produced by all these sources was 10^{-3} volt/cm. This is within the limits of the ambient emf's in an urban environment and, therefore, was the same for target cells and controls. Also, the temperature of the samples, estimated from the vapor pressure of the cooling liquid and the evaporation rate of the bath, was constant to $\pm$ 0.1°K for both target and control cells.

We have also estimated the effect the magnetic field should have on a system of independent magnetic moments of the size of the Bohr magneton. For the fields and temperatures used, no more than 20 percent of the spins would be preferentially aligned with the field.

We conclude that the observed transformations are due to magnetic field effects alone. Also, from the estimate given above of the alignment of noninteracting magnetic dipoles at the temperatures and magnetic fields used, it seems unlikely that these effects could be due to magnetic moment alignments in small portions of the cellular material. Permanent magnetically induced changes in the cellular material would seem to require much larger participation of cellular material because of energy considerations—that is, cooperative magnetic field interactions analogous to the cooperative dielectric phenomena postulated by Fröhlich (*6*). There is some evidence in the literature that such interactions are possible in cellular material such as DNA (*7–9*).

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Thermal Stability of Human DNA and Chimpanzee DNA Heteroduplexes

Abstract. *The base pairing fidelity of heteroduplexes formed from human DNA and chimpanzee DNA has been studied by the criterion of thermal stability to test the evolutionary conservation of repeated DNA base sequences.*

The eukaryotic genome is known to have a regular arrangement of repetitive and single copy sequences (*1*). In general, short repetitive DNA sequences, 300 nucleotides in length, are interspersed with short single copy sequences of a gene-sized length (*1*). This sequence arrangement is highly conserved and has been observed in organisms ranging from mollusks to mammals. The only documented exception to this sequence arrangement is that in *Drosophila* (*2*). In *Drosophila* the average lengths of the repeated and single copy DNA sequence classes are much longer than those in other organisms. It has, therefore, been concluded that the basic interspersion pattern of short repetitive and short single copy sequences is biologically important as suggested by its evolutionary conservation (*1*).

We have found that the DNA sequence organization in humans also follows this pattern (*3*). By an electron microscope method (*2*), we have determined the distribution of lengths of repeated DNA sequences in humans. There is good agreement between this distribution—both its width and mean—and the distribution of corresponding lengths found in *Xenopus* (*4*) by the same electron microscope technique. In contrast to the strong selection which maintains the lengths of the repeated sequences, the base sequence is not, in general, highly conserved. Duplex DNA prepared by renaturing repetitious DNA sequences is known, by the criterion of thermal stability, to have numerous sequence mismatches. The primary structures of the repetitive sequences have diverged since their original repetition, and repetitive sequences are similar but not identical in base sequence (*5*).

These observations suggest two extreme possibilities that we can test. The first is that the biological function of the repetitive DNA sequences is based exclusively on length and arrangement, and that detailed primary structure is not important to this function. One could imagine several structural functions for which this might be the case. The second possibility is that the primary structure is also important to the function of the repetitive sequences. According to this view, any useful mutations in repetitious sequences would be conserved.

These two possibilities can be distinguished by the methods which Kohne, Hoyer, and co-workers developed to compare the extent of divergence of the single copy sequences found in the primate genome (*5*). This method consists of cohybridizing the DNA of two different species to form heteroduplex DNA. In forming this heteroduplex, the DNA of one species, for example, chimpanzee, is present as a radioactive tracer at infinite dilution so that its concentration does not contribute to second order renaturation. The DNA of the other species, for example, human, is present at high concentration so that this component dominates the second order renaturation. Radioactive duplex DNA formed by this procedure is an interspecific DNA heteroduplex. Nonradioactive duplex DNA—monitored by ultraviolet absorbance—is the result of any intraspecific hybridization. The two types of duplexes are next bound to hydroxylapatite under conditions where single-strand DNA elutes. The thermal stability of the duplex DNA is then determined by the temperature at which the DNA elutes from hydroxylapatite as denatured single-strand DNA. The thermal stability of the hybrids depends on the fidelity of base pairing. A 1 percent mismatching of base pairs depresses the thermal stability of a duplex by approximately 1°C (*6*).

The evolution of repeated DNA sequences in rodents has been studied by the method we propose (*7*). Because of the extensive divergence of all sequence classes in rodents, the results of that study are not suitable for the comparison of divergences of sequences in humans and primates. Rice noted that repetitive DNA sequences in primates—rhesus and human—have undergone relatively less divergence than in rodent (*7*). We compared the relative divergence of repetitive and single copy DNA sequences in the primate line. We have chosen human and chimpanzee for this comparison of repetitive DNA sequences. The single copy DNA sequences of primates have been extensively compared (*5*). Furthermore, comparisons of the DNA's of chimpanzees and humans are of interest from an evolutionary viewpoint (*5, 8*).

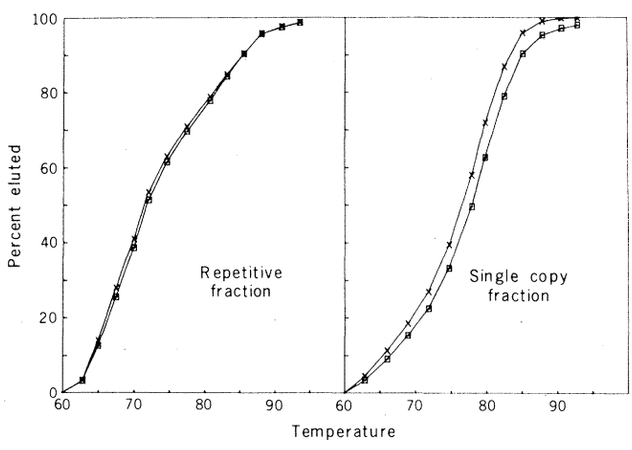
The procedures for DNA renaturation and thermal stability studies are adapted

from our previous study (*3*). All DNA samples were highly sheared, to an approximate length of 500 nucleotides. Human placental DNA was admixed with [^{3}H]thymidine-labeled DNA obtained from cultures of human (*3*) or chimpanzee (*9*) cells. The specific activity of the cell culture DNA was greater than 200,000 count min^{-1} μg^{-1} and the mixing ratio of placental DNA to cell culture DNA was at least 1000 : 1. The admixed samples containing 400 μg of placental DNA and 50,000 count/min of cell culture DNA were denatured in alkali and neutralized by phosphate buffer to give a solution having a concentration of 0.24*M* sodium phosphate (an equimolar mixture of NaH_2PO_4 and Na_2HPO_4). This solution was renatured to C_0t of 21 (C_0t is the concentration of nucleotide in moles per liter multiplied by the time in seconds) at 65°C (*3*) in 0.36*M* Na^+ (about 7 hours). This C_0t value, which corresponds to C_0t 80 in 0.18*M* Na^+, was selected to exclude the renaturation of single copy sequences and to include the renaturation of practically all repetitive sequences (*3*). After renaturation, the sample was adjusted to 0.12*M* sodium phosphate buffer and applied to a 1-cm^3 bed of hydroxylapatite in a jacketed column at 60°C. Duplex DNA binds to hydroxylapatite at this buffer concentration, whereas single-strand DNA elutes. The column temperature was raised by increments, and after equilibration the column was washed with 0.12*M* sodium phosphate buffer to elute melted strands. After completion of the thermal study, normally near 100°C, the column was washed with 0.3*M* sodium phosphate to remove any small amount of residual duplex DNA. The concentration of the placental DNA in the eluate was determined by absorbance at 260 nm. The concentration of cell culture DNA was determined by scintillation counting. The results of these studies are summarized in Figs. 1 and 2. To obtain single copy DNA sequences, the eluates from the 60°C column washings were concentrated and readjusted to 0.24*M* sodium phosphate buffer and further renatured to C_0t 1000. The renatured samples—one containing human cell culture DNA and the other containing chimpanzee cell culture DNA—were again bound to hydroxylapatite under exactly the same conditions described above, and the thermal stability studies were similarly repeated on these samples.

The relative thermal stabilities of renatured repetitious and renatured single copy DNA sequence classes in humans are compared in Fig. 1. The thermal stability that we observed for renatured single copy DNA is less than that reported by others (*5*). This may be the result of differences in our renaturation procedures. The melting temperature of the renatured repetitious duplex DNA is 6°C lower than that of the renatured single copy DNA. This depression in thermal stability corresponds to 6 percent mismatching in the renatured repetitious sequences. As a control for the heteroduplex studies on chimpanzee and human discussed below, we formed DNA hybrids between isotopically labeled human DNA, obtained from cell culture, and the unlabeled human DNA isolated from placenta. The preparation and handling of this labeled human DNA was identical to that of the procedures used on the isotopically labeled chimpanzee DNA. The human sequences from these two sources have nearly identical melting profiles for each sequence class studied (Fig. 1).

The thermal stability of a heteroduplex formed from chimpanzee DNA and human DNA, formed from single copy sequences, is 2.4°C lower than the human DNA homoduplex formed from single copy sequences (Fig. 2). Others have found this difference to be 0.7° to 1.4°C (*5*). Since we collected data at intervals of 2°C this discrepancy in comparable results should not be considered substantial. We find that the repetitive heteroduplexes formed from human and chimpanzee DNA's have a melting profile that is indistinguishable from the melting profile of renatured repetitive human DNA (Fig. 2). It should also be noted that the extent of renaturation is the same for the labeled human and chimpanzee DNA's, 28.7 percent and 29.6 percent, respectively (Figs. 1 and 2).

If the base sequences of repetitive DNA's had formed exclusively before the divergence of human and chimpanzee then a heteroduplex formed from repetitive human and chimpanzee DNA's should have a thermal stability profile identical to that of renatured repetitive human DNA. This predicted re-



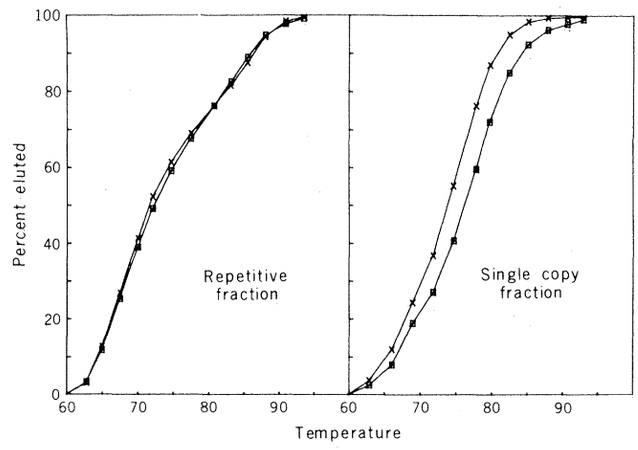


Fig 1 (left). The thermal stability of renatured human DNA. Repetitive DNA was isolated by corenaturing highly sheared (about 500 nucleotides in length) labeled human DNA (×) with a 1000:1 excess of unlabeled human DNA (□) to C_0t 21 (0.36*M* Na^+) at 65°C. The preparation of the DNA as well as the renaturation profile have been described (*3*). The unrenatured and renatured DNA's, were fractionated by hydroxylapatite chromatography at 60°C in 0.12*M* sodium phosphate buffer. For the radioactive DNA 28.7 percent of the genome renatured as repetitive DNA. The thermal elution profiles of the repetitive sequences is shown above. The single copy DNA sequences were isolated by further incubation of the unrenatured fraction to C_0t 1000 in 0.36*M* Na^+ at 65°C, and the renatured single copy sequences—26 percent of the genome for the labeled DNA—were collected by hydroxylapatite chromatography. The elution profiles of these single copy sequences are shown above. Fig. 2 (right). The thermal stability of heteroduplexes formed from human DNA and chimpanzee DNA. The methods and procedures were identical to those of Fig. 1, except that chimpanzee cell culture (*9*) was used a source of isotopically labeled DNA (×) which was corenatured with a 1000:1 excess of unlabeled human DNA (□). The repetitive chimpanzee DNA represents 29.6 percent of the genome, and the renatured single copy chimpanzee DNA represents 21 percent of the genome.

sult is observed in the data of Fig. 2. A second possibility, which is also completely consistent with the data of Fig. 2, is that families of repeated DNA sequences in both human and chimpanzee consist of related base sequences having the same proportion of their bases changed and that these changes occur randomly at any site in the sequence. Again for this case the repetitive DNA heteroduplex of chimpanzee and human should have a thermal stability profile identical to that of renatured repetitive human DNA. Both of these possibilities should be contrasted to the very different case of renatured satellite DNA's from *Drosophila* (*10*). In these sequences specific mutations are located at specific sites in the sequence. As a result, the renaturation product of two different *Drosophila* satellite DNA's has a much lower thermal stability than the self-renaturation product of an isolated satellite.

A complication in these experiments is the existence of a zero-time binding fraction which binds to hydroxylapatite without undergoing second-order renaturation with a second DNA strand (*3*, *11*). At least part of this fraction results from inverted repeated DNA sequences. This sequence class could alter the melting profiles of the repetitious sequence classes, especially since short DNA strands containing this fraction have a high thermal stability (*11*). As a control, we have removed this fraction (*3*) from the isotope-labeled DNA preparations from the human and chimpanzee, 4 and 7 percent, respectively, and we have repeated the experiments described above. Again, the melting profiles of the repetitive labeled human DNA and chimpanzee DNA hybrids were indistinguishable.

A second complication in these experiments is the possible self-renaturation of chimpanzee DNA. To test this possibility labeled chimpanzee DNA was renatured exactly as described for the renaturation of the admixed DNA's to C_0t of 21, except that the placental human DNA was omitted. On binding to hydroxylapatite 11.5 percent of this DNA behaved as duplex so that, excluding the zero-time binding fraction, only 4.5 percent (11.5 − 7 percent) of the chimpanzee DNA would self-renature (with other chimpanzee DNA sequences) during the C_0t 21 incubation. This is, in principle, an upper limit to the amount of chimpanzee DNA which self-renatures since some fraction of this 4.5 percent may also share homology with human DNA sequences. The small extent of self-renaturation of the chimpanzee DNA would not influence the results of the thermal stability study.

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A Specific Population of Gonadotrophs Purified from Immature Female Rat Pituitary

Abstract. *When dispersed pituitary cells from 14-day-old female rats were sedimented in a bovine serum albumin gradient, a fraction was isolated which consisted of almost 90 percent of large cells that stained purple in the periodic acid–Schiff (PAS) reaction. Immunostaining indicated that over 85 percent of these PAS-purple cells were gonadotrophs containing both follicle stimulating and luteinizing hormone. Reproducible cell cultures could be obtained on poly-L-lysine coated dishes. As early as the second day in culture, the secretion of both follicle stimulating and luteinizing hormone was highly stimulated by luteinizing hormone–releasing hormone. The effect on FSH was as marked as that on LH. The data suggest that the isolated gonadotrophs are a specific functional subtype.*

Gonadotrophs are specific cell types of the anterior pituitary gland which secrete the gonadotrophins follicle stimulating hormone (FSH) and luteinizing hormone (LH). There are morphologically distinct subtypes among these cells (*1*). For many years it was thought that a separate gonadotroph subtype secreted LH while another subtype secreted FSH, but recent studies have shown that part of the FSH-type gonadotrophs also contain LH (*2*, *3*). Moreover, hormone distribution over the two types of gonadotrophs has been found to vary with sex, age, and certain physiological conditions, indicating that in addition to morphological heterogeneity there may also be functional heterogeneity (*4*).

Because the occurrence of functional variants of gonadotrophs appears to be directly related to the mechanisms that control gonadotrophin secretion, the characterization of these variants is most important. Most investigators have studied these cells by electron microscopy of fixed tissue. In the study described here, functional heterogeneity was explored in living cells. From dispersed pituitary cells we isolated gonadotrophs to a high degree of purity and with specific functional characteristics. Cells were separated by velocity sedimentation at unit gravity. Using this technique with pituitaries from adult rats, other investigators had already succeeded in obtaining marked enrichments in populations of several pituitary cell types (*5*, *6*). Instead of using adult rats, we used female rats at the age of 14 days, since female rats at this age secrete large amounts of FSH (*7*). We supposed that because of this hypersecretion the gonadotrophs or a certain number among them might be advanced in development and might therefore be larger than other pituitary cells. If so, it should be relatively easy to isolate them by velocity sedimentation.

Monodispersed cells from 14-day-old female Wistar rats were prepared according to a method slightly modified from Hopkins and Farquhar (*8*). The distribution of cell types was monitored by light microscopy after the cells were stained with Alcian blue–periodic acid–Schiff (PAS)–orange G (*9*). Gonadotrophs and thyrotrophs were specifically identified by immunostaining with antiserums to rat LH, FSH, and thyroid stimulating hormone (TSH) prepared in rabbits (*10*). As expected, a considerable number of PAS-purple cells (presumably gonadotrophs) were markedly larger in size than were cells of all other types. Table 1 shows the distribution of cell types after