samples were taken in a Philips Debye-Scherrer type powder camera (114.59 mm diameter) with the use of nickel-filtered copper radiation and exposure times of 2 to 4 hours. Samples were prepared for examination by mounting them in thin-walled glass capillaries (0.3 mm in diameter). The identity of the diffraction lines in the recorded patterns was established in two ways: (i) by visual comparison with the x-ray diffraction patterns of standard materials recorded under identical conditions; (ii) by comparing interplanar spacing, *d*, and the intensities, *I*, of the observed lines against published standards (Powder Diffraction File, American Society for Testing and Materials, Philadelphia, Pa.). For this latter comparison, the angular position of each diffraction line was measured to a precision of 0.05° 2θ and then converted to the corresponding *d*-value through use of appropriate tables [H. E. Swanson, *Tables for Conversion of X-ray Diffraction Angles to Interplanar Spacing*, National Bureau of Standards Applied Mathematics Series, vol. 10 (U.S. Government Printing Office, Washington, D.C., 1950). The relative intensities were estimated visually.

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Informational DNA Synthesis Distinguished from That of Nuclear DNA by Inhibitors of DNA Synthesis

Abstract. *With inhibitors of DNA synthesis used in the presence of ethidium bromide, it has been possible to distinguish between synthesis of informational DNA (I-DNA) and that of nuclear DNA. Hydroxyurea depresses I-DNA synthesis preferentially but does not affect DNA transport between cell compartments. 5-Fluorodeoxyuridine and cytosine arabinoside reduce synthesis of I-DNA to a much lesser degree than that of nuclear DNA.*

The discovery in chick embryos of a class of DNA's, informational DNA (I-DNA), which, it was suggested, represented copies of nuclear genes recovered in the cytoplasm was reported a little over a year ago (*1*). In response to the original paper Fromson and Nemer, on the basis of experiments with sea urchins, said that the finding was artifactual and represented contamination of the cytoplasm with nuclear DNA, which occurred during cell homogenization and fractionation (*2*). The Fromson and Nemer report has been cited uncritically (*3*).

If synthetic processes in developing sea urchin cells are like those in embryonic chick or mouse cells, Fromson and Nemer would have seen virtually *no* I-DNA synthesis during the 3-hour labeling period employed, especially since their experiments were carried out at 18°C, at which temperature rates of biochemical reactions must be substantially lower than those at 37°C. After labeling mouse or chick cells for 3 hours at 37°C, no 16*S* or larger-sized free I-DNA–containing particles (I-somes) can be observed in cytoplasmic extracts. It is therefore unlikely that Fromson and Nemer would have seen I-DNA in their cells, if it was being synthesized.

Nevertheless I have tried to settle the problem of whether the DNA in question arises in the nucleus and belongs there but not in the cytoplasm.

Control experiments which support the reality of I-DNA and describe its functional association with other particles under conditions of relatively high ionic strength, in the cytoplasm, have since been performed (*4–6*). However, to add to the weight of evidence needed to strengthen a heterodox view, we have continued to look for ways to distinguish between nuclear DNA and nonmitochondrial cytoplasmic DNA (I-DNA) of probable nuclear origin.

A new set of experiments has revealed fresh distinctions. By means of inhibitors of DNA synthesis, it has been possible to view separately the synthesis of I-DNA from that of nuclear DNA. It appears that I-DNA is much more sensitive to the inhibitory action of hydroxyurea (HU) than is nuclear DNA. The reverse is true when DNA synthesis is inhibited with 5-fluorodeoxyuridine (FUdR). A third inhibitor, cytosine arabinoside (CA), like FUdR, appears to favor the synthesis of cytoplasmic nonmitochondrial DNA but only of low molecular weight. Cytosine arabinoside blocks the formation of 16*S* and larger DNA-containing particles seen in extracts from control cells.

In general, for all of the experiments reported here, chick breast muscle from 11-day embryos was first incubated with an inhibitor of chromosomal DNA synthesis. [^{3}H]Thymidine was then added and the incubation was continued. Ethidium bromide (EB), which blocks mitochondrial DNA synthesis at a concentration of 1.0 μg/ml (*7*), was present throughout the incubation period. Cells of the tissue pieces were dissociated with trysin, and the washed cells were opened without homogenization (*8*) by means of Triton X-100. Nuclei were separated from the cytoplasm by centrifuging the extract at 10,000 rev/min in a Sorvall centrifuge at 4°C.

Hydroxyurea inhibits the synthesis of I-DNA; it affects the synthesis of nuclear DNA to a much lesser degree. In cells incubated for 5 hours with the inhibitor, the amount of labeled I-DNA that is recovered in the cytoplasm is 18.7 percent of that in the control cells, whereas the amount of labeled nuclear DNA recovered is 67.5 percent of that in the control cells, a 3.5-fold difference (Fig. 1A and Table 1).

In a similar experiment FUdR acts in an opposite sense (Fig. 1B and Table 1) when cells are incubated with the inhibitor continuously. During a 5-hour period of incubation, synthesis of nuclear DNA is reduced to about 9 percent (Table 1) of that in the control cells, while that of I-DNA is reduced to 44 percent of the control

Table 1. Incorporation of radioactivity (count/min) into nuclear DNA. Nuclei from cells used in the experiment described in the legend to Fig. 1 were dissolved overnight in 2 ml of SDS buffer (0.1*M* NaCl, 10^{-3}*M* EDTA, 0.5 percent sodium dodecyl sulfate, 0.01 tris; *p*H 7.4) made 1.0 percent with deoxycholate. Before a portion was taken for determination of radioactivity associated with DNA, the volume was raised to 3 ml with distilled water.

	HU	FUdR	CA
Control	3.74×10^6	2.79×10^6	1.73×10^6
Experimental	2.53×10^6	0.23×10^6	0.02×10^6

Table 2. Ratios of incorporation of [^{3}H]thymidine into nuclear and cytoplasmic DNA fractions in control as compared with that incorporated into inhibitor-treated cells. Cells were treated (pulse) with [^{3}H]thymidine (50 μc/ml) for 2 or 3 hours with an inhibitor present, then washed and returned to incubate with the same inhibitor, but with no isotope, for an additional period to bring the total incubation to 5 hours (chase). Ethidium bromide (1.0 μg/ml) was present throughout with experimental and control tissues.

Inhibitor	Pulse (hr)	Pulse and chase (hr)	Ratios* control/inhibitor
HU	3		0.99
CA	2		150.0
HU		5	0.274
CA		5	34.8
FUdR		5	5.4

* The ratio, control/inhibited = (count/min of nucleus control/count/min in cytoplasm control)/(count/min in nucleus inhibited/count/min cytoplasm inhibited).

(Fig. 1B), a fivefold difference. This I-DNA synthesized in the presence of FUdR as with HU, has the same sedimentation pattern in a sucrose gradient as I-DNA from control cells.

Cytosine arabinoside apparently favors the synthesis of DNA that is recovered in the cytoplasm. After a 2-hour incubation with [^{3}H]thymidine, a small amount of newly made DNA, from a cytoplasmic extract of control cells sedimented in a sucrose gradient, is observed in the region of polyribosomes and toward the top of the gradient. During a successive 3-hour period in Tyrode solution, after the labeling has been stopped, the amount of DNA cosedimenting with polyribosomes increases, and 16*S* as well as larger I-DNA–containing particles (I-somes) appear (control gradient Fig. 1C). These can be resolved by a longer centrifugation into 32, 27, 22, and 16*S* I-DNA–containing particles (*4*). Treatment with cytosine arabinoside eliminates the DNA which cosediments with polyribosomes and blocks the appearance of 16*S* to 32*S* I-somes, which are not seen 3 hours after labeling has been stopped (Fig. 1C). It does not reduce the amount of slowly sedimenting DNA (*4*) seen in a cytoplasmic extract after an incubation for 2 hours in [^{3}H]thymidine even though nuclear DNA synthesis is reduced 100-fold (Table 1).

The precise degree to which each of the inhibitors affects nuclear and I-DNA synthesis, respectively, is summarized in Table 2, in which the results

Table 3. Incorporation of [^{3}H]thymidine into nuclear and cytoplasmic cell fractions. The experimental procedure is described in the legend to Fig. 1. Results typify a large number of experiments.

Fraction	After 2 hours with isotope (pulse) (10^3 × count/min)	After 2 hours with isotope and 3 hours without isotope (pulse + chase) (10^3 × count/min)	Ratio pulse/chase
Nuclear	1455.4	3741.1	1:2.57
Cytoplasm	2.9	56.2	1:19.9

obtained after dividing the ratio of nuclear to I-DNA synthesis in control cells by that in the experimental cells are given. If the inhibitor affects the synthesis of nuclear DNA and I-DNA identically, the ratio would be unity. A ratio of less than one means a more pronounced depression of I-DNA synthesis than nuclear DNA synthesis, while a ratio greater than unity means the opposite.

Hydroxyurea appears to depress I-DNA preferentially, whereas FUdR

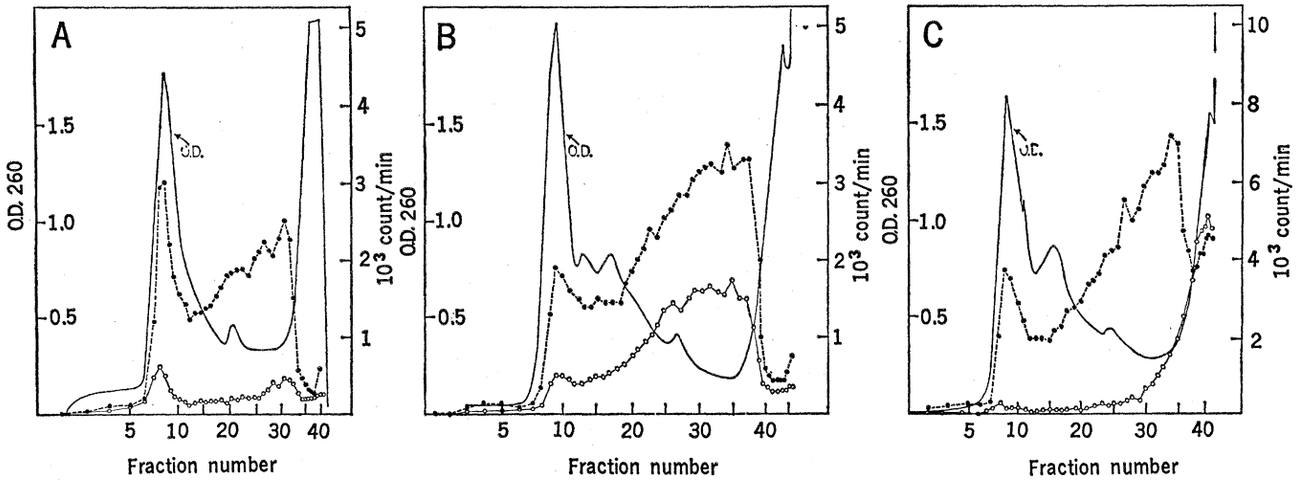

Fig. 1. Pieces of breast muscle from 11-day chick embryos (Spafas SPF) were incubated with ethidium bromide (1.0 μg/ml) (Calbiochem) in Tyrode solution for 30 minutes. The medium was then made $10^{-3}M$ with 5-fluorodeoxyuridine (gift of Hoffmann-La Roche), hydroxyurea (Aldrich), or cytosine arabinoside (Sigma); and [^{3}H]thymidine (Schwarz/Mann), labeled in the methyl group, was added to a concentration of 50 to 75 μc/ml. The labeling period was 3 hours with FUdR or hydroxyurea and 2 hours with CA. After labeling, tissues were washed and returned to isotope-free medium containing EB and one of the inhibitors. Controls contained EB but no other inhibitor. Tissues with FUdR or hydroxyurea were incubated for 2 hours after labeling while those with CA for 3 hours. At the end of the incubation period, tissues were washed and dissociated with 0.05 percent trypsin (NBC 1:300) for 6 minutes at 36°C; trypsin action was then neutralized with an equivalent amount of soybean trypsin inhibitor. Cells were washed, filtered through cheesecloth, sedimented, and resuspended in saline (*4*). Cells were then filtered through flannel and centrifuged. They were resuspended in "M" buffer (*4*), and Triton X-100 was added to a concentration of 0.5 percent. Centrifugation at 10,000 rev/min for 10 minutes (Sorvall SS-34 rotor) sedimented the nuclei. The supernatant was the cytoplasmic extract. Nuclei were not cleaned further since cleaning did not reduce the radioactivity noticeably. Cytoplasmic extracts were layered on 15 to 30 percent sucrose gradients having below them a 2-ml cushion of 60 percent sucrose. DNA associated with polyribosomes or cosedimenting particles or both accumulates at the interface between the cushion and the 30 percent sucrose. After centrifugation for 4.5 hours at 40,000 rev/min (SW-41 Spinco rotor), fractions were collected in a continuous flow-through Gilford spectrophotometer set to record at 260 nm, and the radioactivity in the acid-insoluble fraction was plated by conventional methods. (A) hydroxyurea; (B) FUdR; (C) Cytosine arabinoside; ○ - - ○ control; O.D., optical density.

and CA favor it. The comparatively small effect of HU on nuclear DNA synthesis has been observed in chick erythroblasts (*9*).

Experiments were designed to ascertain whether the reduction in radioactivity in the cytoplasm when cells were incubated continuously with HU was the result of (i) a transport block between the nucleus and cytoplasm in response to the inhibitor, or of (ii) preferential depression of the synthesis of I-DNA. Tissue was labeled with [^{3}H]-thymidine for 3 hours, then washed, and incubated without isotope for 2 hours more at 38°C but with $10^{-3}M$ HU. After the isotope was washed out and incubated for two more hours, not less, about 30 percent more I-DNA as compared to that in the control, was found in a cytoplasmic extract and distributed in a sucrose gradient (15 to 30 percent) in a manner identical to that of the control. Thus, the conclusion that HU affects synthesis and not transport seems strengthened.

The observation that recovery of newly made DNA in the cytoplasm after a chase (a period of incubation of cells in the absence of isotope after the cells have been labeled), is more than 19 times greater than that found after a pulse (the period of cell labeling), while the ratio of the nuclear pulse to chase radioactivity is 1:2.57 (Table 3), suggests that cytoplasmic I-DNA does not consist of short-chain intermediates (*10*) not yet ligated into chromosomes. Because of their size, short-chain intermediates would be expected to leak preferentially from the nucleus, and would be present during labeling in nuclei of cells in "S" phase of the mitotic cycle. Since tissue cells are not synchronized, some cells would be in "S" phase during all periods of labeling.

If I-DNA is an artifact of tissue preparation, "leaked" short-chain intermediates should have been in evidence in a cytoplasmic extract after labeling, but virtually none was; and no 16*S* to 35*S* DNA-containing particles were observed until after the chase. In fact, at the end of the labeling period we see a total of only 5 percent of the amount of labeled DNA recovered in the cytoplasm after the chase. Short DNA segments earmarked for incorporation into chromosomes would be expected to disappear during the chase as they became linked into large-sized DNA.

Thus, I-DNA does not behave as though it consisted of small intermediates; rather, sufficient time is needed for it to be seen in the cytoplasm, suggesting that prior processing is a prerequisite for its appearance. We have ruled out the possibility that nuclei become leaky after a period of culture, although this would account for the efflux of radioactivity during the chase. Radioactivity recoverable in the cytoplasm after a 3-hour labeling period is the same as that recoverable after a 3-hour labeling period preceded by a 3-hour period of incubation with no isotope present (*4*).

The apparent preferential synthesis of I-DNA, as compared with that of nuclear DNA, in the presence of FUdR might be attributed to the fragmentation of DNA, which the inhibitor is known to produce (*11*), and the subsequent real or artifactual loss of fragmented DNA from the nucleus. Rapid, naturally occurring loss or turnover of DNA in healthy, cultured rat tumor cells has been reported (*12*) and may be a common event under certain conditions of cell culture. Perhaps FUdR and CA, as well as other factors (*13*), might bring about the random release from the nucleus of DNA meant for incorporation into chromosomes; but nevertheless a normal mechanism probably exists for the replication release and transport of DNA into the cytoplasm (*2*).

The inhibitors CA or FUdR may produce fragments which the nucleus "sees" as future I-somes meant for delivery to the cytoplasm, and so processes them, or—as an alternative explanation—the inhibitors might synchronize the release of I-somes. The significance of I-DNA as a possible source of template RNA for use in protein synthesis and for cell differentiation has been discussed (*4*).

We have also isolated mitochondrial DNA from the chick; its buoyant density of 1.709 g/cm^3 determined by centrifugation in CsCl gradients is easily distinguishable from that of nuclear DNA which, in our experiments, bands at 1.700 g/cm^3 (*5*). To rule out further the possibility that the DNA recovered in the cytoplasm was mitochondrial, even though cells were labeled in the presence of EB 1.0 μg/ml, which blocks synthesis of mitochondrial DNA, the DNA was banded in CsCl saturated with EB (*14*). A reference marker having a buoyant density less than that of chick mitochondrial DNA was also included. For

Table 4. Effect of various treatments on cytoplasmic extracts from [^{3}H]thymidine-labeled cells. Cytoplasmic extracts were centrifuged in sucrose gradients (15 to 30 percent) after labeling and processing of tissue, as described for control gradients in the legend to Fig. 1. On one pair of gradients, the control and deoxyribonuclease-treated extracts were from homogenized cells not treated with EB. Radioactivity in fractions sedimenting more slowly than 12*S* was excluded, but sediments in the gradients were included in totals. Fractions were collected and treated. For alkaline hydrolysis, the fractions were made 1.0*M* with KOH, heated to 80°C for 1 hour, and neutralized with HCl. Deoxyribonuclease (Worthington DPFF, electrophoretically purified) was used at 100 μg/ml, and ribonuclease was used at 4 μg/ml, each for 30 minutes at 4°C.

	Radioactivity (count/min)		Reduction percentage
	Control	After treatment	
Deoxyribonuclease (homogenized)	144,500	40,500	72
Deoxyribonuclease (Triton X-100)	38,500	13,500	65
Ribonuclease (Triton X-100)	30,000	31,000	0
1.0*M* KOH (Triton X-100)	27,700	26,800	3

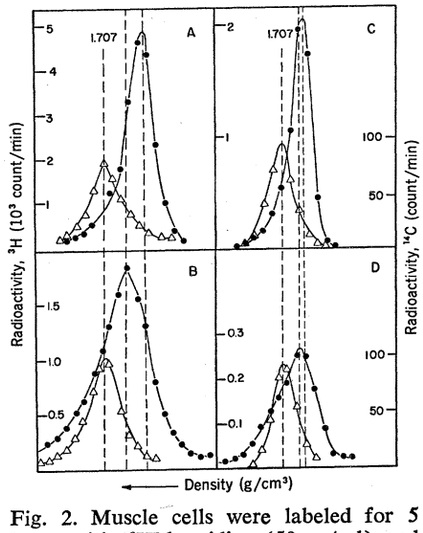

Fig. 2. Muscle cells were labeled for 5 hours with [^{3}H]thymidine (50 μc/ml) and processed as described in Fig. 1, to yield cytoplasmic and nuclear fractions; DNA was extracted from each by a modified Marmur (*16*) procedure. In (A) and (B) one sample of each of nuclear and I-DNA were banded in CsCl without ethidium bromide, as shown by ●——●. In (C) and (D) samples of each were also banded in a CsCl gradient with ethidium bromide (400 μg/ml) (*15*), as is shown by ●——●. A marker DNA which bands at 1.707 g/cm^3 in the absence of ethidium bromide was used as reference (*E. coli* ^{14}C marker △——△).

comparison, a sample of muscle nuclear DNA with marker was also banded. Both the I-DNA and nuclear DNA were less dense than the marker (Fig. 2, C and D). The EB shift (*14*) with circular mitochondrial DNA was not observed. In the absence of EB, chick mitochondrial DNA has a buoyant density of 1.708 g/cm^3 (*15*), that for the marker DNA is 1.707 g/cm^3, and that for the chick nuclear DNA is 1.701 g/cm^3.

The acid-insoluble radioactivity which results from labeling with [^{3}H]thymidine in the experiments reported herein is in DNA: it is ribonuclease insensitive, it resists base hydrolysis, is digestible by deoxyribonuclease (Table 4), and bands as DNA in CsCl (Fig. 2). The ratios of the absorbancies at 260 and 280 nm of purified DNA is 1.95 after deproteinization; treatment with amylase, ribonuclease, and pronase; banding in CsCl; and elution from hydroxyapatite. On denaturation and banding the buoyant density increases (*5*).

Although we have reported that I-DNA and nuclear DNA have the same buoyant densities (*3*), here they differ slightly (Fig. 2, A and B). This result has been observed in three separate experiments and may reflect a difference in base composition between I-DNA and nuclear DNA.

In conclusion, the difference in sensitivity of nonmitochondrial cytoplasmic DNA, which we have called I-DNA, as compared with nuclear DNA, to various inhibitors of DNA synthesis makes it entirely unlikely that I-DNA arises as an artifact of tissue fractionation.

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DDT Residues: Distribution of Concentrations in Emerita analoga (Stimpson) along Coastal California

Abstract. *The total concentrations (tDDT) of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane], and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] in* Emerita analoga *from 19 California beaches reflect tDDT contamination nearby. Animals near the Los Angeles County sewer outfall contain over 45 times as much tDDT as animals near major agricultural drainage areas. Sediments near the outfall probably contain over 100 metric tons of tDDT—a reservoir for input into marine organisms. The effluent from a plant that manufactures DDT is a probable source.*

Marine organisms from the waters off southern California consistently contain more DDT, DDD, and DDE (tDDT) (*1*) than those taken from near Monterey and San Francisco bays, despite the fact that these latter areas receive drainage from extensive agricultural areas (*2*). This observation has been attributed to a general southerly drift of DDT-laden aerial particles coupled with an input from sewer systems (*2, 3*). I here report on a determination of the relative importance of the various inputs of tDDT into the California coastal waters and provide a profile of the extent of tDDT contamination along the coast of California.

Emerita analoga (Stimpson), the common surf-zone sand crab, was selected as an indicator organism because it is a widely distributed particulate filter feeder in which individual range is confined to at most a few kilometers (*4, 5*). Most oceanic tDDT not found in living organisms is adsorbed onto particles in the size range from 4 to 2000 μm selected by *Emerita* (*5, 6*). *Emerita* will also rapidly take up DDT not associated with particulate material. This ability was demonstrated when 25 animals were placed in 25 liters of seawater (filtered through glass fibers) containing 7.8 parts per trillion (ppt) of ring-labeled [^{14}C]DDT and left there for 24 hours. No sand was provided, and therefore the animals were not expected to feed; feeding was not observed during intermittent observations. The DDT was extracted with hexane from an acid digest (*7*) and counted with a scintillation counter (Nuclear-Chicago Unilux II). Each of the animals contained an average of 1016 ppt of [^{14}C]DDT or 325 times the final seawater concentration. This uptake was largely active. In a similar experiment 58 live animals took up an average of 50 times as much [^{14}C]DDT as did ten animals killed by exposure to Dry Ice; the dorsal carapace and telson, major surfaces for passive adsorption, analyzed separately from the rest of the animals in two cases, accounted for less than one-fifth of the label.

Female animals, each weighing over 2 g, with a mean weight of 3.4 g, were collected from 19 beaches between the Golden Gate Bridge in San Francisco and a point 5 km south of Ensenada, Mexico. Since 36 animals, each over 1 g in weight, collected from a single location in October 1970 showed no correlation between the size of animal and the amount of tDDT, no correction factor for size was deemed appropriate.

I blotted the animals dry, removing the eggs when present, and placed them on Dry Ice within 30 minutes of the time of capture. Within 50 hours the samples were weighed and placed in individual DDT-free shell vials for acid digestion, hexane extraction, and subsequent cleanup on a silica-gel column (*7*). The extracts contained compounds with retention times and partition coefficients matching (within 2 to 3 percent) standards of the DDT series. No interfering chromatographic peaks were present, the large aggregate of peaks characteristic of polychlorinated biphenyls was not present, and the base line was always clearly defined (*8*). The results of the gas-liquid chromatograph-