when it is prepared in a Cs_2SO_4-Ag^+ density gradient at pH 9.2 and $r = 0.40$. It has a buoyant density of 1.726 gm/cm^3 in neutral CsCl. Chuang and Saunders (1974) claim that this satellite DNA has many properties consistent with a DNA having ribosomal genes and ribosomal spacers. *In situ* hybridization experiments with cDNA prepared from this satellite show heavy grains clustered at the nucleoli. When unlabeled 18 S + 28 S rRNA was used as a competitor for the *in situ* experiments, all grains in the nucleoli disappeared.

G. Satellites C and D

Not much data is available for these two satellites isolated by Saunders *et al.* (1972a, 1975). Satellite C can be seen as a satellite band on the light side of a Cs_2SO_4-Ag^+ density gradient at pH 9.2 and $r = 0.27$; it has a buoyant density of 1.703 gm/cm^3 in neutral CsCl. For satellite D, see Table V.

In view of the facts just described, and with the data available, it is difficult to correlate our findings on human satellite DNA's with those of other authors. Of the eight satellites studied, four of them, I to IV, are best characterized. Ambiguities in data exist however, between satellites II and III: for example, Saunders *et al.* (1975) reported densities of 1.695 gm/cm^3 and 1.699 gm/cm^3 for satellites II and III; these values are nearer to the values given by Corneo *et al.* (1971, 1972) for their satellites III and IV; the density difference in alkaline CsCl for the separated strands of satellite III reported by Saunders *et al.* (1975), 13 mg/cm^3, is as close to the difference found by Corneo *et al.*, (1972) for their satellite IV, 12 mg/cm^3 as to that for their satellite III, 14 mg/cm^3 (Corneo *et al.*, 1972). Our analysis of human placenta DNA showed the existence of three satellite DNA's of densities in neutral CsCl of 1.6875, 1.6955, and 1.6995 gm/cm^3 (see above). Our 1.6955 gm/cm^3 satellite reinforces the ambiguity between satellites II and III: it has a buoyant density close to that found by Corneo *et al.* (1971, 1972) for their satellite III but it is found on the heavy side of the main band in Cs_2SO_4-Ag^+ density gradients at pH = 9.2 and $r = 0.32$ as is satellite II (Corneo *et al.*, 1970). Melli *et al.* (1975) have shown that there is cross-hybridization between satellites II and III. All this may indicate that these two satellites are either closely related or are the same satellite.

For satellites A, B, C, and D more work should be done for their identification. No strand separation has been observed in any of these satellites; their concentration is not well-defined in total DNA. Satellite B has been associated with ribosomal genes by Chuang and Saunders (1974), even if its buoyant density seems too high in view of the findings of Sinclair and Brown (1971) that *Xenopus* ribosomal RNA hybridizes with HeLa cells DNA at a density of 1.719 gm/cm^3. Satellites C and D show some specificity in *in situ* hybridization (see Section IV), a fact that can argue for their human origin, against the contaminant DNA argument. Satellite A shows no specific hybridization, all chromosomes being labeled.

IV. *IN SITU* HYBRIDIZATION

A. Satellite DNA

Results of *in situ* hybridization of human satellite sequences reported by various authors are contradictory. Moar *et al.* (1975) have recently shown that in *in situ* hybridization experiments, the dispersion of the autoradiographic label throughout the karyotype is dependent on the incubation temperature. As a consequence, all the results of *in situ* hybridization of human satellite sequences should be revised. For example, Jones *et al.* (1973) reported that cRNA prepared from human satellite III hybridizes *in situ* mainly to chromosome 9 and to a lesser extent to chromosomes of the D and G groups. Moar *et al.* (1975) reported for this same satellite III definite sites of hybridization not reported by Jones *et al.* (1973); labeling near the centromere is found in chromosomes 1, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and Y.

TABLE VI
Chromosomal Localization of Human Satellite DNA (*in Situ* Hybridization)

Satellite	Chromosomal localization		References[a]
	Main	Other	
I	Y		1
	Y	1,3,13,14,16,21	2
	Y,9	14,15,21,22	3
II	1	9,16	4
	Y	9	1
	Y,9	1,15,16,17,21,22	3
III	9	D and G groups, 1,16	5
	Y		1
	9,15,Y	13,14,20–22	3
		1,7–11,13–22,Y[b]	6
IV	Y		1
	9,15,Y	13,14,20,21,22	3
A	Not localized		7
B	Nucleoli		8
C	9	D and G groups	9
D	9		10

[a]References:
1. Evans *et al.* (1974)
2. Jones *et al.* (1974)
3. Gosden *et al.* (1975)
4. Jones and Corneo (1971)
5. Jones *et al.* (1973)
6. Moar *et al.* (1975)
7. Chuang and Saunders (1974)
8. Chuang (1974)
9. Saunders *et al.* (1972a)
10. Saunders *et al.* (1975).
11. Tanguay *et al.* (1975).

[b]No distinction was made between main and other localizations.

Table VI shows the chromosomal localization reported for human satellite sequences. These results clearly show the existing confusion. Chromosomes 1 and 9 show systematic labeling with cRNA prepared from 6 of the 8 satellites studied. Chromosome Y shows labeling with 4 of them. In the human karyotype, the largest areas of heterochromatin are found in chromosomes 1, 9, and 16 (and Y) (Arrighi and Hsu, 1971; Yunis *et al.*, 1971; Yunis and Yasmineh, 1972).

B. Repetitive DNA

Only *in situ* hybridization experiments done with cRNA prepared from "Cot fractions" will be discussed here.

By *in situ* hybridization experiments, Saunders *et al.* (1972b) have shown that repetitive DNA is species-specific. A similar result was found by Hearst *et al.* (1973) with "h.a.r.r. DNA" (hydroxyapatite-isolated rapidly renaturing DNA). Interpretation of these results should be made with great care, since the specificity of hybridization may come from satellite sequences preferentially transcribed from repetitive DNA during cRNA preparation. Using two "Cot-fractions", human fast repetitious DNA (Cot 0–0.05) and intermediate repetitious DNA (Cot 0.05–50), Saunders *et al.* (1972b), found that both of them are species-specific. Satellite sequences are expected to be enriched in the "fast repetitious" fraction, but in view of the peculiar renaturation properties of some human satellite DNA (see Section V,B), some satellite sequences are probably found in the "intermediate repetitious" DNA. Using cRNA prepared from two "h.a.r.r. DNA" fractions, 1.703 and 1.714 gm/cm^3, Hearst *et al.* (1973) found species-specific hybridization in *in situ* experiments; this "h.a.r.r. DNA" preparation was free of at least three of the known satellites.

V. RENATURATION PROPERTIES OF HUMAN DNA

A. Total DNA

Renaturation experiments done on a number of highly sheared animal DNA's have shown practically constant proportions of repetitive and nonrepetitive or unique sequence DNA's (Davidson *et al.*, 1975); unique sequences usually

account for 60–75% of the genome. The repetitive sequences can be subdivided into two classes: the fast repetitive sequences (a fraction that usually includes the highly repetitive or satellite sequences) and the intermediate or middle-repetitive sequences. A third class of sequences, the "foldback" or inverted sequences, have not been extensively studied, although they have been identified in some organisms (Hearst *et al.*, 1973; Wilson and Thomas, 1974; Schmid *et al.*, 1975; Schmid and Deininger, 1975).

Renaturation studies on the human genome have shown that the relative amounts of repeated and unique sequences are the same as for other animal DNA's, as expected. Saunders *et al.* (1972b) reported 35% repetitive and 65% unique sequence DNA; essentially the same figures were found by Mitchell (1974) and Schmid and Deininger (1975).

Even if the relative amounts of repeated and unique sequence DNA's are fairly constant in different animals, the arrangement of these sequences within the genome seems to follow two different patterns of organization, the so-called *Xenopus* and *Drosophila* patterns (Davidson *et al.*, 1975). The "*Xenopus* pattern" is characterized by the interspersion of single copy sequence segments of lengths from 800 to several thousand base pairs, with repetitive sequence segments about 300 base pairs long (Davidson *et al.*, 1973, 1974). The "*Drosophila* pattern" is characterized by the existence of single copy sequence elements about 40,000 base pairs long with no interruptions by repetitive sequences; the middle repetitive sequences (nonsatellite sequences) are of an average length of 5000 base pairs (Manning *et al.*, 1975).

Saunders *et al.* (1975) have reported that about 10% of human DNA is repetitive, arranged as repetitive sequence segments, 1100 base pairs long, and interspersed with less repetitive sequences longer than 2500 base pairs.

Schmid and Deininger (1975) studied the sequence organization of the human genome. Basically, the human genome follows the "*Xenopus* pattern". Repetitive sequence classes are distributed throughout 80% of the genome. About one-half of the genome is made up of short single copy sequences, 2000 base pairs long, interspersed with very short repetitive sequences of an average length of 400 base pairs. A second fraction of the genome is composed of an interspersed arrangement of very long unique sequences with short repeated sequences.

The organization of the inverted sequences in human DNA has been studied by Hearst *et al.* (1973), Wilson and Thomas (1974), and Schmid and Deininger (1975). About 3% of the genome can be isolated as "foldback" DNA (Hearst *et al.*, 1973). Wilson and Thomas (1974) have shown that these inverted sequences ("palindromes") are found in clusters of two to four, these clusters being sparsely distributed; the palindromes are not randomly located, specific sequences being adjacent to them. Schmid and Deininger (1975) suggested that the inverted repeat sequences should not be considered a subclass of repeated

sequences. According to these authors, inverted repeat, repetitious, and single copy sequences are mutually interspersed. The arrangement of repeated and single copy sequences near an inverted repeat is similar to the arrangement found elsewhere in the genome.

A different approach to the study of the sequence organization in the human genome had been used by several authors. This approach consists of the study of the buoyant properties in CsCl of renatured DNA fragments, previously fractionated according to their renaturation properties ("Cot fractionation"), thermal stability, or MAK column chromatography (Saunders *et al.*, 1972b; Hearst *et al.*, 1973; Corneo *et al.*, 1975). This kind of work allows the isolation of different classes of molecules among a "Cot" family; some of these classes of molecules can be associated with satellite DNA (Hearst *et al.*, 1973), others reflect the true nonsatellite repetitious molecules.

B. Satellite DNA

Renaturation data on human satellite DNA is scarce. Chuang and Saunders (1974) studied the renaturation kinetics of human satellite A, sonicated to an average length of 250 nucleotides. The renaturation curve was biphasic: 22% of the DNA had an apparent $Cot_{1/2}$ of 1.3×10^{-2} and 54% an apparent $Cot_{1/2}$ of 8.3. Thus the first component can be classified in the highly repetitive and the second one as an intermediate repetitive DNA, the fast component being 1600 times more frequently repeated than the slow component. This satellite was found to be intermolecularly homogeneous by the author. If that is the case, the renaturation kinetics reflect a particular intramolecular heterogeneity.

At least three of the five classes of human "h.a.r.r." DNA molecules studied by Hearst *et al.* (1973) can be associated with satellite DNA. For two of them, 1.687 and 1.700 gm/cm^3, renaturation analysis was done, showing a biphasic renaturation curve, the first one with 32% of a kinetic complexity of 20 base pairs and 68% with a kinetic complexity of 530 base pairs; for the second one the values are 40% of 35 base pairs and 60% of 700 base pairs.

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3

Human Gene Localization by RNA:DNA Hybridization *in Situ*

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I. Comments on Isolating and Labeling RNA . . . 60
II. Methods of *in Situ* Hybridization . . . 62
A. Introduction . . . 62
B. RNase . . . 63
C. Denaturation of Chromosomal DNA . . . 64
D. Time, Concentration, and Temperature . . . 65
E. Conditions of Hybridization . . . 65
III. Gene and Genome Evolution in Primates . . . 66
IV. Ribosome Genes . . . 68
A. 5 S rRNA . . . 68
B. 18 S and 28 S rRNA Genes on the D and G Group Chromosomes . . . 73
C. D and G Group Chromosomes in Rosette Association . . . 75
V. Clinical Applications of *in Situ* Hybridization . . . 78
VI. Localizing Genes at Interphase . . . 80
VII. Messenger and Transfer RNA . . . 82
References . . . 84

This article is not intended to be a complete review of gene localization by *in situ* hybridization, but rather a restricted coverage involving the human genome. Since very few loci have been located directly, it is necessary to examine a variety of organisms in order to organize a strategy for the more difficult studies