Deoxyribonucleic Acid Sequences Complementary to Ribosomal Ribonucleic Acid in a Crustacean*

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ABSTRACT: Three per cent of the deoxyribonucleic acid of the land crab *Gecarcinus lateralis* is comprised of a (G + C)-rich satellite that has a base composition similar to, if not identical with, the deoxyribonucleic acid which codes for ribosomal ribonucleic acid in other species. Although, as shown by saturation experiments, about 2.5% of the genome of *Gecarcinus* is specific for ribosomal ribonucleic acid, the hybridization does not occur with the (G + C)-rich satellite, but rather with deoxyribonucleic acid slightly denser than the peak of the main-band deoxyribonucleic acid. These data imply that the cistrons for ribosomal ribonucleic acid are present in multiple copies but are not tightly clustered.

The DNA of the land crab, *Gecarcinus lateralis*, contains two satellites in addition to the main component (Skinner, 1967). One of the satellites, comprising 3–4% of the total DNA, is rich in deoxyguanylate and deoxycytidylate residues and has a density in CsCl of 1.721 g/cm^3. Such d(G + C)-rich satellites have also been reported in other organisms (M. Birnstiel and J. G. Gall, personal communications). The (G + C)-rich satellite of *Gecarcinus* appears to be very similar in composition to the DNAs containing the rRNA cistrons (rDNA)[1] in *Xenopus laevis* (Wallace and Birnstiel, 1966; Birnstiel *et al.*, 1968; Brown and Weber, 1968a,b; Gall, 1968).

The base composition of crab rRNA is (G + C)-rich (Skinner, 1968) as is that of *Xenopus* (Birnstiel *et al.*, 1968) and HeLa cells (Zimmerman *et al.*, 1963). Moreover, the rRNA content of certain crab tissues increases as much as eightfold while the animal is preparing for ecdysis (Skinner, 1968, and unpublished observations). In certain other organisms there is an amplification of the rRNA cistrons preceding periods of heightened ribosome synthesis in the oocytes (Miller, 1964, 1966; Brown and Dawid, 1968; Gall, 1968). It therefore seemed possible that the demand for increased numbers of ribosomes in the premolt period of the crab might require a large percentage of its DNA to be composed of rRNA cistrons, and that these might be localized in the d(G + C)-rich satellite, even though the proportion of the DNA comprising the satellites does not change very markedly at any time in the molt cycle (D. E. Graham and D. M. Skinner, unpublished observations). Accordingly, we have determined the association of crab rRNA with total crab DNA and with DNA that has been fractionated in CsCl isopycnic density gradients. From the former experiments we find that some 1.1–1.4% of the total DNA may be hybridized with rRNA, implying that rRNA cistrons (including the complementary DNA strands) make up about 2.5% of the total DNA. From the latter experiments, however, we find that the rRNA does not associate with the d(G + C)-rich satellite, but with the dense side of the main DNA peak. The results suggest that the rRNA cistrons, although present in multiple copies, are not clustered together as they are in some other organisms (Birnstiel *et al.*, 1968; Brown and Weber, 1968a,b).

Materials and Methods

Animals. Specimens of the land crab *Gecarcinus lateralis* were obtained from the Bermuda Biological Station and maintained as described previously (Skinner, 1962). Only intermolt animals were used.

Reagents. [5-C-^{3}H]Uridine (16–20 Ci/mmole) was obtained from New England Nuclear Corp. or Schwarz BioResearch, Inc.; [*methyl*-^{3}H]thymidine (6.7 Ci/mmole) and carrier-free ^{32}P (as P_i) were obtained from New England Nuclear Corp. Electrophoretically pure DNase and beef pancreatic RNase were obtained from Worthington Biochemical Corp. Pancreatic RNase was heated to 90–100° for 10 min. Bac-T-Flex B7 (S-S) filters were obtained from Schleicher & Schuell Co. Dialysis tubing was prepared according to Roblin's (1968) method.

Isolation of RNA. 1. *Gecarcinus* rRNA. Crabs were injected with 5–10 mCi of ^{32}P; 4 days later, ^{32}P-labeled RNA was isolated from the midgut gland (hepatopancreas) with cold phenol as described (Skinner, 1968). From this, rRNA was precipitated with molar NaCl (Crestfield *et al.*, 1955), washed in molar NaCl, and fractionated into 28S and 18S components by centrifugation through linear sucrose gradients (Skinner, 1968); 28S and 18S rRNAs were precipitated with 66%

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[1] rDNA is the DNA sequences homologous to rRNA.

ethanol in 0.1 M NaCl, solubilized in one-tenth standard saline citrate, adsorbed to methylated albumin kieselguhr columns, and eluted with linear gradients of 0.2–1.2 M NaCl–0.05 M sodium phosphate (pH 6.7) (Brown and Weber, 1968a). Each purified rRNA was dialyzed overnight against water and its volume was reduced. Before use, it was filtered one to three times through S-S filters soaked in standard saline citrate (Gillespie, 1968).

2. *Xenopus* rRNA. *Xenopus* liver cells were grown as previously described (Regan *et al.*, 1968). [^{3}H]Uridine was introduced into the medium to a final concentration of 8 μCi/ml. After a labeling period of 8 hr, the radioactive medium was replaced with fresh medium containing 0.3 M nonradioactive uridine; 35 hr later (the extended "chase" period was considered necessary to eliminate labeled mRNA) the cells were washed three times in 66% Hanks' medium and the final pellet was frozen at −70°. rRNAs (28S and 18S) were prepared by Brown and Weber's (1968a,b) method except that phenol was removed by two precipitations from 0.01 M potassium acetate (pH 5) with 66% ethanol in 0.1 M NaCl instead of passage through Sephadex.

3. HeLa rRNA. HeLa cells were grown in medium 199 (Morgan *et al.*, 1950) with 10% calf serum until the cells formed a nearly confluent monolayer. They were labeled with [^{3}H]uridine and chased with cold uridine in the same manner as *Xenopus*, except that the period of labeling was 24 hr followed by an 18-hr chase. rRNA was isolated with cold phenol (Hiatt, 1962) from purified ribosomes (Attardi and Smith, 1962; Salb and Marcus, 1965). The purity of the rRNA was checked by centrifugation on a sucrose gradient. All *Gecarcinus*, HeLa and *Xenopus* rRNA preparations were subjected to MAK chromatography, since in at least some organisms, mRNA is eluted at a different salt concentration from rRNA (Ingle, 1965).

DNA was isolated from crab testes as described (Skinner, 1967). [^{3}H]Thymidine-labeled DNA was isolated from crabs that had been injected with a total of 400 μCi of [^{3}H]thymidine on 4 consecutive days. DNA was fractionated by centrifugation for 66–70 hr at 25° in CsCl isopycnic density gradients in an angle head rotor at 33,000 rpm (Flamm *et al.*, 1966).

Hybridization was performed essentially according to Gillespie and Spiegelman (1965). In all experiments the DNA was fractionated on CsCl density gradients as described above. Each fraction of the CsCl gradient was denatured with alkali (0.15 N NaOH) for 10 min at room temperature, neutralized, brought to six-times standard saline citrate, and immobilized on a S-S filter (which had been soaked in six-times standard saline citrate for 1–2 hr) by passage through a Fisher filtrator at a rate of 1 ml/min or less. In the experiments with 28S and 18S crab or *Xenopus* rRNA, equal aliquots of each DNA fraction of an individual CsCl gradient were immobilized on two filters. The DNA-impregnated filters were dried and exposed to rRNA in two- or six-times standard saline citrate as described (Gillespie and Spiegelman, 1965). At the end of the incubation period, filters were twice treated with pancreatic RNase (30–50 μg/ml) for 1–1.5 hr at room temperature. They were dried and counted in toluene containing 4 g of 2,3-bis[2-(5-*t*-butylbenzoxasolyl)]thiophene/l. Each sample was counted to a minimum of 10,000 counts or, when the radioactivity was low, for 1 hr. Counts bound to blank filters which were incubated with equal amounts of radioactive RNA as were the experimental filters were subtracted from counts bound to the experimental filters. The same lot of filters was used in all experiments.

Most of the hybridization experiments were run in six-times standard saline citrate. Since it has been reported that such high salt concentrations promote nonspecific binding (Church and McCarthy, 1968), we ran several experiments in two-times standard saline citrate for comparison. After subtraction of the RNA blanks, the extent of saturation and the position of the hybridization peak were the same in both salt concentrations (*cf.* Figure 5a,b *vs.* 5c).

Determination of the Molecular Weight of Gecarcinus DNA. The molecular weight of the DNA was determined either from the width of DNA peaks in CsCl isopycnic density gradient centrifugations in the Model E analytical ultracentrifuge (see Skinner, 1967; Thomas and Pinkerton, 1962) or by sedimentation velocity in 5–20% sucrose gradients (Burgi and Hershey, 1963). Varying amounts (from 6–30 μg) of *Gecarcinus* DNA were run with less than 1 μg of marker DNA isolated from ^{3}H-labeled λ phage in 5 M neutralized $NaClO_4$ (Freifelder, 1967). Since 1 M $NaClO_4$ floats on 5% sucrose–1 M NaCl (Freifelder, 1967) and since the sedimentation of native DNA is independent of ionic strength between 0.01 and 1 M (Studier, 1965), gradients were made 1 M in NaCl.

Results

Preparation of rRNA. As shown by acrylamide gel electrophoresis and sucrose gradient analyses, the two components of crab rRNA have sedimentation coefficients of 28 and 18 S (Skinner, 1968). In this respect, as well as in their base composition, the two species of crab rRNA correspond closely to those of HeLa cells (Skinner, 1968; Zimmerman *et al.*, 1963). Sucrose gradient analysis of the radioactive crab rRNA used in the present hybridization experiments shows the ^{32}P label in the 28S and 18S regions (Figure 1). The preparations contain no measurable quantities either of heavy precursors of rRNA or of 23S and 16S RNAs which would be indicative of bacterial contamination.

In some experiments, in order to remove any methylated albumin contaminating the preparations (Gillespie, 1968), we filtered the rRNA sequentially through three filters soaked in standard saline citrate at the same concentration used in the subsequent hybridization. The filters were then dried and counted. The first filter invariably contained more counts (by as much as four-fold) than the other two. In all cases, the second and third filters contained less than 2% of the total counts filtered. After we increased the number of prefiltrations from one to three, the counts in the RNA blanks were constant over an input range of 2.5–100 μg (see Figure 3 and unpublished observations).

Binding of DNA to Filters. The binding of denatured

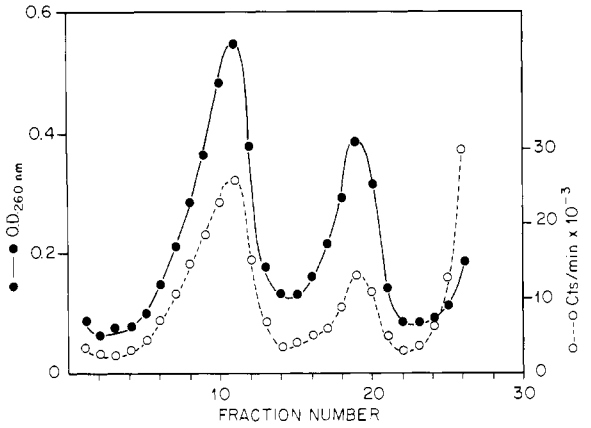

FIGURE 1: Sucrose gradient pattern of ^{32}P-labeled midgut gland RNA. RNA was not precipitated with molar NaCl; therefore, small molecular weight material was present in the 5–20% sucrose gradient containing 0.1 M NaCl, 0.01 M EDTA, 0.001% sodium dodecyl sulfate, and 0.01 M potassium acetate (pH 5). This was centrifuged in a Spinco SW65 rotor for 5.5 hr at 38,000 rpm, 4°. Sedimentation was from right to left. (●—●) OD_{260nm} and (○---○) ^{32}P.

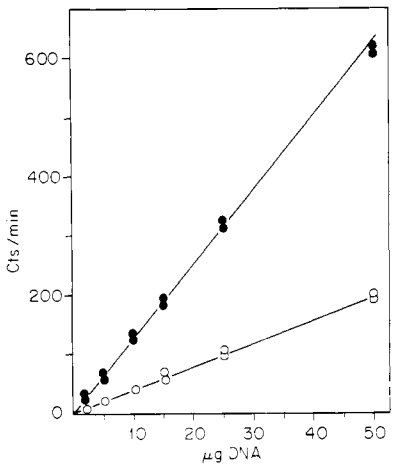

FIGURE 2: DNA binding to filters. DNA was immobilized on S-S filters that were incubated in six-times standard saline citrate for 20 hr at 66° and counted as experimentals. (●) Main-band DNA and (○) total DNA.

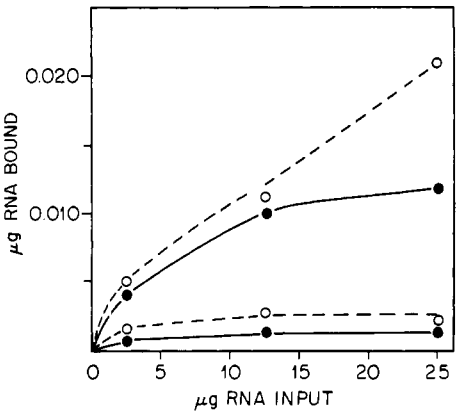

FIGURE 3: Saturation curve of crab rRNA with crab DNA before and after chromatography on methylated albumin kieselguhr. DNA was fractionated on a CsCl gradient. Fractions including main-band DNA and both satellites (see tubes 7–28, Figure 5) were pooled, denatured, and immobilized on filters (1 μg of DNA/filter). Upper 2 curves: 1 μg of DNA/filter; lower 2 curves: no DNA on filters; (●—●) rRNA (2250 cpm/μg) which had been subjected to chromatography on methylated albumin kieselguhr; (○---○) aliquot of same preparation of rRNA (2320 cpm/μg) which had not been subjected to chromatography on methylated albumin kieselguhr.

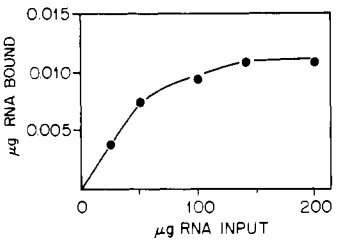

FIGURE 4: Saturation curve of crab rRNA with crab DNA. DNA purified by CsCl centrifugation as in Figure 3. Increasing amounts of crab rRNA (840 cpm/μg) which had been chromatographed on methylated albumin kieselguhr were added in two-times standard saline citrate.

DNA to nitrocellulose filters is shown in Figure 2. Either purified main-band (upper curve) or total DNA (lower curve), from different preparations labeled with [^{3}H]thymidine at different specific activities, was denatured, neutralized, and filtered. The filters were dried (Gillespie and Spiegelman, 1965), then incubated in six-times standard saline citrate for 20 hr at 70°. In both cases, the binding was linear from 2.5 μg to at least 50 μg/filter, which is well above the maximum amount of DNA in any one fraction used in the subsequent experiments. Moreover, more than 95% of the DNA was retained.

Figure 3 compares the saturation curves of a single preparation of RNA, half of which has been chromatographed on a MAK column and the other half of which has not. At the lower values of input RNA the two curves are very similar. At higher values the binding of the material which has been through MAK columns begins to level off whereas the binding of the unchromatographed RNA continues to increase linearly. These data suggest that (1) MAK chromatography removes from the RNA a small amount of material (mRNA?) which has sequences homologous to DNA and which continues to bind at very high values of input RNA; (2) at low inputs of RNA, this material contributes very little to the amount bound; and (3) basic protein (*i.e.*, methylated albumin), which can potentially give spurious results in hybridization experiments by binding to filters and contributing, in turn, nonspecific RNA binding sites (Gillespie, 1968), is not a problem in the present experiments. The third point follows from the observation that both the RNA blanks and the experimental points reach saturating levels; if basic protein

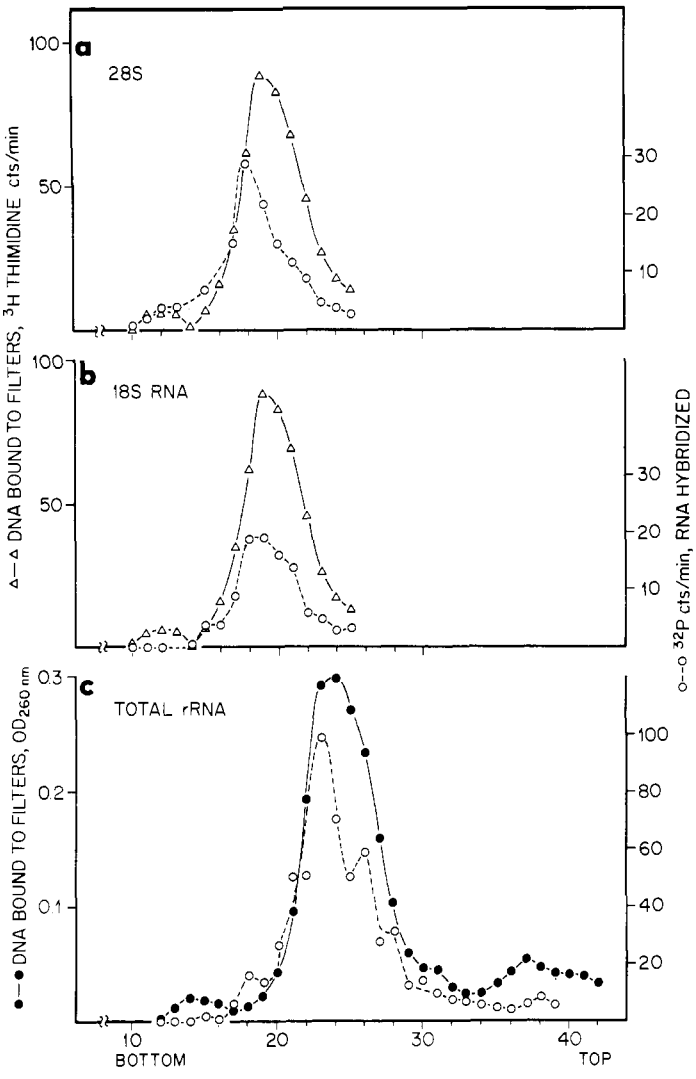

FIGURE 5: Hybridization of crab rRNA with crab DNA [^{3}H]thymidine- (150 μg) labeled DNA were centrifuged in a CsCl isopycnic density gradient. (a) Half of each fraction (10–25) of a CsCl gradient was immobilized on filters; to one set of filters in six-times standard saline citrate was added 43 μg of crab 28S rRNA (1000 cpm/μg); to the other set was added (b) 28 μg of 18S rRNA (950 cpm/μg). Incubation time was for 22 hr at 70°. (○---○) ^{32}P and (△—△) ^{3}H. (c) Half of each fraction (12–39 of a second CsCl gradient) was immobilized on filters; 40 μg of rRNA (3500 cpm/μg; 28 plus 18 S) in two-times standard saline citrate were added to each tube. (●—●) OD_{260nm} and (○---○) ^{32}P.

contaminants were present, the increased RNA input would be accompanied by an increased contaminant input and binding would increase indefinitely.

Saturation Experiments. Figure 4 shows a curve of DNA saturation with crab rRNA in a typical hybridization experiment. The curve was obtained under conditions such that more prolonged incubation would not yield higher values nor would repeated posthybridization treatment with RNase yield lower values. In this instance, the DNA was saturated when 1.1% was associated with rRNA. Since hybridization presumably occurs with only one strand of the denatured DNA, allowance for the complementary strand indicates that 2.2% of the total DNA is complementary to rRNA. Five such experiments gave values ranging from 2.2 to 2.8%. This value is an order of magnitude greater than the values previously obtained for somatic tissues from other species (McConkey and Hopkins, 1964; Ritossa and Spiegelman, 1965; Merits *et al.*, 1966; Huberman and Attardi, 1967; Brimacombe and Kirby, 1968; Brown and Weber, 1968a; Steele, 1968).

RNA Hybridization with Fractionated DNA. DNA-containing fractions from a preparative CsCl gradient were immobilized on filters and exposed to a fixed, subsaturating amount of rRNA. Under these conditions, the peaks of hybridization serve to identify that component of DNA containing the ribosomal cistrons (Wallace and Birnstiel, 1966; Birnstiel *et al.*, 1966; Brown and Weber, 1968a). The conditions of the individual experiments are given in the legends to the relevant figures (Figures 5–7).

Total rRNA (combined 28 and 18S) from the crab hybridizes with DNA which is slightly denser than the main component DNA (Figure 5c). Using the equation of Sueoka (1961) and a density of 1.701 g/cm^3 for main-band DNA (Skinner, 1967), we calculate the density of the DNA with which peak binding occurs to be 1.704 g/cm^3. There is no preference for the d(G + C)-satellite (fractions 12–17). That the two rRNA species are individually associating with DNA in this position may be seen in Figure 5a,b. In this experiment a DNA sample was fractionated as usual and the two halves of each fraction were immobilized on separate filters that were incubated with purified 28S and 18S crab RNA, respectively. Although the number of counts was low, each peak is defined by at least seven points. As before,

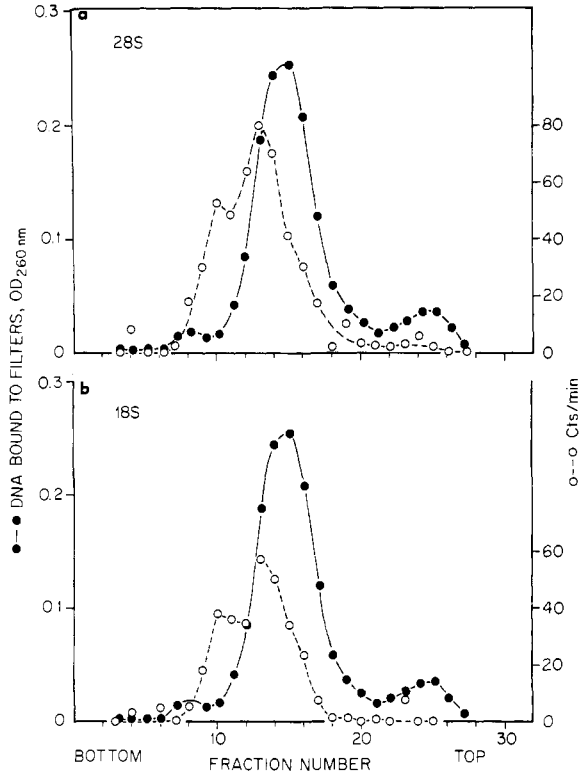

FIGURE 6: Hybridization of *Xenopus* rRNA with crab DNA. The experiment was carried out as in Figure 5, except that (a) 7.5 μg of 28S rRNA (19,000 cpm/μg) and (b) 5.1 μg of 18S rRNA (18,000 cpm/μg) from *Xenopus* were added to the two sets of filters impregnated with unlabeled crab DNA. (●—●) OD_{260nm} and (○---○) ^3H.

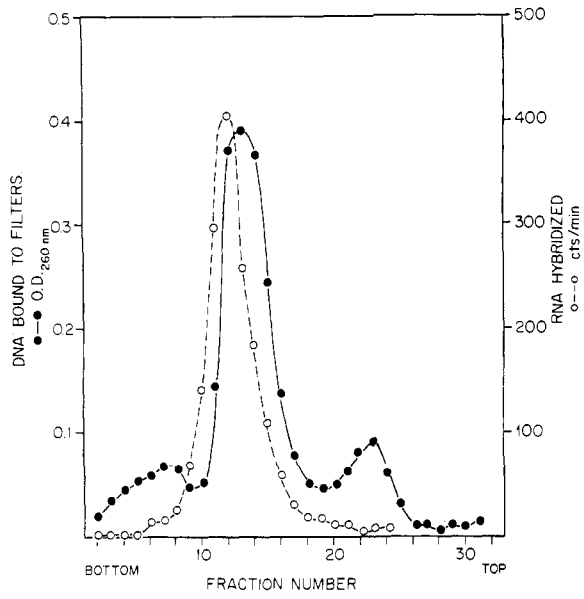

FIGURE 7: Hybridization of HeLa rRNA with crab DNA. The experiment was carried out as in Figure 5, except that 6.3 μg of total rRNA (32,000 cpm/μg) from purified HeLa ribosomes was added to filters impregnated with unlabeled crab DNA from fractions 2–24 of a CsCl density gradient. (●—●) OD_{260nm} and (○---○) ^3H.

the peak association of both RNA species was with DNA with a density of 1.704 g/cm^3. Similar observations were made by Steele (1968) for the hybridization of rat rDNA with rat rRNA.

Because there is believed to be at least partial homology between rRNAs of various metazoans (Brown *et al.*, 1967) and because the rDNA of *Xenopus laevis* has a density almost identical with that of the d(G + C)-rich satellite of *Gecarcinus*, we have measured the hybridization of fractionated crab DNA with *Xenopus* rRNA, isolated from cultured liver cells by the method of Brown and Weber (1968b). Again, the peak association occurs with DNA of density 1.704 g/cm^3 (Figure 6). Although for both the 28S and 18S *Xenopus* RNAs there was a shoulder on the denser side of the peak, there was no indication of either a peak or a shoulder in the region of the d(G + C)-rich satellite.

Methods for the isolation of purified ribosomes have not been devised for either *Gecarcinus* or *Xenopus*. Such methods, however, are available for HeLa cells (Attardi and Smith, 1962; Salb and Marcus, 1965) and they yield cleaner preparations of rRNA. In order to avoid contamination of our radioactive rRNA with radioactive ribosomal-bound mRNA, we purified ribosomes from HeLa cells which had been labeled for one generation with [^{3}H]uridine and subsequently subjected to a chase for a second generation time with 1000 times as much unlabeled uridine. Finally, 28S and 18S rRNA were isolated from the purified ribosomes. When CsCl-fractionated crab DNA was incubated with total HeLa rRNA, the peak association occurred once again in the fraction of density 1.704 g/cm^3 (Figure 7).

In comparison of Figures 6 and 7 with Figure 5 it is obvious that the *amount* of rRNA bound by the crab DNA is greater for the homologous than either of the heterologous rRNAs. Although interpretations of such data at subsaturating levels are uncertain, these results are what would be expected if there are only partial interspecific homologies between rDNA and rRNA.

In summary, rRNAs from three different phylogenetic classes bind to the DNA of *Gecarcinus*, a fact which is consistent with the earlier observation of Brown *et al.* (1967) that at least partial homologies exist between the rRNAs of very diverse species. Of principal interest to the present work is the fact that, in all three cases, these RNAs hybridize with crab DNA of the same density (ρ = 1.704 g/cm^3) and show no special affinity for the d(G + C)-rich satellite which has density characteristics similar to the rDNAs of *Xenopus*.

Molecular Weight of the Crab DNA Used in These Experiments. In order to determine whether clustering of rRNA cistrons occurs in *Gecarcinus*, it was necessary to measure the molecular weight of the DNA. In the preparation of the DNA for these experiments, we used methods aimed at yielding a pure product; no special effort was made to avoid shearing the DNA. Assuming that the main component of the DNA is homogeneous, we estimate from the width of the peaks of a typical preparation in analytical centrifugation in CsCl that

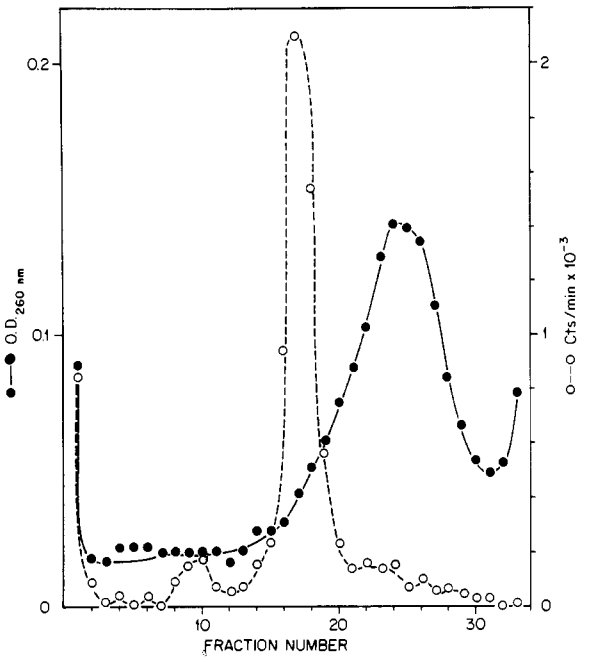

FIGURE 8: Sucrose gradient pattern of [^{3}H]thymidine-labeled λ DNA cosedimented with unlabeled *Gecarcinus* DNA. Crab DNA (30 μg) was mixed with an optically indetectable amount of [^{3}H]thymidine-labeled λ DNA on top of a 5–20% sucrose gradient which was 1 M in NaCl and 0.05 M sodium phosphate (pH 6.7). Centrifugation was done in an SW-65 rotor at 40,000 rpm for 3 hr at 10°. Sedimentation was from right to left. (●—●) OD_{260nm} and (○---○) ^{3}H.

the molecular weight of the DNA is on the order of 2×10^7. The existence of heterogeneity in this peak would tend to make this a minimum estimate. The heterogeneity is apparent from the sedimentation pattern of another DNA preparation centrifuged through a 5–20% sucrose gradient with ^{3}H-labeled λ DNA. The broad peak of the crab DNA (Figure 8) contains pieces ranging in molecular weight (Studier, 1965) from approximately 5–20 $\times 10^6$.

Discussion

We believe that the observed binding is specifically by rRNA and not mRNA contamination for the following reasons: (1) We would expect the peak of association of crab mRNA to coincide with the peak of its homologous DNA, and not be skewed toward higher densities as is observed; on the other hand, we would expect the rRNA association to be found at the higher densities (see below). (2) It seems unlikely that the mRNAs of *Gecarcinus*, *Xenopus*, and human cells would be so similar (see Figures 5–7); on the other hand, homologies between rRNAs of widely divergent metazoan species do exist (Brown *et al.*, 1967). (3) In our RNA preparations the specific activities of the two rRNA peaks were very similar to each other. In the experiments in which 28S and 18S crab rRNA isolated from a single preparation were hybridized separately with paired fractions from a single CsCl gradient (Figure 5a,b), the ratio of counts bound to DNA was the same as the ratio of radioactivity in the two rRNA peaks on a sucrose gradient. Such a result could be due to mRNA contamination only if the labeled mRNA were distributed throughout the sucrose gradient in a manner identical with the distribution of the rRNA. (4) In the cases of both *Xenopus* and HeLa cells, actively growing cultures were subjected to extended "chases" with unlabeled uridine for a full generation after the incorporation of radioactive precursor; in the case of HeLa cells, the rRNA was isolated from previously purified ribosomes. Under the conditions used in these experiments, at the end of one generation, less than 5% of the label in HeLa cells should be in non-rRNA (Girard *et al.*, 1965). Since the subsequent chase period equaled more than 5 half-lives of mRNA in HeLa (Penman *et al.*, 1963), the amount of label in mRNA should be reduced to less than 0.15% of the total. This figure, which is a *maximum*, is based on the assumption that the isolation methods are optimal for extracting all RNAs, whereas the extraction methods used here were selected for the preferential extraction of rRNA. The amount of binding in Figure 7 exceeds 0.15% of the input; that is, it exceeds the maximum amount of mRNA present even without assuming that 100% of the mRNA could bind to the heterologous crab DNA.

The molecular weight of the combined 28S and 18S rRNAs is on the order of 2×10^6. If more than 2% of the DNA codes for rRNA, as it appears to do, there must be many copies of the rRNA cistrons in each nucleus, although we cannot calculate the exact number without a reliable estimate of the amount of DNA per cell. If, in the extreme case, the rRNA cistrons were tightly clustered; *i.e.*, if they existed sequentially on the DNA, we would expect to find in this sheared material that the rDNA would have a density roughly corresponding to the d(G + C)-content of the rRNA. Such is the case in *Xenopus* (Brown and Dawid, 1968; Birnstiel *et al.*, 1968). *Gecarcinus* rRNA is 58% d(G + C) (Skinner, 1968); thus, a DNA of equivalent composition would have a density of 1.722 g/cm^3 in CsCl. Since the rRNA does not associate with DNA of this density, or of a density even close to this value, ribosomal cistrons do not appear to be tightly clustered in *Gecarcinus*.

The alternative extreme is that each piece of DNA containing rDNA contains, *at most*, one cistron for 28S and one cistron for 18S RNA (many pieces may, of course, contain only fragments of rDNA). In this case, some 10% of the DNA piece of molecular weight 2×10^7 would be 58% d(G + C). If we assume the remaining 90% of the piece has a base composition like the peak of the main DNA component [38% d(G + C)], the total composition of such pieces will be approximately 40% d(G + C), and they have a buoyant density in CsCl of 1.703 g/cm^3. Although these calculations are only approximate, the assumption of no more than one cistron per DNA piece fits our observations much more closely than the assumption that ribosomal cistrons are clustered. Whether each 28S gene is closely linked to an 18S gene cannot be ascertained from the present data.

A third extreme possibility is that each rRNA cistron is adjacent to a DNA segment of high d(A + T) content such that the total base composition of the cistron and the adjacent segment is 40% d(G + C). In the same category there exists the possibility that rDNA contains a high percentage of bases other than adenylate, cytidylate, guanylate, and thymidylate with the result that the buoyant density does not directly reflect the G + C content (Schildkraut *et al.*, 1962). In either case, clustering could not be recognized in these experiments.

Although these experiments do not define the biological role of the d(G + C)-rich satellite, they show that it does not contain the cistrons for rRNA.

Acknowledgments

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