and glucuronidated MOPEG are found in about equal concentrations of 6 to 10 ng/ml in human plasma (*13*), whereas the sulfate and glucuronide conjugates of MOPEG predominate in equal concentrations of 0.5 to 0.7 μg/ml in human urine (*14*). Rat urine, like human urine, contains little free MOPEG, but the concentration of the sulfate conjugate (3 μg/ml) is about six times that of the glucuronide conjugate (0.5 μg/ml) (*14*). This radioimmunoassay is thus sufficiently sensitive to quantify MOPEG in tissue, cerebrospinal fluid, plasma, and urine and can therefore be used in many areas of biomedical research. For example, several groups of investigators (*15*) have suggested that the urinary levels of MOPEG prior to treatment are predictive of the therapeutic response to tricyclic antidepressants in some depressed patients. A recent prospective study supports this hypothesis (*16*). Only preliminary studies of cerebrospinal (*17*) and urinary levels of MOPEG in hypertension (*18*) and myocardial infarction (*19*) have been conducted, and the diagnostic and therapeutic implications of changes in the plasma concentration of MOPEG in these and other diseases have yet to be determined (*20*).

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Two Classes of Single-Stranded Regions in DNA from Sea Urchin Embryos

Abstract. *Native DNA from sea urchin embryos contains single-stranded regions (gaps) of up to 3000 nucleotides. The longer gaps (more than 1400 nucleotides) are nonrandomly distributed and are rich in histone gene sequences, other moderately repetitive sequences, and polypyrimidines. The shorter gaps are associated with DNA replication. A method for isolation of the two classes of single-stranded DNA pieces is reported.*

Isolated native eukaryotic DNA of diverse origins contains single-stranded regions (gaps) interspersed throughout the double helical DNA (*1-13*). Some of these single-stranded regions have been associated with DNA replication sites that were active in vivo at the time that the DNA was isolated. Other single-stranded regions may not be associated with replication and may be at nonrandom positions on the genome (*1, 4-6*). We now report some experiments that distinguish and characterize two classes of gaps in sea urchin embryo DNA; one is rich in replication forks or regions, and the other is rich in histone gene DNA sequences, with the gaps therefore nonrandomly distributed.

Under conditions in which single-stranded DNA is not cut, we have used the double-stranded DNA specificity of certain prokaryotic restriction endonucleases to generate a set of DNA pieces that include those containing the naturally occurring gaps. In some experiments, DNA from the morula-stage embryos of the sea urchin *Strongylocentrotus purpuratus* was isolated and cut into specific pieces with Hind III endonuclease. These pieces were separated by column chromatography with benzoylated naphtholated diethylaminoethyl cellulose (BND) into double-stranded pieces without gaps (eluted early from the column and shown in Fig. 1 as peak I) and pieces containing internal single-stranded regions of 20 nucleotides or more (*4, 14*). These single-stranded pieces of DNA were selectively retained on the column and then eluted by a caffeine gradient according to the length of the internal single strand for each DNA piece; the gaps ranged from 20 to 3000 bases per DNA piece, as eluted by a 0 to 1 percent caffeine gradient. The BND columns had been calibrated according to the total length of the single-stranded regions per DNA piece eluted at different concentrations of caffeine; the length of the double-stranded portions of any piece containing gaps was shown not to influence this calibration (see legend to Fig. 1). Figure 1 shows that the single-stranded pieces elute as two major peaks; one peak at about 0.15 percent caffeine (peak II) contains short gaps of about 500 nucleotides (the total length of single-stranded regions per DNA piece), and the other peak at about 0.5 percent

Table 1. DNA replication positions relative to gap length in DNA pieces. ^{3}H-labeled DNA from morula-stage sea urchin embryos was isolated, treated with restriction enzymes, and fractionated as described in the legend to Fig. 1, except that the embryos were exposed to [^{3}H]thymidine (1.0 μCi per milliliter of seawater medium; 20 Ci/mmole) for 3 minutes before the DNA isolation. Fractionation of the restriction pieces yielded those without gaps (< 20 bases per gap; fractions 3 to 7, in the vicinity of peak I), those with short gaps (20 to 500 bases per gap; fractions 9 to 12, in the vicinity of peak II), and those with long gaps (1400 to 3000 bases per gap; fractions 14 to 27, in the vicinity of peak III). Incorporation of ^{3}H was determined by scintillation counting. Unfractionated DNA (10^4 count/min; 100 percent) was treated with restriction enzymes and applied to a BND column. Means ± range for three experiments are shown.

DNA pieces	Percent of ^{3}H in DNA pieces labeled in vivo
Short gaps	69 ± 5
Long gaps	12 ± 3
Without gaps	13 ± 3
Unfractionated	100

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caffeine (peak III) contains long gaps of about 1500 nucleotides. Essentially the same elution profile as shown in Fig. 1 was obtained when restriction endonucleases other than Hind III were used to cut the native DNA before fractionation on BND.

Hybridization of fractions of labeled histone gene DNA that eluted under these conditions (Fig. 1) shows that the long gaps (peak III and the fractions eluting at the higher concentrations of caffeine) are rich in histone gene sequences. Labeled sea urchin ribosomal RNA (18*S* plus 28*S* ribosomal RNA's in equal molar amounts, isolated from sea urchin gastrulas) shows no hybridization preference for those restriction pieces of DNA that contain either short or long gaps, compared with an equivalent amount of unfractionated genomic DNA or with restriction fragments having no gaps (data not shown). In these hybridization experiments and in the experiment shown in Fig. 1, the fractionated fragments or unfractionated DNA's were denatured before hybridization to the labeled nucleic acid. The hybridization results are consistent with a nonrandom distribution of the longer gaps according to certain nucleotide sequences along the genome and strengthen earlier indications in both sea urchin embryos (*1*) and mammalian tissue culture cells (*4*) that some of the gaps are nonrandomly positioned. In addition, we have found, using the criteria of renaturation kinetics, that the long gaps are rich in sequences that are moderately repetitive and also for polypyrimidine sequences of 60 to 70 nucleotides (*1*).

Labeling of morula DNA in vivo with [^{3}H]thymidine for a 3-minute period predominantly labels the DNA replication sites or forks (*15*). Since mainly those DNA restriction fragments containing the shorter gaps (Table 1 and the vicinity of peak II shown in Fig. 1A) are labeled during this short period, the shorter gaps or the flanking double-stranded sections on the short-gap restriction pieces must be rich in sequences that are, or have recently been, active in DNA synthesis. These short gaps must therefore be random sequences if the replication sites or forks are randomly distributed, as is presumably the case for these mitotically asynchronous cultures of morulae.

Our results suggest that there are two functionally different classes of single-stranded regions in the DNA of the sea urchin morula. The positioning of long gaps at particular locations in the genome suggests that these gaps may be present as a feature or consequence of the state or utilization of the DNA in morulae; at the gastrula stage of sea urchin embryogenesis, DNA gaps are essentially absent (*1–3*). Clough (*16*) isolated from human cells a site-specific endonuclease that could account for the formation in vivo of gaps at specific positions in otherwise double-stranded DNA. Clough's data suggest that in vitro this endonuclease creates gaps of variable length after initial single-strand cuts at specific positions on bacteriophage lambda DNA. An enzyme with a similar activity has been isolated from *Chlamydomonas* by Burton *et al.* (*17*). These eukaryotic gap-generating enzymes are strikingly similar in function to *Escherichia coli rec BC* gene products (*16*). We therefore suggest that the long single-stranded regions we have described, if in register on homologous chromosomes of these rapidly dividing, differentiating cells, might bring about recombination between homologous sequences in these diploid cells. Unequal recombination by selective scrambling of sequences of unequal single-stranded DNA could lead to differences in the genomes of daughter

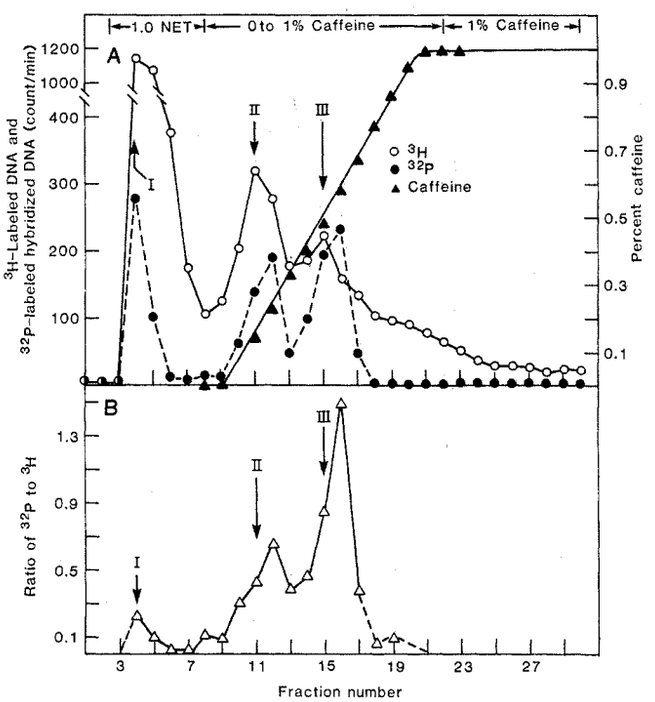

Fig. 1. Fractionation of Hind III restriction fragments of DNA from sea urchin morulae by chromatography with benzoylated naphtholated diethylaminoethyl cellulose (BND). The DNA from sea urchin morulae was labeled with ^{3}H (2×10^4 cpm/μg) by growing *S. purpuratus* embryos from fertilization in the presence of 2.0 μCi of [^{3}H]thymidine (22.7 Ci/mmole) per milliliter of seawater (*18*). After complete restriction under standard conditions of 20 μg of this DNA, the mixture of DNA fragments generated was adjusted to contain 0.3*M* NaCl, 1 m*M* EDTA, and 10 m*M* tris, at *p*H 7.4 (0.3*M* NET) and applied to a BND column (1 by 5 cm). The column was washed with 15 ml of 0.3*M* NET. Double-stranded fragments and those with gaps totaling less than 20 bases were selectively removed with 15 ml of 1.0*M* NaCl, 1 m*M* EDTA, and 10 m*M* tris, at *p*H 7.4 (1.0*M* NET). Fragments with gaps totaling 20 to 3000 bases were removed from the column according to gap length by elution with 40 ml of a 0 to 1 percent caffeine gradient in 1.0*M* NET. The columns were calibrated as described below. Finally the column was washed with 1 percent caffeine in 1.0*M* NET. Fractions from the column were denatured and applied to 24-mm nitrocellulose filters (B6, Schleicher and Schuell) (*19*). The filters were incubated for 6 hours at 68°C in Denhardt's solution (*20*) made up in double-strength SSC (SSC is 0.15*M* NaCl and 0.015*M* sodium citrate). Denatured histone gene DNA that had been labeled with ^{32}P was added to the filters, and incubation was continued overnight. The filters were washed in double-strength SSC, dried, and assayed for ^{3}H and ^{32}P by scintillation spectrometry. For these hybridizations, ^{32}P-labeled histone gene DNA was prepared by amplification (in *E. coli* HB101 cells) of plasmid pSR1, which bears H2A and H3 sea urchin histone genes on PMB9; $H_3{}^{32}PO_4$ (neutralized, carrier-free) was added to 2 μCi per milliliter of medium at a culture density of 3×10^8 cells per milliliter. At the same time chloramphenicol (250 μg/ml) was added (*21*) and, after growth overnight, the amplified ^{32}P-labeled plasmid DNA was isolated from Brij lysates on CsCl–ethidium bromide density gradients (*22*). The BND columns were calibrated according to the length of the single strand on DNA pieces eluted at increasing concentrations of caffeine. This was done by treating SV40 DNA with restriction enzymes Hae III or Eco RII, followed by electrophoresis; the separated gel products were then annealed to various lengths of single-stranded SV40 DNA. In this way, gaps of different lengths were created for various lengths of double-stranded DNA; with SV40 DNA, a range of 5000 bases of single-stranded DNA to 5000 base pairs of double-stranded DNA was obtained. Fragments eluted from the column according to the individual lengths of the single strands; the length of the double-stranded portion of any piece did not influence the concentration of caffeine required for elution of a particular single-stranded length of DNA. (A) Fractionation on BND of ^{3}H-labeled DNA restriction fragments and hybridization of ^{32}P-labeled histone gene-bearing plasmid DNA to the ^{3}H-labeled fractions. (B) Relative proportion of the eluted DNA fragments that are identifiable as histone gene DNA; this is shown as the ratio of ^{32}P to ^{3}H calculated from the data in (A).

cells forming differentiated embryo tissue. The long single-stranded regions might also be related to transcription of specific genes; the formation of gaps may be part of the mechanism by which histone genes are regulated during the mitotic cycle and early development.

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Regulation of Cutaneous Previtamin D_3 Photosynthesis in Man: Skin Pigment Is Not an Essential Regulator

Abstract. *When human skin was exposed to simulated solar ultraviolet radiation, epidermal 7-dehydrocholesterol was converted to previtamin D_3. During prolonged exposure to simulated solar ultraviolet radiation, the synthesis of previtamin D_3 reached a plateau at about 10 to 15 percent of the original 7-dehydrocholesterol content, and previtamin D_3 was photoisomerized to two biologically inert isomers, lumisterol$_3$ and tachysterol$_3$. Increases either in skin melanin concentration or in latitude necessitated increases in the exposure time to simulated solar ultraviolet radiation required to maximize the formation, but not the total content, of previtamin D_3. In order of importance, the significant determinants limiting the cutaneous production of previtamin D_3 are (i) photochemical regulation, (ii) pigmentation, and (iii) latitude.*

Although the photochemical pathway by which 7-dehydrocholesterol (7-DHC) in the skin is converted to previtamin D_3 (preD_3) by exposure to sunlight is established, little is known about the factors that directly influence the cutaneous photosynthesis of preD_3 (*1–4*). We exposed hypopigmented and hyperpigmented human skin to simulated solar ultraviolet radiation for various times and determined the photoproducts of 7-DHC in order to (i) study the sequential photochemical events quantitatively; (ii) examine regulatory processess, if any; (iii) investigate the role of melanin in the production of preD_3 in the skin; and (iv) determine the effect of latitude on preD_3 formation in the skin. In these experiments, the formation of preD_3 in hypopigmented skin reached a plateau after a short (15 minute) exposure to simulated noon equatorial ultraviolet radiation; further irradiation increased only the biologically inactive photoisomers, lumisterol$_3$ and tachysterol$_3$. As the melanin concentrations in skin increased, the time of exposure necessary to maximize the formation, but not the total content, of preD_3 also increased.

Surgically obtained hypopigmented (type III) and hyperpigmented (types V and VI) human skin specimens (*5*), obtained from different areas of the body with subcutaneous fat removed, were cut into samples (6.25 cm^2) and immersed in a water bath (60°C) for 30 seconds according to the method of Blank *et al.* (*6*). The skin was blotted dry, and the stratum-corneum side was exposed for various times to simulated solar ultraviolet radiation. The radiation source was a 2.5 kW xenon arc lamp (Schoeffel) coupled with a dichroic mirror and appropriate filters. Conditions were adjusted to approximate the ultraviolet radiation that reaches Earth in June at sea level at either the equator (0° latitude) or in Boston (42°20′N) at noon. Immediately after irradiation, the skin was separated into a top layer (stratum corneum, granulosum, and stratum spinosum) and a bottom layer (stratum basale and dermis). The basal cells (stratum basale) were mechanically scraped from the dermis. Control skin samples maintained in an ultraviolet-free environment for the same periods of time were similarly separated. The skin layers were confirmed histologically. The separated layers of skin were extracted with 8 percent ethyl acetate in *n*-hexane for 24 hours at −20°C. The extracts were centrifuged, and the supernatant was collected, taken to dryness under N_2, and weighed. A portion of each sample was then chromatographed in duplicate to determine the concentrations of 7-DHC, preD_3, lumisterol$_3$, and tachysterol$_3$ (Fig. 1, A to D).

The chromatography of the lipid extracts from skin was performed in ethyl acetate (8 percent) in *n*-hexane on a high-performance liquid chromatograph (HPLC) equipped with a radial compression module containing a Radial-Pak-B column (10 by 0.8 cm) coupled with an ultraviolet absorption detector at 254 nm (Waters Associates, model 440) and a printer-plotter Data Module Integrator (Waters Associates). This chromatographic system permits complete resolution to baseline of 7-DHC, preD_3, lumisterol$_3$, and tachysterol$_3$ (*2*). Quantitation of the concentration of 7-DHC, preD_3, lumisterol$_3$, and tachysterol$_3$ in skin lipid extracts was based on standard curves that were generated by plotting the integrated area under the peak as a function of known concentration of pure compound.

As standards for the chromatography we used [3α-^{3}H]7-DHC, [3α-^{3}H]-preD_3, [3α-^{3}H]tachysterol$_3$, and [3α-^{3}H]-lumisterol$_3$; each compound had a specific activity of 4.8 Ci/mmole and was synthesized as previously described (*2*). Five milligrams of 7-DHC were exposed to ultraviolet radiation (*2*) to generate sufficient quantities of preD_3, lumisterol$_3$, tachysterol$_3$, and D_3 for the vitamin-D binding-protein (DBP) assay and for the standard concentration curves. Identification of the skin lipid peaks shown in Fig. 1 was based on cochromatography studies with radioactive tracers (*2*). In addition, 7-DHC was structurally characterized (*3*), and lumisterol$_3$ was isolated in pure form from surgically obtained human thigh epidermis that was exposed to 3 hours of simulated equatorial solar ultraviolet radiation. Determination of the structure of lumisterol

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