McCarthy, B. J., and Duerksen, J. D. (1970), *Cold Spring Harbor Symp.* (in press).
McCarthy, B. J., and Hoyer, B. H (1964), *Proc. Nat. Acad. Sci. U. S. 52*, 915.
McCarthy, B. J., and McConaughy, B. L. (1968), *Biochem. Genet. 2*, 37.
McConaughy, B. L., and McCarthy, B. J. (1970), *Biochem. Genet. 4*, 425.
Pardue, M. L., and Gall, J. G. (1970), *Science 168*, 1356.
Paul, J., and Gilmour, R. (1966), *J. Mol. Biol. 15*, 242.
Paul, J., and Gilmour, R. (1968), *J. Mol. Biol. 34*, 305.
Paul, J., and Gilmour, R. (1969), *J. Mol. Biol. 40*, 137.
Schwartz, M. N., Trautner, T. A., and Kornberg, A. (1962), *J. Biol. Chem. 237*, 1961.
Skinner, D. M., Beattie, W. G., Kerr, M. S., and Graham, D. E. (1970), *Nature (London) 227*, 837.
Smith, K. D., Church, R. B., and McCarthy, B. J. (1969), *Biochemistry 8*, 4271.
Smith, M. (1963), *Biochem. Biophys. Res. Commun. 10*, 67.
Southern, E. M. (1970), *Nature (London) 227*, 794.
Sueoka, N. (1961), *J. Mol. Biol. 3*, 31.
Taper, H., Wooley, G. W., Teller, M. N., and Lardis, M. P. (1966), *Cancer Res. 26*, 143.
Teng, T. Y., and Sueoka, N. (1964), *Science 143*, 1442.
Waring, M., and Britten, R. J. (1966), *Science 154*, 791.
Yasmineh, W. G., and Yunis, J. J. (1969), *Biochem. Biophys. Res. Commun. 35*, 779.
Yunis, J. J., and Yasmineh, W. G. (1970), *Science 168*, 263.

A Comparison of the Primary Structures of 28S and 18S Ribonucleic Acid from HeLa Cells and Different Human Tissues*

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ABSTRACT: The primary structures of 28S and, respectively, 18S RNA from HeLa cells have been compared to those of the high molecular weight rRNA species from different human tissues by several methods at different levels of resolution: base composition analysis, oligonucleotide mapping after pancreatic RNase digestion, analysis of the pattern of degradation by mild digestion with pancreatic or T1 RNase, and RNA–DNA hybridization.

By all these methods no significant difference could be detected between homologous ribosomal RNA components from HeLa cells and various human tissues. It is concluded that the 28S RNA and, likewise, the 18S RNA preparations from different human sources analyzed in the present work are very similar, if not identical. The significance of these results with respect to the problem of the heterogeneity of rRNA genes is discussed.

A redundancy in the genetic information for the two high molecular weight rRNA components and for 5S rRNA has been demonstrated by RNA–DNA hybridization in bacteria and, of two or more orders of magnitude greater, in eukaryotic cells (see review by Attardi and Amaldi, 1970). In HeLa cells, a line of human origin, the number of genes has been found to be about 1000/cell for each of the two high molecular weight components, 28S and 18S RNA (Jeanteur and Attardi, 1969), and about 7600 for the 5S RNA (Hatlen and Attardi, 1971). This redundancy of information raises the problem of the possible variability of the rRNA genes.

The existence of such variability in a higher organism could result either in heterogeneity of the populations of each of the rRNA components within the same cell, or, if all rRNA genes are not equally expressed in different cell types of the same organism, in differences between rRNA preparations from different tissues or developmental stages of the same organism.

Reich *et al.* (1963) had reported substantial differences in base composition between samples of unfractionated rRNA from different tissues of the same animal species, more so than between RNA samples prepared from the same tissues of different animal species. These results, however, were not confirmed by Hirsch (1966), who found the same base composition in 28S RNA, and, respectively, in 18S RNA, purified from various rat and rabbit tissues. Likewise, other investigators could not detect any difference in base composition between preparations of the two high molecular weight rRNA components or of unfractionated rRNA isolated from different developmental stages of the same organism (Lerner *et al.*, 1963; Henney and Storck, 1963; Brown and Gurdon, 1964; Slater and Spiegelman, 1966; Tata, 1967; Grummt and Bielka, 1968). Gould *et al.* (1966) analyzed by polyacrylamide gel electrophoresis the products of limited digestion by T1 ribonuclease of rRNA (unfractionated) prepared from different organisms and from two cell types, reticulocytes and liver cells, of the same organism (rabbit): while they found differences in the pattern of degradation of the rRNA from different organisms, the rRNA from the two rabbit cell types analyzed gave identical results. Similarly, RNA–DNA hybridization experiments failed to show any difference in sequence between high molecular weight rRNA components prepared from rabbit reticulocytes and liver (Di Girolamo *et al.*, 1969), or from different developmental stages of sea urchin (Mutolo and Giudice, 1967).

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The above-mentioned negative results could conceivably reflect the insensitivity of the analytical methods employed. In order to approach the problem at a finer level of analysis, in the present work, the method of oligonucleotide mapping previously described (Amaldi and Attardi, 1968) has been used to compare the distribution of nucleotide sequences released by pancreatic RNase in 28S and 18S RNA prepared from HeLa cells and from different human tissues. The results obtained by this method as well as by other methods (base composition studies, sedimentation analysis after limited RNase digestion, and RNA–DNA hybridization) indicate that, even at the highest level of resolution employed here, there are no detectable differences between the 28S RNA and, likewise, 18S RNA preparations analyzed.

Materials and Methods

Cells and Tissues. Reference is made to a preceding paper (Amaldi and Attardi, 1968) for the method of growth of HeLa cells.

Human tissues were mainly autoptic material, and in a few cases surgical material, derived from male and female individuals (from 6 months to 67 years in age). The tissues were frozen as soon as possible and kept at −70° until used for RNA extraction.

Labeling Conditions. The method utilized to label HeLa cell rRNA to a high specific activity with [^{32}P]orthophosphate has been described previously (Attardi *et al.*, 1965b).

RNA Extraction. ^{32}P-labeled 28S and 18S RNA were extracted from the purified 50S and 30S ribosomal subunits, respectively; nonradioactive HeLa rRNA was isolated from total cells; rRNA of human tissues, on the contrary, was extracted from the monomer–polysome fraction. The methods of isolation of ribosomal subunits, extraction and fractionation of rRNA into the 28S and 18S components have been previously described (Amaldi and Attardi, 1968).

Base Composition and Sequence Analysis. The procedure utilized for base composition analysis and oligonucleotide mapping after pancreatic RNase digestion have been reported in detail in a preceding paper (Amaldi and Attardi, 1968).

Hybridization Experiments. RNA–DNA hybridization and isolation of the hybrids have been carried out by RNase digestion and Sephadex chromatography as described previously (Attardi *et al.*, 1965a). In some experiments, the RNA–DNA hybrids were collected directly on Millipore membranes after RNase digestion (30 μg/ml, 30 min at 37°) and washed with 100 ml of 0.5 M KCl–0.01 M Tris buffer (pH 7.0) at 60°.

Partial Degradation of RNA by Mild Digestion with Pancreatic or T1 Ribonuclease. The nonradioactive RNA to be analyzed (about 100 μg) was mixed with a small amount of ^{32}P-labeled HeLa 28S RNA in a volume of 0.4 ml of SSC (SSC: 0.15 M NaCl–0.015 sodium citrate). Treatment with pancreatic RNase (five-times crystallized, Sigma Chemical Co., heated at 80° for 15 min) was carried out at 2° for 20 min with 0.005 μg of enzyme; in some experiments, the limited digestion was carried out with 1 unit of T1 RNase (Sankyo Co., Ltd., Tokyo). At the end of the digestion, sodium dodecyl sulfate was added to a final concentration of 0.5%, and the sample was layered on a 5–20% sucrose gradient in SSC and centrifuged in the SW25.3 rotor of the Spinco L ultracentrifuge at 25,000 rpm for 20 hr at 2°. The collected fractions were analyzed for ultraviolet absorption and for ^{32}P acid-precipitable radioactivity, as previously described (Attardi *et al.*, 1966).

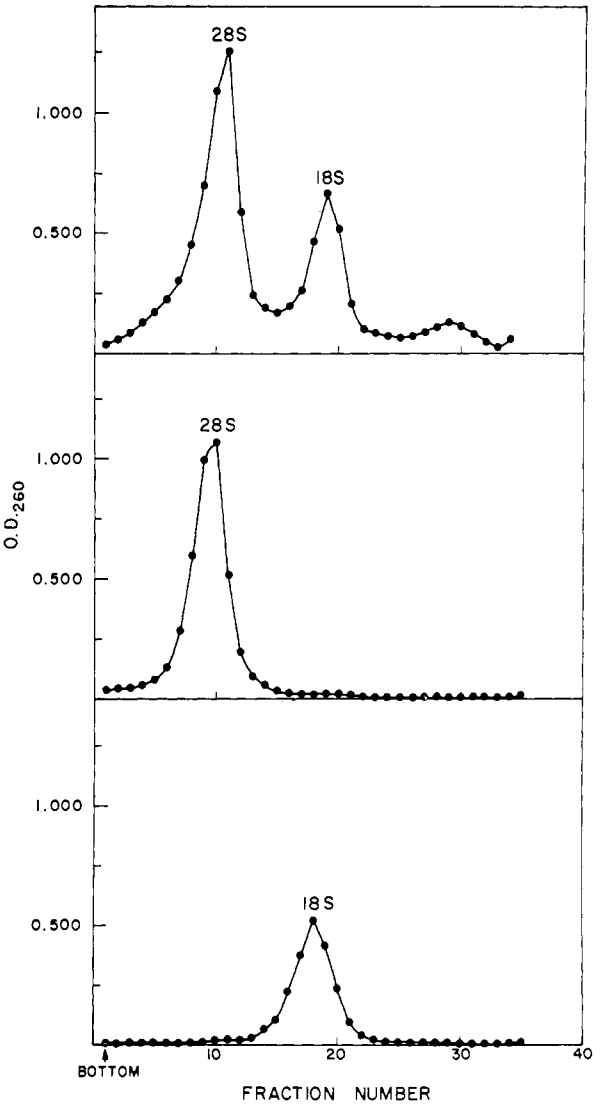

FIGURE 1: Sedimentation pattern of rRNA extracted from the monomer–polysome fraction of human liver. The RNA was extracted from this fraction with sodium dodecyl sulfate–phenol (Attardi *et al.*, 1965b) and separated into 28S and 18S components by two cycles of centrifugation in 5–20% (w/w) sucrose gradient in 0.01 M acetate buffer (pH 5.0)–0.1 M NaCl (16 hr at 23,000 rpm, 2°, in the SW 25-1 Spinco rotor).

Results

Isolation of 28S and 18S rRNA from HeLa Cells and from Human Tissues. The isolation and the criteria of purity of 28S and 18S RNA (^{32}P labeled and nonlabeled) from HeLa cells have been discussed previously (Amaldi and Attardi, 1968).

The RNA of human tissues was mainly prepared from autoptic material; for this reason it was expected that in many cases it might have undergone some degradation. As criteria to distinguish intact from slightly degraded RNA preparations a ratio of 28S to 18S RNA in the first sucrose gradient centrifugation of about 2.5 and the lack of material between the 18S and the 4–5S peaks were used (Amaldi and Attardi, 1968). Only those tissue RNA preparations which satisfied these criteria have been utilized in the present work. As an example, Figure 1 shows the sedimentation pattern of an undegraded rRNA preparation from human liver.

Base Composition. Table I summarizes the results obtained for the base composition of the two rRNA components ex-

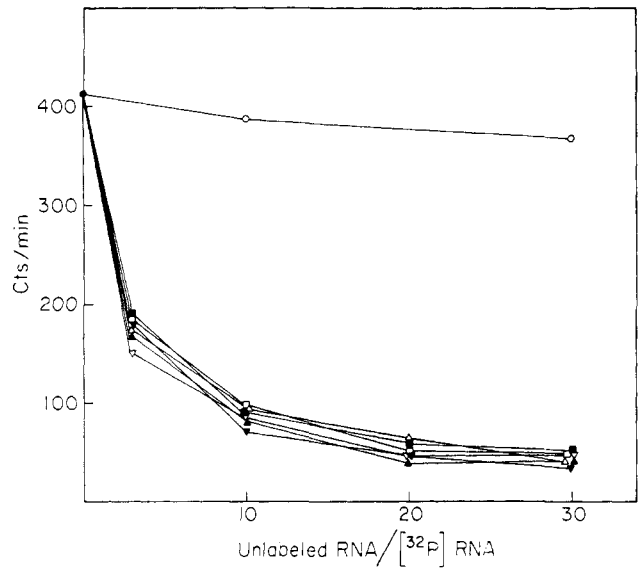

FIGURE 2: Tests of competition for sites in HeLa DNA between ^{32}P-labeled HeLa cell 28S RNA and unlabeled 28S RNA from HeLa cells and different human tissues. Each incubation mixture contained, in a volume of 3 ml of 2X SSC, 50 μg of HeLa DNA, 1.0 μg of ^{32}P-labeled HeLa 28S RNA, and variable amounts of unlabeled 28S RNA from different source. The incubation was carried out at 70° for 4 hr and the RNA–DNA hybrids collected on Millipore membranes after RNase treatment and washed as described in Materials and Methods. (○) *E. coli* 235 RNA; (□) Hela 285 RNA; (■) human liver 285 RNA; (△) human spleen 285 RNA; (▲) human kidney 285 RNA; (▽) human pancreas 285 RNA; (▼) human brain 285 RNA.

TABLE I: Nucleotide Composition of 28S and 18S RNA from HeLa Cells and Different Human Tissues.[a]

RNA Source	Moles % Cp	Ap	Up + ψp	Gp	No. of Determinations
28S RNA					
HeLa cells	32.1	15.8	17.0 (1.1)	35.1	2
Human liver	32.3	15.6	16.4	35.7	2
Human spleen	32.2	15.9	16.7	35.2	2
Human kidney	32.6	15.6	16.9	34.9	2
Human pancreas	32.6	15.6	16.6	35.2	2
Human brain	30.8	16.6	17.0	35.6	1
18S RNA					
HeLa cells	27.8	19.9	21.3 (1.5)	31.0	5
Human liver	28.0	19.9	21.6	30.5	2
Human spleen	28.5	20.5	21.5	30.5	2
Human kidney	27.9	19.7	21.4	31.0	2
Human pancreas	28.1	19.8	21.5	30.6	2
Human brain	28.2	19.9	21.5	30.4	1

[a] The nucleotide composition of the unlabeled rRNA components from HeLa cells and different human tissues was determined from the optical density measurements on the basis of the extinction coefficients of the four 2′,3′-nucleotides (The extinction coefficients reported by Beaven *et al.* (1955) were utilized here, after correction for the different pH of the individual elution media.)

tracted from different human tissues and from HeLa cells. It appears that the base compositions of all the 28S RNA and, respectively, all the 18S RNA preparation analyzed are, within experimental error, identical.

The amount of pseudouridylic acid in the rRNA from human tissues has not been precisely determined, but it appears to be present in about the same proportion as in HeLa cells (Amaldi and Attardi, 1968).

Partial Sequence Analysis. The analysis of frequencies of mono-, di-, and trinucleotides released by pancreatic RNase digestion has been carried out as described in detail in a preceding paper (Amaldi and Attardi, 1968). The method of the relative specific activity has been utilized here. In brief, this method consists in carrying out the RNase digestion and DEAE chromatography on a mixture of a relatively large amount of an unlabeled RNA species and a small amount of a ^{32}P-labeled RNA species and in determining the specific activity of the individual RNase digestion products (up to trinucleotides), and then normalizing it to the average specific activity (thus obtaining the relative specific activity). By using either the unlabeled or labeled RNA as an internal standard, one can compare different labeled or, respectively, unlabeled RNA samples. Although this method does not give absolute values but only relative values for the frequencies of the products of RNase digestion, it is very accurate and can reveal small differences between various RNA preparations.

^{32}P-labeled HeLa 28S RNA has been used in the present work as an internal standard to compare the nonradioactive preparations of 28S RNA (and 18S RNA) from HeLa cells and from different human tissues. Table II summarizes the results obtained by this analysis. In the table the results are expressed as ratios between the relative specific activities obtained in experiments utilizing ^{32}P 28S RNA from HeLa cells and nonradioactive 28S RNA (or 18S RNA) from different human tissues and the relative specific activities obtained in experiments utilizing ^{32}P 28S RNA both and nonradioactive 28S RNA (or 18S RNA) from HeLa cells. The values thus obtained give the frequencies of the oligonucleotide sequences in the HeLa cell RNA relative to those found in the tissue RNA. It appears that the frequencies of the oligonucleotides (up to trinucleotides) released by pancreatic RNase digestion are very similar in all the 28S RNA preparations analyzed and, likewise, in all the 18S RNA preparations. The data for 18S RNA show a somewhat higher variability than those for 28S RNA, but always within the experimental error. This is probably due to the difficulty to obtain a completely undegraded rRNA from human tissues; as a consequence the 18S RNA from this source is slightly contaminated by fragments of 28S RNA (to a different extent in different preparations).

Hybridization Experiments. As a different approach to the problem of the relationship between rRNA from different cell types of the same organism, RNA–DNA hybridization experiments were performed between HeLa cell DNA and ^{32}P-labeled HeLa 28S RNA (or 18S RNA) in the presence of an excess of nonradioactive 28S RNA (or 18S RNA) from HeLa cells (as a control) or human tissues.

Figure 2 shows one of these experiments utilizing 28S RNA. It appears that the 28S RNA from all the tissues analyzed competes with ^{32}P HeLa 28S RNA for hybridization with the rRNA sites in the DNA to the same extent as unlabeled HeLa 28S RNA.

Similar results have been obtained in hybridization competi-

TABLE II: Ratios of Frequencies of Partial Nucleotide Sequences in 28S, and, Respectively, 18S RNA, from HeLa Cells and Different Human Tissues.[a]

RNase Digestion Product	28S RNA				18S RNA			
	Liver (9)[b]	Spleen (4)	Kidney (5)	Pancreas (4)	Liver (4)	Spleen (4)	Kidney (4)	Pancreas (3)
Cp	0.998 ± 0.017	0.990 ± 0.021	1.006 ± 0.013	0.998 ± 0.015	0.989 ± 0.016	0.989 ± 0.015	0.985 ± 0.016	0.979 ± 0.025
Up	1.009 ± 0.015	1.006 ± 0.027	1.026 ± 0.026	1.019 ± 0.020	1.037 ± 0.044	1.013 ± 0.023	1.032 ± 0.053	1.035 ± 0.056
ApCp	0.998 ± 0.020	0.993 ± 0.016	0.990 ± 0.021	0.995 ± 0.013	0.982 ± 0.018	0.971 ± 0.032	0.984 ± 0.042	0.963 ± 0.044
ApUp	1.044 ± 0.038	1.041 ± 0.042	1.053 ± 0.036	1.043 ± 0.042	1.071 ± 0.016	1.039 ± 0.059	1.081 ± 0.105	1.107 ± 0.056
GpCp	1.001 ± 0.013	0.994 ± 0.013	0.989 ± 0.019	0.983 ± 0.021	0.972 ± 0.031	0.979 ± 0.071	0.970 ± 0.035	0.960 ± 0.044
GpUp	1.016 ± 0.017	1.026 ± 0.039	1.007 ± 0.023	1.010 ± 0.019	1.032 ± 0.033	1.019 ± 0.043	1.020 ± 0.033	1.030 ± 0.065
ApApCp	1.013 ± 0.032	1.025 ± 0.033	0.991 ± 0.025	1.010 ± 0.028	1.069 ± 0.067	1.062 ± 0.082	1.089 ± 0.093	1.059 ± 0.053
ApApUp	1.046 ± 0.038	1.044 ± 0.045	0.996 ± 0.050	1.043 ± 0.062	1.024 ± 0.064	1.013 ± 0.083	1.056 ± 0.088	1.069 ± 0.127
GpApCp + ApGpCp	0.986 ± 0.018	0.984 ± 0.033	0.968 ± 0.019	0.976 ± 0.029	1.031 ± 0.043	1.035 ± 0.036	1.036 ± 0.048	1.046 ± 0.050
GpApUp	1.008 ± 0.032	1.017 ± 0.044	0.984 ± 0.023	1.008 ± 0.038	1.032 ± 0.086	1.068 ± 0.140	1.068 ± 0.072	1.099 ± 0.084
ApGpUp	0.983 ± 0.030	0.984 ± 0.042	0.963 ± 0.037	0.965 ± 0.039	1.044 ± 0.110	0.934 ± 0.068	0.995 ± 0.056	1.027 ± 0.067
GpGpCp	0.982 ± 0.018	1.008 ± 0.012	1.016 ± 0.017	1.018 ± 0.019	0.988 ± 0.075	1.039 ± 0.077	0.990 ± 0.049	0.989 ± 0.029
GpGpUp	1.000 ± 0.016	1.015 ± 0.031	0.998 ± 0.018	1.011 ± 0.011	1.078 ± 0.026	1.064 ± 0.031	1.061 ± 0.065	1.043 ± 0.049

[a] The data summarized in this table were derived from various experiments involving the use of ^{32}P-labeled HeLa cell 28S RNA prepared from the 50S subunits and unlabeled 28S or 18S RNA from HeLa cells and from different human tissues, isolated, respectively, from total cells and from the monomer–polysome fraction. The figures reported here represent the average ratios between relative specific activities (rsa) of pancreatic RNase digestion products obtained in experiments utilizing HeLa ^{32}P 28S RNA and unlabeled 28S RNA (or 18S RNA) from different human tissues and the relative specific activities obtained in experiments utilizing HeLa ^{32}P 28S RNA and unlabeled HeLa 28S RNA (or 18S RNA): as explained in the test, each ratio represents the ratio of the frequencies of a given nucleotide sequence in HeLa cell RNA and tissue RNA. (See a previous paper (Amaldi and Attardi, 1968) for the procedure utilized for the calculation of relative specific activities.) Each mean is given with its standard error multiplied by a factor corresponding to a probability of 95%, as determined from the t distribution (Fisher, 1950). The standard error of the ratios was calculated according to the formula: $S_{\bar{x}/\bar{y}} = [(\bar{x}^2(S_{\bar{y}})^2 + \bar{y}^2(S_{\bar{x}})^2)/\bar{y}^4]^{1/2}$, where $S_{\bar{x}}$ is the standard error of $\bar{x}$ and $S_{\bar{y}}$ that of $\bar{y}$. [b] The number in parentheses indicates the number of tissue preparations tested; the averages of 8 rsa values for HeLa ^{32}P-28S-HeLa unlabeled 28S mixtures and 5 rsa values for HeLa ^{32}P 28S-HeLa-unlabeled 18S mixtures were used in the calculation of the ratios in the table.

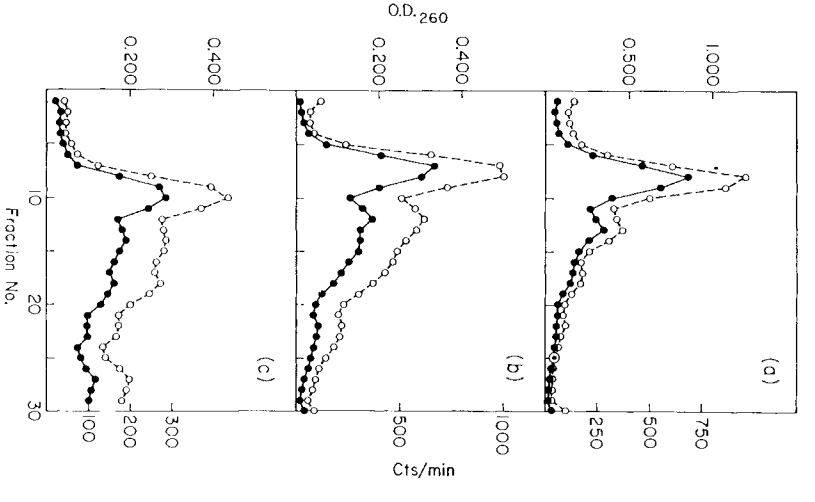

FIGURE 3: Sedimentation analysis in sucrose gradient of 28S RNA from HeLa cells (a), human liver (b), and human spleen (c) after mild digestion with pancreatic RNase. A mixture of nonradioactive RNA from different source (about 100 μg) and ^{32}P-labeled HeLa 28S RNA (<1 μg) was digested with pancreatic RNase and analyzed in sucrose gradient as described in Materials and Methods. (O---O) OD_{260} and (●—●) cpm of ^{32}P.

tion experiments carried out with ^{32}P HeLa 18S RNA and an excess of nonradioactive 18S RNA from HeLa cells or different human tissues.

RNA Degradation by Mild RNase Digestion. Another approach has been attempted to reveal small differences between 28S RNA preparations from various human sources. It was reasoned that minor differences in nucleotide sequences of these RNA molecules could give rise to significant differences in secondary structure, such as to be revealed by changes in sensitivity to RNase. For this reason, the RNA samples were treated, under very mild conditions, with pancreatic or T1 RNase, and the partially degraded material was analyzed by sucrose gradient centrifugation. A similar approach, involving the analysis of the electrophoretic pattern of the resistant fragments after limited T1 RNase digestion, has been utilized to compare rRNA samples from various sources (Gould *et al.*, 1966; Pinder *et al.*, 1969). In the present work, all the experiments have been performed by mixing, before the enzyme treatment, a small amount of ^{32}P HeLa 28S RNA with the nonradioactive RNA under study. The ^{32}P-labeled internal standard was necessary as a control for variations in the degree of RNase digestion in different experiments. The 28S RNA preparations from various human tissues were compared by this method with 28S RNA from HeLa cells and with each other. In most cases, no obvious differences in susceptibility to RNase were found among human liver, spleen, pancreas, kidney and HeLa 28S RNA preparations (Figure 3). Some preparations of human liver 28S RNA showed reproducibly a somewhat higher resistance to the RNase digestion than HeLa 28S; the reason for this behavior is not known and has not been investigated further.

Discussion

The purpose of this work has been to compare the primary structures of 28S and, respectively, 18S RNA preparations obtained from different human tissues. Critical to the results of this analysis was the purity of the RNA preparations utilized and the intactness of the molecules (such as to exclude the loss of any piece caused by degradation). The purity of the ^{32}P-labeled 18S and 28S RNA components, as isolated in the present work, has been discussed previously (Amaldi and Attardi, 1968). As to the unlabeled HeLa cell rRNA species, which were purified from total RNA, the only significant source of contamination was presumably the heterogeneous nuclear RNA and cytoplasmic mRNA sedimenting in the regions of 28S and 18S RNA, and the mitochondrial 16S RNA (Attardi *et al.*, 1970). Since the heterogeneous nuclear RNA represents *in toto* about 2% of total cell RNA, and the mRNA about 3% (Soeiro *et al.*, 1968), it can be estimated that the possible fraction of these heterogeneous RNA species cosedimenting with either 28S or 18S RNA would correspond to less than 2% of each rRNA component; this level of contamination would not affect appreciably the results obtained here. Somewhat more significant, although still low, would be the possible contamination of the 18S RNA by the 16S mitochondrial RNA (estimated to be at most 5%). The unlabeled rRNA species from human tissues, which were isolated from the monomer–polysome fraction, were presumably not appreciably contaminated by either heterogeneous nuclear RNA or mitochondrial RNA.

As concerns the requirement of intactness of the rRNA molecules for a meaningful analysis of primary structure, in the present work it has been reasonably well satisfied by utilizing only RNA preparations which showed a normal sedimentation pattern with the expected ratio of 28S to 18S RNA (about 2.5) and without any indication of slower sedimenting degradation products.

The comparison among the various 28S RNA and, respectively, 18S RNA preparations has been carried out with various methods at different levels of resolution: (1) base composition; (2) analysis of the frequencies of mono-, di-, and trinucleotides released by pancreatic RNase digestion; (3) RNA–DNA hybridization; and (4) pattern of degradation by mild digestion with pancreatic or T1 RNase.

The first three methods proved unable to discriminate between 28S RNA and, respectively, 18S RNA samples from various sources. The fourth method, utilized only with the 28S RNA, gave similar results in most cases. A few liver 28S RNA preparations showed a higher resistance to the RNase digestion; however, the lack of reproducibility makes the interpretation of these results very difficult.

From the data presented in this work it can be concluded that all the 28S and, likewise, 18S RNA preparations analyzed are very similar, if not identical. This conclusion, which reinforces the available evidence from other eukaryotic systems, can be interpreted in different ways. (1) The genes coding for rRNA in each cell are heterogeneous, and differentially expressed in various cell types or stages of development, so that the RNA preparations studied here are indeed different from each other; however, the differences are smaller than the resolving power of our methods of analysis; (2) the rRNA genes are heterogeneous, but they are equally expressed in different cell types; as a consequence, equally heterogeneous populations of molecules would have been analyzed in this work; and (3) there is in the cell a special mechanism to keep all the rRNA genes homogeneous or nearly so during evolution.

Very little is known about the degree of homogeneity of the high molecular weight rRNA population within a given bacterial or eukaryotic cell. The available evidence points to homogeneity in sedimentation and chromatographic properties. Furthermore, homogeneity in primary structure of the high molecular weight rRNA has been suggested, in *Escherichia coli*, by the molar yield of unity or a multiple thereof of methylated sequences (Fellner and Sanger, 1968), and, in rabbit reticulocytes, by the near to unity molar yield of RNA fragments obtained by mild digestion with T1 RNase (Gould, 1967). A limited degree of heterogeneity in primary structure has, on the contrary, been shown to exist in 5S RNA from *E. coli* and strongly hinted at in 5S RNA from human source (Hatlen *et al.*, 1969).

As concerns the question of the possible heterogeneity of the rRNA genes, it should be mentioned that the rapid initial rate with which rRNA hybridizes with rDNA (Birnstiel *et al.*, 1968), the apparently regular and complete hydrogen bonding of rRNA molecules to rDNA sites in these hybrids (Jeanteur and Attardi, 1969), and the renaturation kinetics of rDNA (Birnstiel *et al.*, 1969) suggest a close similarity in sequence, if not an identity of rRNA genes. As to the mechanism which may operate in keeping low the variability of the highly redundant rRNA genes in eukaryotic cells, one may think of a mechanism like that proposed by Callan (1967), involving one "master" gene and multiple "slave" copies, which are matched against the master and corrected for mistakes due to mutation or recombination once per life cycle of the organism or even per cell division. Alternatively, one can postulate that gameles represent a "bottleneck," where replication of a new family of rRNA genes proceeds from a master gene with destruction of the old replicas. Experimental evidence speaks,

on the contrary, against a selective replication of a master gene at each cell division (Amaldi *et al.*, 1969).

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References

Amaldi, F., and Attardi, G. (1968), *J. Mol. Biol. 33*, 737.
Amaldi, F., Zito-Bignami, R., and Giacomoni, E. (1969), *Eur. J. Biochem. 11*, 419.
Attardi, G., Aloni, Y., Attardi, B., Ojala, D., Pica-Mattoccia, L., Robberson, D., and Storrie, B. (1970), *Cold Spring Harb. Symp. Quant. Biol. 35, 599.*
Attardi, G., and Amaldi, F. (1970), *Annu. Rev. Biochem. 39*, 183.
Attardi, G., Huang, P. C., and Kabat, S. (1965a), *Proc. Nat. Acad. Sci. U. S. 53*, 1490.
Attardi, G., Huang, P. C., and Kabat, S. (1965b), *Proc. Nat. Acad. Sci. U. S. 54*, 185.
Attardi, G., Parnas, H., Hwang, M.-I. H., and Attardi, B. (1966), *J. Mol. Biol. 20*, 145.
Beaven, G. H., Holiday, E. R., and Johnson, E. A. (1955), *in* The Nucleic Acids, Vol. 1, Chargaff, E., and Davidson, J. N., Ed., New York, N. Y., Academic Press, p 493.
Birnstiel, M., Grunstein, M., Speirs, J., and Hennig, W. (1969), *Nature (London) 223*, 1265.
Birnstiel, M., Speirs, J., Purdom, I., Jones, K., Loening, M. E. (1968), *Nature (London) 219*, 454.
Brown, D. D., and Gurdon, J. B. (1964), *Proc. Nat. Acad. Sci. U. S. 51*, 139.
Callan, H. G. (1967), *J. Cell Sci. 2*, 1.
Di Girolamo, A., Busiello, E., and Di Girolamo, M. (1969), *Biochim. Biophys. Acta 182*, 169.
Fellner, P., and Sanger, F. (1968), *Nature (London) 219*, 236.
Fisher, R. A. (1950), Statistical Methods for Research Workers, Edinburgh, Oliver & Boyd.
Gould, H. J. (1967), *J. Mol. Biol. 29*, 307.
Gould, H. J., Bonanou, S., and Kanagalingham, K. (1966), *J. Mol. Biol. 22*, 369.
Grummt, F., and Bielka, H. (1968), *Biochim. Biophys. Acta 161*, 253.
Hatlen, L. E., Amaldi, F., and Attardi, G. (1969), *Biochemistry 8*, 4989.
Hatlen, L. E., and Attardi, G. (1971), *J. Mol. Biol.* (in press).
Henney, H. R., and Storck, R. (1963), *Science 142*, 1675.
Hirsch, C. A. (1966), *Biochim. Biophys. Acta 123*, 246.
Jeanteur, Ph., and Attardi, G. (1969), *J. Mol. Biol. 45*, 305.
Lerner, A. M., Bell, E., and Darnell, J. E. (1963), *Science 141*, 1187.
Mutolo, V., and Giudice, G. (1967), *Biochim. Biophys. Acta 149*, 291.
Pinder, J. C., Gould, H. J., and Smith, I. (1969), *J. Mol. Biol. 40*, 289.
Reich, E., Acs, G., Mach, B., and Tatum, E. L. (1963), *in* Informational Macromolecules, Vogel, H. J., Bryson, V., and Lampen, J. O., Ed., New York, N. Y., Academic Press, p 317.
Slater, D. W., and Spiegelman, S. (1966), *Biophys. J. 6*, 385.
Soeiro, R., Vaughan, M., Warner, J. R., and Darnell, J. E. (1968), *J. Cell Biol. 39*, 112.
Tata, J. R. (1967), *Biochem. J. 105*, 783.

Role of Ferrichrome as a Ferric Ionophore in *Ustilago sphaerogena**

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ABSTRACT: The smut fungus, *Ustilago sphaerogena*, possesses a highly specific iron transport system in which the trihydroxamic acid–iron chelate, ferrichrome, serves as an iron carrier, or ferric ionophore. Intracellular removal of the metal from the chelate permits egress of the ligand, desferriferrichrome, which can then sequester extracellular iron and again shuttle the metal into the cells. In the presence of excess iron, ferrichrome is concentrated by the cells. The transport system has an optimum pH of 7 and optimum temperature of 30°, and it is inhibited by azide, *N*-ethylmaleimide, or anaerobiosis. Growth of the cells in medium containing 10 μM iron suppresses the iron transport system. Ferrichrome is a specific iron carrier for *Ustilago*. No other iron chelates tested, including natural hydroxamic acid analogs of ferrichrome, can substitute. Conversely, many other di- and trivalent metals chelated with the ligand, desferriferrichrome, are not taken up by the cells. The aluminum and gallium chelates, however, are active in the system. The specificity data indicate that conformation of the chelate, rather than charge or solubility, is the basis for recognition by the transport system.

A fundamental problem of cellular biology is the mechanism by which water-soluble extracellular nutrients enter the cell through lipophilic membranes. Specific transport systems appear to be present in most cell membranes. The transport of amino acids and sugars has been investigated, but the mechanism of the process remains unsolved. According to

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