Table 2

Phage	Yield of phage in complementation test**	Yield of Wt phage in recombination test***
T4 No. 4	5×10^7	–
T4 No. 20	6×10^7	–
T4 am H626*	4×10^6	–
T4 am H28*	2×10^6	–
T4 No. 4+T4 am H626	4×10^7	1.2
T4 No. 4+T4 am H28	5×10^9	2.8
T4 No. 4+T4 No. 20	3×10^9	3.4
T4 No. 20+T4 am H626	2×10^9	2.5
T4 No. 20+T4 am H28	7×10^7	0.005
T4 am H626+T4 am H28	9×10^9	4.1

*am H626 is located in gene 8; am H28 is located in gene 53; **the crosses were performed on the non-permissive strain B sup⁻. The progeny phage yield was assayed on the permissive strain K12 sup⁺; ***the crosses were performed on the permissive strain K12 sup⁺. Total phage progeny was assayed or K12 sup⁺ and wild type recombinant phage on B sup⁻. The value given is the percentage of wild type phage present in the burst.

closely linked to amH462 (0.005% recombination) and that the T4 No. 20 mutation is very closely linked to amH28 (0.005% recombination). The 8 and 53 gene products of phage T4 are known to be components of the outer wedges of the bacteriophage baseplate structure[7]. We have considered 2 types of explanation to account for the existence of the T4 No. 4 and T4 No. 20 mutations. The first type suggests that the structural gene products of genes 8 and 53, induced by T4 No. 4 and T4 No. 20 respectively, are different in the 2 hosts. This could be due to differences in the translational apparatus of K12 and B cells as, for example, in the specificity of the tRNA. The second type of explanation derives from previous observations of host involvement in T4 phage morphogenesis[8–10], and in T4 tail fibre function[11,12]. It suggests that the 8 and 53 altered gene products are able to interact effectively with a K12 host component involved in baseplate assembly, but cannot do so with the corresponding B component. The elegant electron micrographs of Simon[13], which show that T4 baseplate formation takes place at or near the bacterial membrane, support such an explanation. It is interesting to note that the T4 mutant HL626, originally thought to be an amber mutation in gene 60, has been shown[14] to be analogous to T4 No. 4 and T4 No. 20 in that it grows on all K12 strains tested but not on all B sup⁻ or B sup⁺ strains tested. The gene 60 product is thought to associate with the bacterial membrane to promote proper phage DNA replication[15].

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Heterogeneity of HeLa cell DNA as evidenced by CsCl density gradient centrifugation

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Summary. HeLa cell DNA was analyzed through CsCl density gradient centrifugation and thermal denaturation. Neutral centrifugation without ionic treatment allowed the resolution of a main peak with density 1.699 g/ml and of 3 satellites DNAs with densities 1.683, 1.710 and 1.720. First derivatives of melting curves showed the presence of 4 DNA families, whose G+C content calculated from T_m values corresponded almost exactly to the G+C content expected from the previously densities. The extraction method seems particularly suitable for quantitative separation of DNA classes.

The existence of the heterogeneity of human DNA was demonstrated by Corneo et al.[2–4], who showed the presence of satellite DNAs after fractionation in Ag^+–Cs_2SO_4 density gradients. In order to obtain quantitative yields of all human DNA components, a method has been developed whose application in plant DNA extraction has showed remarkable results[5,6]. This paper presents the results obtained in the resolution and fractionation of HeLa DNA components using neutral CsCl density gradients.

Materials and methods. DNA was obtained from whole cells suspended in a solution containing $5\cdot10^{-2}$ M Tris buffer, pH 7.8, $8\cdot10^{-2}$ M EDTA, 4% Na-dodecylsarcosinate and lysed by gentle stirring for 1 h at room temperature. NaCl was added to a 2 M final concentration and the solution was stirred for 1–2 h. After dialysis at 4 °C against 1×SSC (0.15 M NaCl and 0.015 M trisodium citrate), RNase (100 μg/ml preheated at 90 °C for 10 min) and α-amylase (150 μg/ml) were added directly in the dialysis tube. After incubation at 37 °C for 1 h, pronase (500 μg/ml, self digested for 2 h at 37 °C) was added and allowed to act for 2 h at 37 °C. The solution was then centrifuged at 18,000 rpm for 15 min at 4 °C: to the supernatant CsCl was added to a refractive index of 1.4000. The CsCl solution containing the DNA was centrifuged to equilibrium in a 30 rotor of a Spinco L 2-65 B ultracentrifuge at 20 °C and 25,000 rpm or in a 40 rotor at

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33,000 rpm alternatively. Gradients were fractionated under continuous scanning at 257 nm in a LKB Uvicord II.

Fractions containing DNA were extensively dialyzed against 1×SSC in Ultrahülsen UH 100 membranes (Schleicher-Schüll) and further characterized. Analytical ultracentrifugation was performed according to Schildkraut et al.[7] in a Spinco E ultracentrifuge for 20 h at 44,770 rpm at 25 °C. The DNA amounts per analytical cell ranged from 3 to 5 μg; DNA from Bacillus subtilis phage ϕ_e (buoyant density 1.742 g/ml) was added as a standard. UV photographs were taken and photoplates examined in a Joyce-Loebl recording microdensitometer. DNA molecular weight was determined according to Eigner and Doty[8].

Thermal denaturation was carried out in a UNICAM SP 800 spectrophotometer equipped with a SP 876 temperature control programmer. Temperature increase was 0.5 °C/min and melting curves were recorded directly by a Philips X-Y recorder PM 8120.

Results and discussion. The estimate of the mol.wt of our DNA samples gives values greater than 10^7 daltons. Curves of DNA distribution are given in figure 1. DNA satellite components with a buoyant density of 1.683 and 1.710 are present in all preparations, while the heavy satellite (ϱ 1.720) is not always present. The light satellite clearly accounts for more than 1% as previously reported[2]. It is worth noting that its absorbance spectrum is shifted towards higher wavelengths with a maximum at 270 nm. Such a property could be tentatively attributed to the presence of complexes with divalent ions[9]. On the other hand, Corneo et al.[4] observed that repetitive human satellite DNAs display peculiar properties related to anomalous binding with Ag^+ and Hg^{++} ions. More recently Sissoeff et al.[10] made evident the presence of divalent ions complexed with satellite DNAs extracted from several sources. This heterogeneity of HeLa cell DNA was confirmed from denaturation profiles (figure 2). The Δt (or width of thermal transition) is 7.55 °C and the thermal denaturation curve is polyphasic. First derivatives of melting curves show the presence of 4 major DNA families (solid arrows) whereas other 2 (dashed arrows) are not clearly distinguishable.

In order to establish a possible correlation between the DNA denaturation classes and the density gradient components, we report in the table the G+C percent calculated both from the T_m of each family[11] and from the buoyant densities[7]. The relative importance or percent of the DNA components were calculated by determination of area of the separate components from the microdensitometer tracing. A clear correspondence was found between derivative classes and CsCl gradient fractions, with the exception of family 1: on the other hand, if the assumption of a cation-light satellite complex is true, the high T_m value could be attributed to an increased thermal stability of the complex.

Denaturation derived curve			Analytical ultracentrifugation		
Peak No.	T_m (°C)	Percent (G+C)	Density (g/ml)	Percent (G+C)	RI%*
I	82.5	31.18	1.683–4	25.2	10
II	85.8	39.23	1.699	39.4	76
III	90.8	51.42	1.710	51.02	12
IV	95	61.67	1.720	61.22	2

* Relative importance.

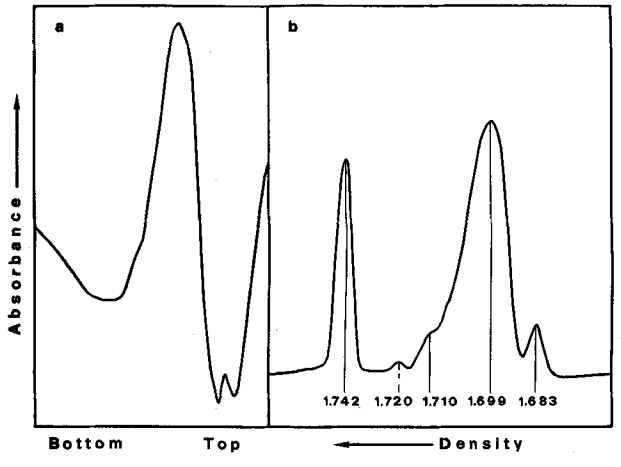

Fig. 1. Preparative (a) and analytical (b) CsCl density gradients of whole HeLa cells DNA.

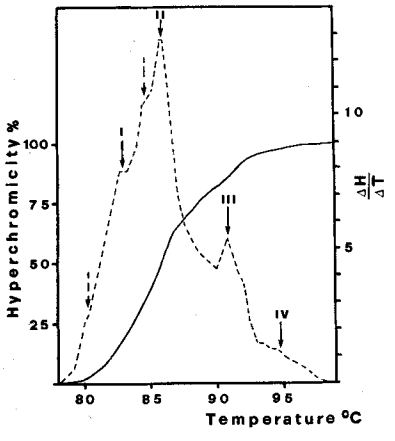

Fig. 2. Thermal denaturation profile of HeLa cells DNA in 1×SSC (—) and derivative melting curve (---) obtained by graphical differentiation using 1 degree intervals.

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