hibited the precipitating antibodies involved in the cross-reaction. The reassociation of the reduced subunits resulted in the recovery of some precipitation with the antiserum (Fig. 1). These observations suggest that the involved antigenic determinants could be uni- or bivalent in the IgM subunits. In this respect, one might recall the recent findings of Onoue *et al.* (*3*) which suggest that the subunits of IgM from rabbit antibody are univalent.

For both antiserums, the precipitin reaction with the homologous myeloma globulin is confined to the Fab-fragment and requires the combination of heavy and light chains. The heavy and light chains were prepared by reduction-alkylation and gel filtration (*4*); the Fab- and Fc-fragments were prepared by papain digestion by the Porter method (*5*). The Fc-fragment did not precipitate with the absorption-A antiserums, nor did the isolated light and heavy chains, although they reacted with the unabsorbed antiserums (Fig. 2). The homologous chains, recombined in the weight ratio of two heavy to one light, gave a reaction of complete identity with the native myeloma globulin (Fig. 2) and removed, by absorption, all reactivity with this myeloma globulin. Similar results concerning the localization of individual specific antigens of some myeloma globulins were obtained by Grey *et al.* (*2*).

The cross-reactions with Waldenström IgM-globulins were not inhibited by the homologous myeloma light chain (5 mg per milliter of antiserum). Among the five Waldenström macroglobulins which cross-reacted with the absorbed antiserum to Du myeloma globulin (type L), two were of antigenic type K.

These observations demonstrate the existence of similar antigenic determinants, shared by some IgG and IgM molecules, which depend upon the conformational structure of the molecule. The importance of conformational structure for the antigenic specificity of several proteins (*6*) and for the Gm(bw) and Gm(f) factors of IgG-globulins (*7*) has been pointed out. That interaction between heavy and light chains of IgG is necessary in order to observe the present reaction revealing the common antigenic determinants suggests that both chains could be involved in the antigenic sites; each of the chains could contain amino acid residues contributing to these antigenic sites. However, another possibility is that these antigenic determinants common to IgG- and IgM-globulins are located on a single chain but that they become exposed or able to react with the antibody only when the double (heavy-light) chain configuration exists.

The demonstration that the antigenic structures are common to an IgG-globulin of the type L and to an IgM-globulin of the type K probably excludes any role of the carboxy-terminal part of the light chain (*8*). These common antigenic determinants could possibly be localized on the Fd-fragment of the heavy chain and depend on its tertiary structure. There is evidence that identical antigenic determinants are present on the Fd-fragment of two distinct classes of immunoglobulins. In the rabbit, the allotypic antigens Aa 1, 2, and 3, which are present on the Fd-fragment of IgG-globulin, have been demonstrated in IgM-globulin (*9*). The Fd-fragments of guinea pig γ_1- and γ_2-immunoglobulins have similar antigenic determinants (*10*). Antiserums prepared against pepsin-digested IgG-globulin from normal serum and absorbed with light chains react with certain IgA myeloma proteins and Waldenström macroglobulins (*11*). However, three antiserums prepared against pepsin-digested Sa myeloma globulin failed to cross-react with any of the 50 Waldenström IgM-globulins.

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Nucleotide Sequence Repetition: A Rapidly Reassociating Fraction of Mouse DNA

Abstract. *The separated complementary strands of a minor component in mouse DNA reassociate with each other much more rapidly than do the complementary strands of other DNA's including those of the principal part of mouse DNA. This difference in capacity of the strands to reassociate can be used to effect a preparative separation of the minor component from the principal fraction. The rate constant for reassociation of the minor component, compared with those of viral and bacterial DNA's, indicates that the minor component consists of a short nucleotide sequence present in about one million copies.*

High-molecular-weight DNA can be fractionated on the basis of nucleotide composition by centrifugation to equilibrium in concentrated CsCl solutions (*1*). When the DNA of higher organisms is analyzed in this way it is found to be heterogeneous in composition. In many cases (*2, 3*) small discrete (satellite) bands are observed, more-or-less well-resolved from a broad principal band. Mouse DNA exhibits a relatively intense band (*3*) with a density of 0.01 g/cm^3 less than that of the center of the principal band. This component has been found in native DNA from all mouse tissues that have been examined and from mouse cell lines cultured in vitro.

We now report that, when the complementary strands are separated by heating (denatured) and incubated under appropriate conditions, they reassociate (*4*) with each other, quickly finding appropriate partners and re-form-

ing helical structures which are reasonably close approximations of the original double-stranded helices. Partial reassociation of animal DNA (*5–7*) and of special fractions (*7, 8*) has been observed recently.

Mouse DNA (*9*) was centrifuged to equilibrium in CsCl after various treatments. Native DNA (Fig. 1A) exhibits the two previously observed bands separated in the density gradient by 0.01 g/cm^3. Denatured DNA (Fig. 1B) exhibits a single broad band centered at a density about 0.015 g/cm^3 higher than the original principal band. After incubation (C) under conditions that lead to reassociation (18 hours, 60°C, 0.4*M* Na+, *p*H 7), sharp bands appear at the density of native satellite DNA and at the density of denatured principal band DNA. Extremely sharp bands (Fig. 1C) are typical of denatured incubated DNA from higher organisms. Imperfect associations between many partially complementary strands lead to the formation of particles or networks which may be readily sedimented by low-speed centrifugation (*6*). The sharp bands are due to the large size of the network particles, which reduces the band width due to diffusion and apparently also the band width due to heterogeneity in composition.

The densities and relative proportions of the two components in Fig. 1C indicate that the minor fraction is made up of satellite DNA which has renatured almost completely during the incubation at 60°C and that the major band is derived from the principal fraction of mouse DNA. The observation that the principal band still maintains the density of denatured DNA indicates that only a few of its nucleotides are involved in network formation. The proportion of helical double-stranded structure in the minor band is large, while that in the principal band is small.

Networks prepared from DNA sheared at 800 atm (*10*) give rise to a band showing a decrease in density due to a greater proportion of helical regions in the principal component (Fig. 1D). The satellite band is broader but still lies at "native" density, while the principal band is now much broader and is skewed toward the lighter density side. Evidently, breakage of the DNA into smaller pieces permits more extensive base-sequence matching in the principal fraction (*6*).

Because the bands formed by the principal and satellite components are sharper and better separated after denaturation and reassociation than in the native state, preparative separation of the components in a CsCl density gradient becomes possible. For this purpose a suspension of networks was prepared by denaturing (10 minutes, 100°C) and incubating (16 hours, 60°C, 0.4*M* Na+, 0.5 mg/ml) unsheared mouse embryo DNA. The network particles were harvested by centrifugation (90 percent of DNA was sedimented) and vigorously resuspended, and portions were mixed with a CsCl solution in varying proportions. These suspensions were layered to give a step density gradient approximating that to be reached after centrifugation (20°C, SW 39 rotor, 20,000 rev/min, 72 hours). In this way a large quantity of DNA was conveniently centrifuged without an early pelleting or flotation of the particles. At the end of the run, the largest network particles were visible as sharp, light-scattering bands separated by a distance of about 8 mm. As estimated from the absorbancy profile, the satellite contained about 10 percent of the total DNA. DNA sheared at 800 atm behaved similarly, except that the contrast in density was somewhat less, as expected from Fig. 1, C and D.

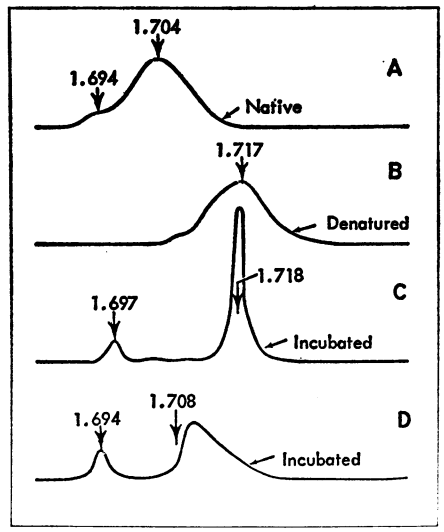


Fig. 1. Analytical density-gradient centrifugation of mouse-embryo DNA. (A) Native high-molecular-weight DNA; (B) same DNA denatured for 10 minutes at 100°C; (C) DNA sheared lightly (33 atm), denatured at 100°C, incubated for 18 hours at 60°C in SET buffer (*11*), diluted, and dialyzed to low ionic strength (HMP, *11*) before adding to CsCl solution; (D) DNA strongly sheared (800 atm) and treated as for third curve.

Evidence for a high degree of helical structure in the reassociated satellite DNA was obtained from the thermal-denaturation profiles of the satellite and principal fractions. The hyperchromic change in satellite material was high, approaching that of unfractionated native mouse DNA, while the melting temperature (T_m) was 55°C compared with 62°C for total native DNA (Fig. 2). A lower melting temperature is to be expected for satellite DNA since its buoyant density in CsCl (native or reassociated) indicates a base composition lower in guanine-plus-cytosine (G + C) content than that of the principal component. The hyperchromic effect given by network particles of the principal band was very much smaller and showed no distinct melting temperature. Similar results were obtained with networks prepared from low-molecular-weight sheared DNA; again the satellite networks gave a large and fairly sharp hyperchromic transition while the absorbancy of the principal band networks simply rose steadily between 40°C and 70°C. However, the principal networks from sheared DNA gave a total hyperchromic change about double that seen in Fig. 2. This evidence and the CsCl buoyant density measurements indicate that base-sequence matching in the principal fraction can occur to a greater extent when the DNA is broken down into smaller pieces (*6*). Such an effect is not observed for satellite DNA.

Denatured mouse satellite DNA, therefore, reassociates to yield a double-stranded molecule with a helical structure and degree of base pairing similar to that of native DNA. The striking feature of the reassociation process, however, is its rapidity. The kinetics of the process (Fig. 3) were measured from the reduction in absorbancy at 260 mμ (hypochromicity) of relatively pure preparations of satellite DNA. The reaction was initiated in the spectrophotometer cell by addition, with stirring, of a concentrated salt solution to previously denatured DNA dissolved in a buffer of low ionic strength, HMP (*11*).

Because of the rapidity of the process it might be argued that the density change (Fig. 1) and the absorbancy changes (Figs. 2 and 3) result from some kind of internal rearrangement of individual molecules of satellite DNA. This possibility is eliminated by the two experiments of Fig. 3. The measured points fit closely the hyper-

bolic curve expected for a second-order reaction (*12*). A fourfold lowering in the concentration at which the DNA is incubated leads to a fourfold increase in the time scale of the reaction. These properties show that the development of the hypochromic effect is a truly bimolecular reaction involving collision between pairs of reactive elements, as expected for the process of DNA reassociation. The exact correspondence between the experimental points and the theoretical curve also shows that the reaction occurring over the period of the measurements is not greatly heterogeneous.

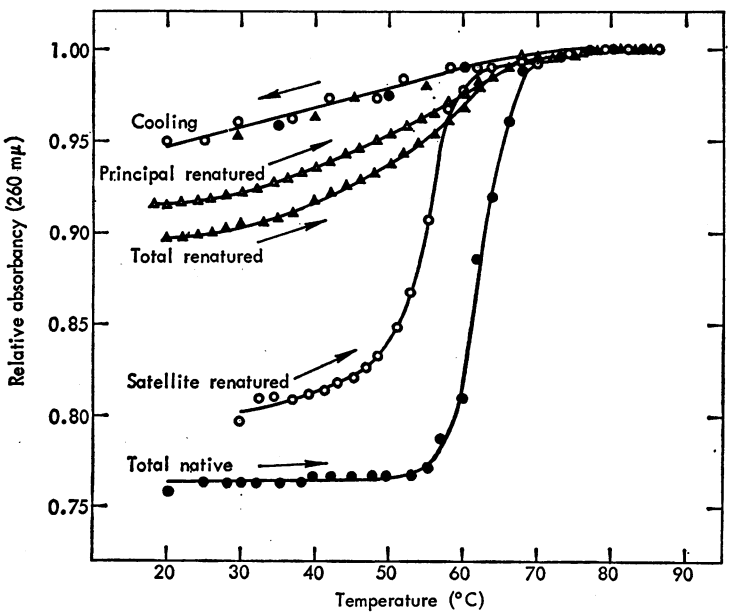
The 8-percent reduction in absorbancy is not at variance with the larger increase with temperature (Fig. 2). The difference is accounted for by the immediate drop in absorbancy, which results from addition of salt to a solution of denatured DNA. This immediate change is due to *intra*molecular interactions in single DNA strands which are distinct from the *inter*molecular interactions between complementary strands resulting from the reassociation.

Analysis of denatured mouse DNA on hydroxyapatite columns yields a fraction which Walker and McLaren (*13*) identify as "stable." It occurs in about the same quantity and has thermal-denaturation characteristics similar to our material prepared by CsCl density-gradient centrifugation. Experiments performed during a visit of P. M. B. Walker to this laboratory show that most of the material prepared by either method is identical and reassociates by a collision-dependent reaction, but a small subfraction (*14*) does exhibit a large reversible absorbancy change with temperature in buffer of very low ionic strength ($10^{-3}M$ Na^+) which indicates that it has quite different properties.

The rate of reassociation is a measure of the number of different nucleotide sequences in a given sample of DNA. Only collisions between complementary strands lead to reassociation. Therefore, it is the concentration of each kind of sequence that establishes the rate, and this concentration is determined by the number of different kinds of sequences present (for a given total DNA concentration). Measurements (*6, 7*) under controlled conditions of DNA fragment size, salt concentration, and temperature show that the rate of reassociation of DNA is just inversely proportional to the DNA content per bacterial cell or virus particle. The number of significantly different fragments is just the haploid DNA complement divided by the average length to which the DNA has been sheared.

The rate of satellite DNA reassociation is much higher than that of any other type of DNA measured under similar conditions (*15*), whether animal, bacterial, or viral (*6, 7*). It reassociates 15 times faster (*16*) than the DNA from simian virus 40 (SV40) (*17*) which contains a length of DNA per particle of only about 6000 nucleotide pairs. Mouse satellite DNA therefore is composed of a short nucleotide sequence that has been copied an enormous number of times with relatively little variation.

Since the satellite makes up about 10 percent of the DNA of mouse tissues we may estimate that there are more than 1 million copies of a segment 300 or 400 nucleotide pairs long. Later measurements might well adjust these numbers by a factor of two or so. The fact that a narrow satellite band is observed in native DNA in CsCl equilibrium centrifugation shows that the segment of DNA in which it occurs is at least several million in molecular weight. Further, since reassociated satellite DNA prepared from high-molecular-weight DNA has virtually the density of the native satellite, the segments must be almost entirely made up of satellite-type DNA. In other words, the repeating sequences appear to be strung end-to-end in large numbers.



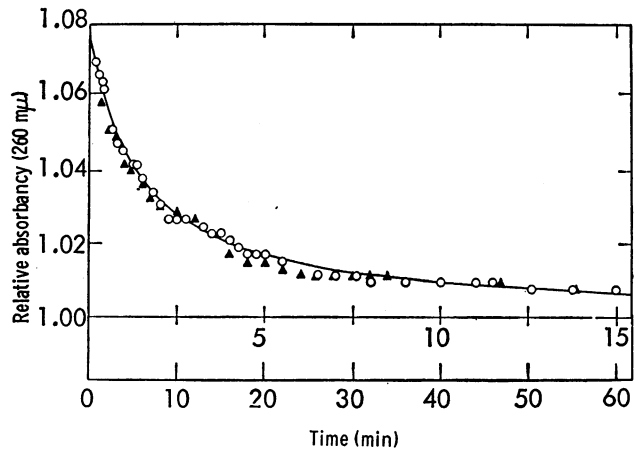


Fig. 2 (left). Absorbancy-temperature profiles of satellite and principal fractions of high-molecular-weight mouse-embryo DNA. The measurements were made in HMP buffer (*11*). Closed circle, unfractionated native DNA; closed triangle, unfractionated networks prepared by denaturing DNA at 100°C for 5 minutes, followed by incubation for 18 hours at 60°C in SET (*11*); open circle, satellite fraction of networks; open triangle, principal fraction of networks, isolated by preparative CsCl density gradient centrifugation. Absorbancy changes for different materials during cooling were closely similar; only a few representative points have been plotted. Fig. 3 (right). Kinetics of renaturation of mouse satellite DNA. The DNA, dissolved in HMP buffer (*11*), was denatured by placing it at 100°C for 4 minutes; it was then returned to room temperature. At time zero, salt concentration was brought to $0.09M$ with respect to Na^+, and the resulting hypochromic change with time was followed. Closed triangle (upper time scale), DNA concentration, 24 μg/ml; absorbancy readings made with a 1-cm light path. Open circle (lower time scale), DNA concentration, 6 μg/ml; readings made with a 4-cm light path. Curve is theoretical, calculated for ideal second-order reaction.

The biological role of the extremely repetitive mouse satellite DNA remains a mystery. We have observed it in DNA prepared from the liver of "germ-free" mice and in that prepared from male and female mouse tissues. It has been observed in many tissues and strains (*3*) including European wild mice. It is probably of nuclear origin since our mouse-embryo DNA was extracted from a moderately good preparation of nuclei (*9*), and it is not mitochondrial DNA (*8*). It is an extreme example of the repetition of nucleotide sequences which appears to occur universally in the DNA of higher organisms (*7*).

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9. The DNA was prepared from embryos (about 15 days old) of Swiss white mice. The DNA was extracted from a nuclear preparation [B. J. McCarthy and B. H. Hoyer, *Proc. Nat. Acad. Sci. U.S.* **52**, 915 (1964)] by a modification of the method of Berns and Thomas (Abstr., Biophysical Society Meeting, Chicago, February 1962).
10. Quantitative interpretation of the rate of reassociation requires control of the size of the fragments of DNA as well as of the salt concentration and temperature. Passage of a solution of DNA through the needle valve of a French pressure cell [B. J. McCarthy and E. T. Bolton, *J. Mol. Biol.* **8**, 184 (1964)] at a few milliliters per minute with a pressure drop of 800 atm yields a relatively homogeneous population with an average length of about 1200 nucleotide pairs.
11. The SET buffer contained 0.3*M* NaCl and 0.001*M* tris-HCl buffer, *p*H 7.4. The HMP buffer contained 0.001*M* EDTA (ethylenediaminetetraacetate), 0.0075*M* sodium phosphate buffer, *p*H 6.8, total Na^+ concentration 0.013*M*.
12. Under reasonable conditions and except at the later stages, reassociation is a second-order collision-dependent process. It is interfered with by high salt or low temperature, which give excessive secondary structure to the single-stranded regions. For high-molecular-weight DNA the pattern (interspersion in a variety of different orders) of repeated sequences may interfere with the reaction. For sheared DNA without repeated sequences (viral or bacterial), long chains of molecules are formed at the later stages of the reaction by pairing of single-stranded ends of reassociated molecules (*7*). None of these complications affect the conclusions drawn here and probably do not seriously affect the numerical estimate of the length of the repeated nucleotide sequence.
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15. A mixture of polyU and polyA [P. O. Ross and J. M. Sturtevant, *J. Amer. Chem. Soc.* **84**, 4503 (1962)], reassociates much more rapidly than mouse-satellite DNA.
16. The reassociation rate constants (K, liter mol^{-1} $second^{-1}$) at 50°C and 0.08*M* Na^+ for 800 atm sheared DNA are: SV40, $K = 12$; satellite, $K = 180$. Measurements with DNA sheared at 3300 atm and satellite purified on hydroxyapatite yield at 60°C and 0.12*M* Na^+ the following: *Escherichia coli*, $K = 0.040$; satellite, $K = 625$. On the assumption that SV40 contains 6×10^3 and *E. coli* 5×10^6 nucleotide pairs, the calculations give, respectively, 400 and 320 nucleotide pairs as the length of the repeated sequence of the satellite DNA.
17. We thank Drs. B. Hoyer and D. Axelrod of the NIH for discussion, mouse embryos, germ-free mouse DNA, and SV40 DNA; our colleagues at the Department of Terrestrial Magnetism for discussion; and M. Chamberlin for technical assistance.

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Thyrocalcitonin: Effect on Idiopathic Hypercalcemia

Abstract. *Identical twins having idiopathic hypercalcemia were treated with repeated doses of thyrocalcitonin extracted from porcine thyroid glands. The treatment produced a marked change in the amount of calcium and phosphate in the plasma and in the excretion of urinary calcium.*

Thyrocalcitonin (TCT) causes a decrease in the amount of calcium in the plasma in animals (*1*). When purified porcine TCT is administered to normal subjects (*2*), the effect is rapid and of short duration. However, the long-acting effect of the hormone when administered in repeated doses and in a slowly absorbing vehicle is not known. Evidence for a long-acting effect is provided by our study of 3-month-old male identical twins afflicted with the mild form of idiopathic hypercalcemia, associated with loss of appetite, vomiting, constipation, loss of weight, and increased bone density; hypocalcemic and hypophosphatemic effects were produced.

Thyrocalcitonin was extracted from porcine thyroid glands, purified, and prepared for injection (*2*). One dose of 100 units (*3*) of TCT in a buffered acetate solution was administered intravenously to test the hypocalcemic activity of the preparation (Fig. 1). The maximum response was reached after 1 hour. For example, in the plasma of subject F.C. the concentration of calcium decreased from 12.6 ± 0.25 to 11.5 ± 0.23 mg/100 ml, and that of subject S.C. decreased from 12.5 ± 0.25 to 10.9 ± 0.22 mg/100 ml. After 2 hours the values were: F.C., 11.7 ± 0.23; S.C., 11.0 ± 0.22. Within 4 hours the concentrations of plasma calcium returned to those observed before injection of thyrocalcitonin: F.C., 12.3 ± 0.25; S.C., 12.6 ± 0.25. To overcome this transient effect and to insure a hypocalcemia that would last from 8 to 12 hours, TCT was prepared with a slowly absorbing vehicle (17 percent gelatin), and at days 5 through 10, daily doses of 100 units of TCT were given intramuscularly. The plasma calcium dropped abruptly after the initial injection; it continued to fall during the period of administration and reached normal levels (Fig. 1). After TCT was discontinued there was a gradual increase in the plasma calcium, but the initial hypercalcemia was never attained during the entire period of observation. After 30 days the plasma calcium concentrations were 11.7 (F.C.) and 11.1 (S.C.) mg/100 ml.

Foster *et al.* (*4*) administered, by infusion, high doses of porcine TCT to three patients with hypercalcemia complicated by disseminated malignant disease. The plasma calcium concentrations were lowered but they never became normal.

Hypercalcemia in infancy can be mild or severe (*5*). The identical twins studied had a mild form of the disease, which is attributed by Kenny *et al.* (*6*) to abnormal metabolism of vitamin D. Forfar *et al.* (*7*) have suggested that the severe form of the disease results when the mild form is not treated. Since hypercalcemia can lead to nephrocalcinosis, it seemed particularly important to treat the condition by administering TCT to subjects maintained on a normal diet, rather than by administering cortisone or a low-calcium diet, or both, which would affect growth. Repeated TCT administra-