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Effect of Heat Shock on the Synthesis of Low Molecular Weight RNAs in Drosophila: Accumulation of a Novel Form of 5S RNA

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Summary

The synthesis and stability of low molecular weight RNAs following heat shock in Drosophila melanogaster cell cultures have been examined. When cultures are raised from 25°C to 37°C, the synthesis of tRNA and at least two other low molecular weight RNAs continues at the 25°C rate. 5.8S ribosomal RNA and most of the low molecular weight nuclear RNAs are not synthesized. The synthesis of 5S ribosomal RNA is greatly reduced. A large amount of an RNA of about 135 nucleotides in length accumulates at 37°C. Nucleotide sequence analysis reveals that this RNA is a novel form of 5S RNA with approximately 15 additional nucleotides at its 3′ end.

Introduction

Brief exposure of Drosophila to elevated temperatures induces rapid and profound changes in RNA and protein synthesis in many tissues of the animal (Ritossa, 1963; Ashburner, 1970; Ellgaard and Clever, 1971; Tissières, Mitchell, and Tracy, 1974; Lewis, Helmsing, and Ashburner, 1975). Recent experiments have shown that cell lines derived from D. melanogaster embryos undergo similar changes in macromolecular synthesis when raised from 25°C to 37°C. Preexisting polysomes rapidly decay and are replaced by polysomes which synthesize a small number of proteins (McKenzie, Henikoff, and Meselson, 1975). Analysis by in situ hybridization of cytoplasmic RNA synthesized at 37°C reveals a drastic alteration in the pattern of gene expression. Most of the ongoing transcription is turned off, and approximately 7 new messenger RNAs are synthesized in large amounts (Spradling, Penman, and Pardue, 1975).

Low molecular weight RNAs have been suggested as regulatory elements in several models for the control of eucaryotic gene expression (for reviews, see Britten and Davidson, 1969; Monahan and Hall, 1974; Robertson and Dickson, 1974). Approximately 10% of the nuclear RNA in mammalian cells consists of low molecular weight RNAs that range in length from 60 to 300 nucleotides and divide into at least 10 distinct species (Weinberg, 1973). Similar RNAs have been observed in the nuclei of D. melanogaster cells (G. M. Rubin, unpublished results). If such RNAs are indeed involved in the control of gene expression, one might expect heat shock to have a dramatic effect on their synthesis or stability. We therefore examined the effect of heat shock on these RNAs in D. melanogaster cell cultures, and in the process discovered a novel form of 5S RNA.

Results

Incubation of D. melanogaster Cells at 37°C Changes the Pattern of Low Molecular Weight RNA Synthesis

^{32}P-phosphate was added to a log-phase spinner culture of D. melanogaster cells 15 min after the incubation temperature was raised from 25°C to 37°C. RNA was extracted from aliquots of the culture at various times thereafter and fractionated by polyacrylamide gel electrophoresis. The total mass and radioactivity of each RNA species were monitored by staining the gel with ethidium bromide and by autoradiography, respectively (Figure 1). Although the mass of most of the RNAs remains approximately constant following the shift to 37°C, the RNA in 6 bands (a, b-doublet, d, e, and h) appears to be degraded, while that in band f accumulates in large amounts (Figure 1, left panel).

The effect of the temperature jump on the synthesis of new RNA is more clearly seen when the amount of radioactivity accumulating in each band is examined (Figure 1, right panel). As expected, the RNAs in bands a, b, d, e, and h (that is, those that disappear in the stained gel) do not accumulate significant radioactivity at 37°C, as is also the case for the RNAs in the other bands between c and f. Hence, most of the low molecular weight RNAs formed at 25°C either are not synthesized at 37°C, or turn over so rapidly at 37°C that they are not detected.

The g band consists of a mixture of the 5S and 5.8S ribosomal RNAs (see legend to Figure 2). The appearance of an autoradiographic band at this position (g′ band) indicates that at least one of these RNAs is formed at 37°C. However, densitometric measurements of this g′ band and of the corresponding band from RNA formed at 25°C (not shown) demonstrate that the rate of accumulation of these RNAs at 37°C is several fold less than at 25°C. Similar measurements of the c′ and i′ bands and of the tRNA region indicate that these RNAs accumulate at approximately the same rate at both temperatures.

The most striking of the changes caused by the temperature jump, however, is the accumulation of a large amount of the f′ RNA. Indeed, densitometric measurements indicate that this RNA is accumulated at least 20 fold more rapidly at 37°C then at 25°C.

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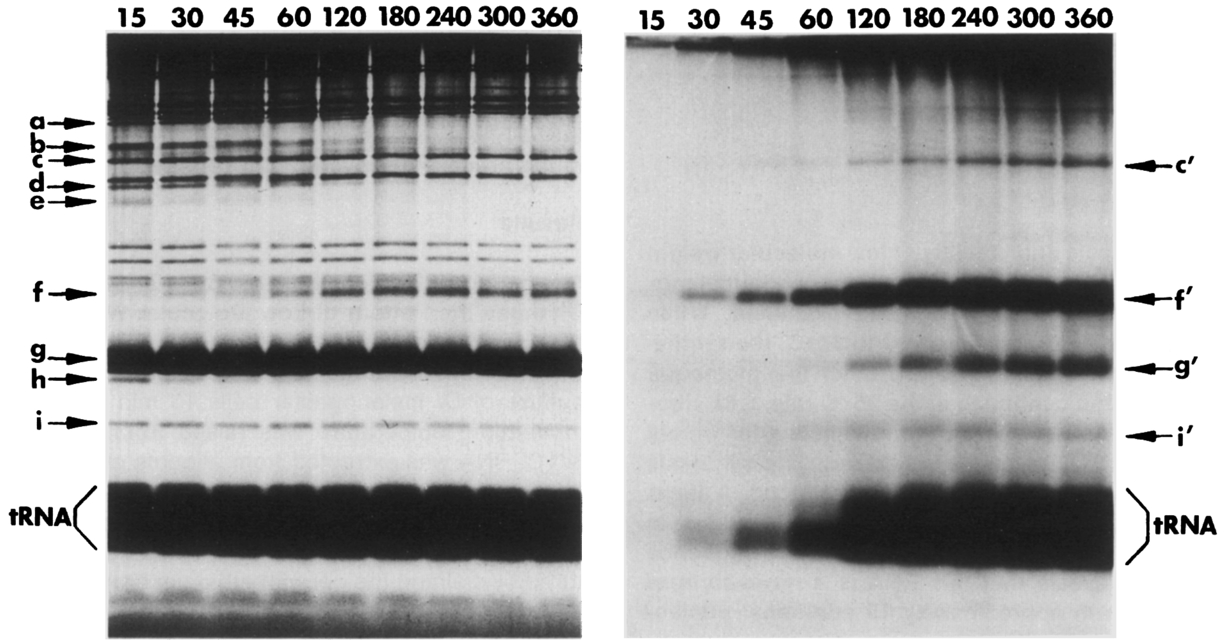

Figure 1. Polyacrylamide Gel Electrophoresis of RNA Extracted from Log-Phase D. melanogaster Cells at Various Times after Raising the Temperature from 25°C to 37°C

15 min after shifting the temperature, 10 mCi of ^{32}P-phosphate were added to a 200 ml spinner culture containing 5×10^6 cells/ml. 5 ml aliquots were removed at times after the addition of the ^{32}P-phosphate that are indicated in minutes at the top of each gel pattern. RNA was extracted from these aliquots and fractionated by electrophoresis on a 10% polyacrylamide gel (Experimental Procedures).

Left panel: ethidium bromide stained gels. Right panel: autoradiograph of the same gel. Bands designated by a common letter in both panels have the same mobilities; primed letters are used in the autoradiograph to designated components synthesized at 37°C.

If instead of labeling at 37°C the cells are exposed to 37°C for 20 min and then labeled at 25°C, the same qualitative change in the accumulation of the above RNAs is observed. In particular, large amounts of the f′ RNA still accumulate.

The f′ RNA Is a Novel Form of 5S RNA

The structure of the f′ RNA formed at 37°C was investigated to determine if it is related to other previously defined RNAs in D. melanogaster. Figure 2A shows a two dimensional fractionation of a T1 RNAase digest of f′ RNA. A comparison of this oligonucleotide map with those of several other low molecular weight RNAs synthesized in D. melanogaster cells at 25°C (G. M. Rubin, unpublished results) revealed a strong similarity with only one RNA, the 5S ribosomal RNA (Figure 2B). Only 3 oligonucleotides (spots 20, 21, and 22) are present in the digest of f′ RNA but not in the digest of 5S RNA, and the only oligonucleotide in the 5S RNA digest not present in the f′ RNA digest is C-C-U$_{OH}$ from the 3′ end of the 5S RNA.

All of the other oligonucleotides appear to be common to both RNAs. They have the same relative mobilities in both dimensions of the fractionation procedure and yield the same products when digested with pancreatic RNAase. Furthermore, digestion of each of the oligonucleotides from the T1 RNAase digests of the f′ RNA with T2 RNAase, and fractionation of the products by electrophoresis on Whatman 3MM paper at pH 3.5 and by chromatography on cellulose thin layers with isopropanol:HCl (Barrell, 1971) did not reveal any modified nucleotides.

Table 1 presents a structural analysis of the oligonucleotides in spots 20, 21, and 22 (Figure 2A), as determined by the products obtained with T2 and pancreatic RNAase digestion. Since oligonucleotides 21 and 22 do not contain G, they must derive from the 3′ end of the f′ RNA; and because the above digests of each differ only by a single U, we suppose that they derive from the same gene product that is frayed at the 3′ end.

A comparison of the pancreatic RNAase digestion products of the f′ and 5S RNAs is shown in Figures 3A and 3B, respectively. The oligonucleotide maps of these RNAs are qualitatively identical, but quantitation of the relative molar yields of the oligonucleotides reveals several differences. The data are presented in Table 2. These data, the appearance of oligonucleotides 20, 21, and 22 in the T1 digests of f′ RNA, the absence of the C-C-U$_{OH}$ 3′ terminal oligonucleotide of the 5S RNA from such digests, and the identity of the other T1 oligonu-

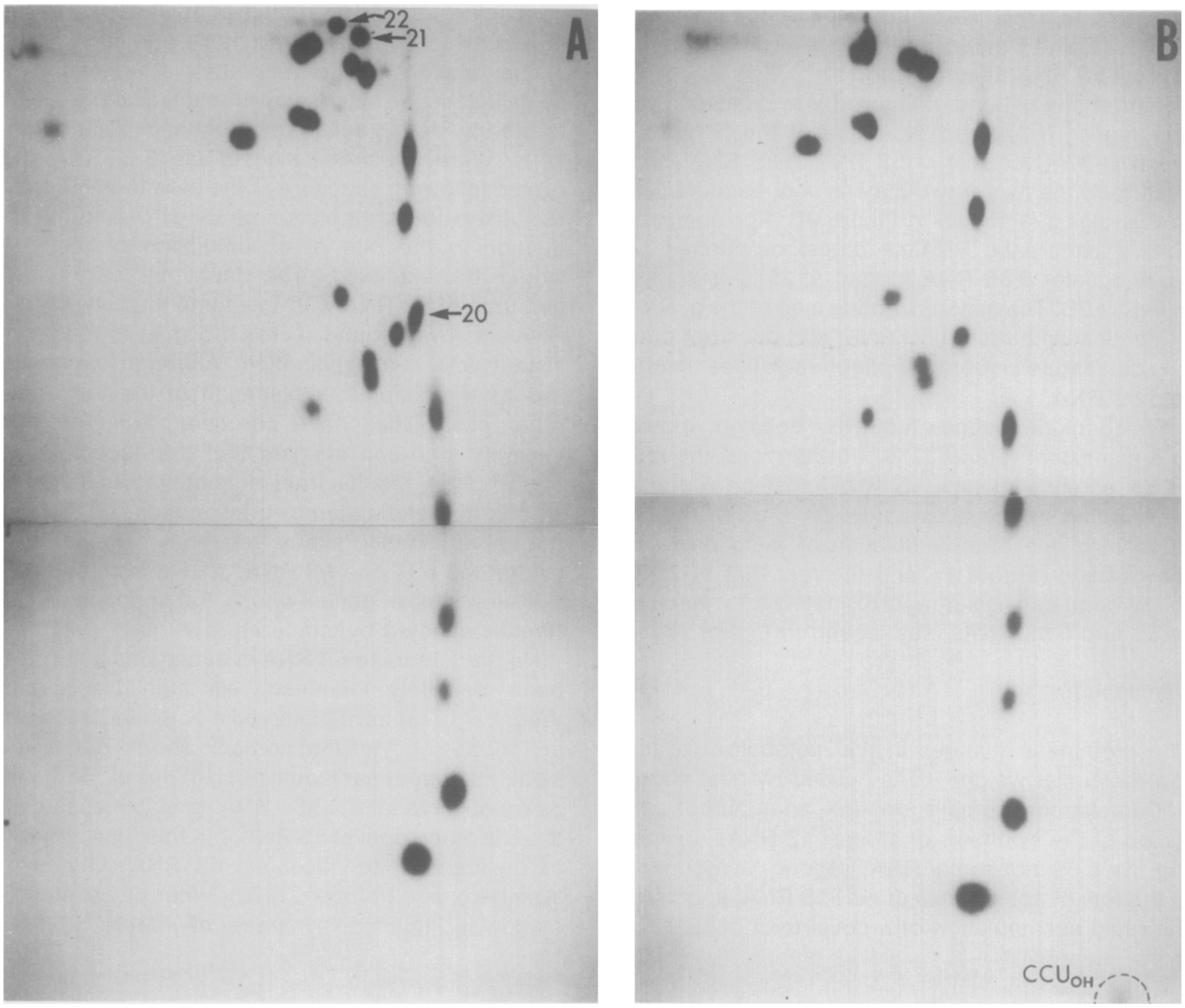

Figure 2. Autoradiograph of Two-Dimensional Fractionations of T1 RNAase Digests of (A) f′ RNA and of (B) 5S RNA

Electrophoresis was from right to left on cellulose acetate at pH 3.5 in 7 M urea, and from top to bottom on DEAE paper in 7% formic acid (Barrell, 1971). The f′ RNA was fractionated by polyacrylamide gel electrophoresis as indicated in Figure 1. The 5S RNA was fractionated in a similar manner from RNA extracted from cells grown at 25°C, except that the electrophoresis was at 2–4°C instead of room temperature. At the lower temperature, the g band (Figure 1) divides into two approximately equal bands of which that with the lower mobility is the 5S ribosomal RNA, and the other is the 5.8S ribosomal RNA. These two RNAs have been identified by the observation that the 5.8S RNA, but not the 5S RNA, hybridizes to the DNA of the hybrid plasmid pDm103 that contains the gene for the 18–28S rRNAs of D. melanogaster (Glover et al., 1975). In addition, the presence of a triphosphate at the 5′ terminus of the 5S RNA (Table 2) and of modified nucleotides in the 5.8S RNA (G. M. Rubin, unpublished results) is consistent with these assignments.

cleotides from the f′ and 5S RNA can be explained most simply by the following model for the structure of the f′ RNA:

$$\underbrace{\text{pppGC}\ldots\ldots\ldots\text{GC}}_{\text{5S RNA}}\!\!\!\!\!\!\!\!\overbrace{\text{CU}(\text{C}_{1-2})\text{G}}^{20}\overbrace{\text{U}(\text{AAC, AC, C}_2\text{, U}_{3-5})_{\text{OH}}}^{\text{21 or 22}}$$

(approximately 120 nucleotides)

The T1 oligonucleotides 21 or 22 are placed at the 3′ end for the previously indicated reason; the GU sequence bridging the T1 oligonucleotides 20 and 21 or 22 accounts for the extra GU dinucleotide in the pancreatic digest of f′ RNA (Table 2, 9); this placement of oligonucleotide 20 accounts for the absence of C-C-U$_{OH}$ among the T1 oligonucleotides of f′ RNA and is consistent with the identity of the other T1 oligonucleotides from f′ and 5S RNA, as well as the observation that both contain pppGC (or ppGC) at their 5′ ends (Table 2). The values for the other products of pancreatic RNAase digestion of the f′ and 5S RNAs also conform to this structure (the observed difference of 2.1 Cs is not significantly different from the expected value of 3 to 4, since it represents the difference between two large numbers).

The Shift to 37°C Stops the Appearance of New 5.8S RNA and Decreases the Rate of Appearance of New 5S RNA Several Fold

RNA from the g′ band of Figure 1 was digested with pancreatic RNAase to determine if the RNAs labeled at 37°C consist of 5S RNA, 5.8S RNA, or a mixture of the two. A two-dimensional fractionation of this digest is shown in Figure 3C. For comparison, a pancreatic RNAase digest of purified D. melanogaster 5.8S RNA formed at 25°C is shown in Figure 3D. The oligonucleotide map of the g′ RNA is identical with that of 5S RNA, and does not contain detectable amounts of oligonucleotides unique to 5.8S RNA.

We conclude that new 5.8S RNA does not appear at a significant rate at 37°C. Furthermore, the rate of appearance of new 5S RNA must be reduced several fold by the temperature shift, given the reduction in rate of accumulation of radioactivity in the g′ band (Figure 1), and the fact that at 25°C the g′ band consists of approximately a 1:1 mixture of 5S and 5.8S RNAs (see legend to Figure 2).

Discussion

The prominent changes in the metabolism of the low molecular weight RNAs caused by heat shock of D. melanogaster cells are: first, an apparent cessation of the synthesis of at least 12 RNAs, including the 5.8S ribosomal RNA; second, a reduction in the rate of appearance of new 5S RNA; and third, the rapid accumulation of a novel form of 5S RNA containing about 15 additional nucleotides at the 3′ end, which we choose to name 5S$^+$ RNA.

The relationship between their structures suggests that the 5S$^+$ RNA may function as a precursor to the 5S RNA in cells grown under normal conditions. Were the processing of the 5S$^+$ RNA disrupted following exposure of the cells to 37°C, then the observed accumulation of 5S$^+$ RNA and the reduction in the rate of accumulation of 5S RNA would be expected. The fact that very small amounts of an RNA with the same mobility as 5S$^+$ RNA are always found in cells grown at 25°C is consistent with this explanation. Although we prefer this as the simplest explanation of the 5S$^+$ RNA, other possibilities have not been excluded. For example, one can imagine that the accumulation of 5S$^+$ RNA results from a temperature-sensitive step in the termination of transcription, so that the normal termination site is frequently skipped over, or that the 5S$^+$ and 5S RNAs derive from different but very similar genes whose transcription is differently affected by temperature.

No precursors to 5S RNA in eucaryotic cells have been definitely identified, although Denis and Wegnez (1973) have observed a potential precursor in Xenopus oocytes that contains approximately the same number of additional nucleotides at the 3′ end as we observe for the 5S$^+$ RNA. In mammalian cells there is a free pool of 5S RNA, but this RNA appears to be identical to ribosomal 5S RNA (Leibowitz, Weinberg, and Penman, 1973). When ribosome formation is inhibited, synthesis of mature 5S RNA

Table 1. Analysis of T1 Oligonucleotides 20, 21, and 22

	RNAase T2 Digestion Products[b]				Pancreatic RNAase Digestion Products[c]		
Oligonucleotide[a]	C	A	G	U	Product	Yield	Deduced Sequence
20	3.3		1.0	1.0	G	1.0	C_{3-4}, U, G
					C	3.7	
					U + U!	1.1	
21	4.1	3.0		5.1	AAC	1.0	AAC, AC, C_2, U_{4-5}
					AC	1.1	
					C	2.0	
					U + U!	3.8	
22	3.9	3.0		6.3	AAC	0.9	AAC, AC, C_2, U_{5-6}
					AC	1.2	
					C	2.2	
					U + U!	4.8	

[a] The numbers refer to the oligonucleotides shown in Figure 2A.

[b] The base compositions are expressed as the number of moles of each nucleotide relative to 1.0 moles of G for oligonucleotide 20, and to 3.0 moles of A for 21 and 22.

[c] The yields of the digestion products are expressed as the number of moles of each product relative to 1.0 moles of G for oligonucleotide 20, and relative to 5.0 moles of phosphate in AAC + AC for 21 and 22.

continues and no precursor is seen to accumulate (Perry and Kelley, 1968).

Whether the apparent cessation in the synthesis of other low molecular weight RNAs after heat shock, in particular of the 5.8S RNA, results from disruption of processing or more directly from a cessation of transcription remains to be determined. If the block in the formation of the low molecular weight nuclear RNAs is in the processing of high molecular weight precursors, heat shock may provide a means for determining the origin and, possibly, the function of this class of RNAs.

None of the low molecular weight RNAs that we have discussed exhibits characteristics that make them likely candidates for regulator elements controlling the changes in gene expression associated with heat shock. However, autoradiographs obtained by longer exposure of the gels in Figure 1 reveal some bands (for example, between c′ and f′) that are not observed in RNA synthesized at 25°C. These represent very small amounts of RNA and have not yet been examined in any detail.

Experimental Procedures

Cell Culture

Eschalier's K_c line of D. melanogaster cells (Eschalier and Ohanessian, 1970; Dolfini, 1971), adapted to grow in the absence of serum, was obtained from W. Gehring. The cells were grown at 25°C in a low phosphate medium D20-P, either in monolayer culture (T-flasks) or in suspension (spinner flasks). D20-P medium is prepared in the same manner as D20 medium (Eschalier and Ohanessian, 1970), except that the NaH_2PO_4 and serum are omitted, and the

Table 2. Analysis of the Pancreatic RNAase Digestion Products of 5S and f′ RNAs

		Molar Ratios[c]		
Oligonucleotide[a]	Sequence[b]	5S	f′	Difference
1A	U!	12.1	16.5	+4.4
1	U			
2	C	14.8	16.9	+2.1
3	AC	6.3	7.1	+0.8
4	AAC	1.0	1.9	+0.9
5	GC	4.7	4.7	
6	AU	2.3	2.1	
7A	GAC	1.1	1.1	
7B	AGC			
8	AAGC	0.9	0.9	
9	GU	6.1	7.1	+1.0
10	GGC	1.1	1.2	
11A	GAU	1.0	1.0	
11B	AGU	1.1	1.1	
12A	GAAU	1.0	1.0	
12B	AGAU			
13	GAAAU	0.8	0.8	
14	GGU	2.6	2.6	
15	GGGU	0.9	1.1	
16	GGGAAC	0.7	0.8	
17A	pppGC	1.1	1.2	
17B	ppGC			
17[d]	$G_{5-6}C$	0.4	0.7	

[a] The numbers refer to the numbered spots in Figure 3B.

[b] The sequences of the oligonucleotides were determined by analysis of the products of their digestion with T1 RNAase.

[c] The molar ratios were calculated by dividing the radioactivity found in each spot by the product of the number of phosphates in the oligonucleotide and the radioactivity per phosphate. The radioactivity per phosphate was determined by dividing the sum of the radioactivities in oligonucleotides 7A, 7B, 8, 11A, 11B, 12A, and 12B by 24. Differences in the relative molar yields of f′ and 5S RNAs of ±0.3 moles or greater are given in the last column. The data for 5S RNA indicate a total of 120 nucleotides in this RNA if the nearest integral value is taken for the relative molar ratios (it is assumed that spot 18 is G_6C and has a ratio of 1 rather than 0; see below). The same process applied to f′ RNA yields a value of 134.

[d] The efficiency of transfer of this oligonucleotide from the cellulose acetate strip to the DEAE paper was variable and low. This behavior has been observed previously with oligonucleotides in pancreatic RNAase digests containing several consecutive G residues (Rubin, 1973).

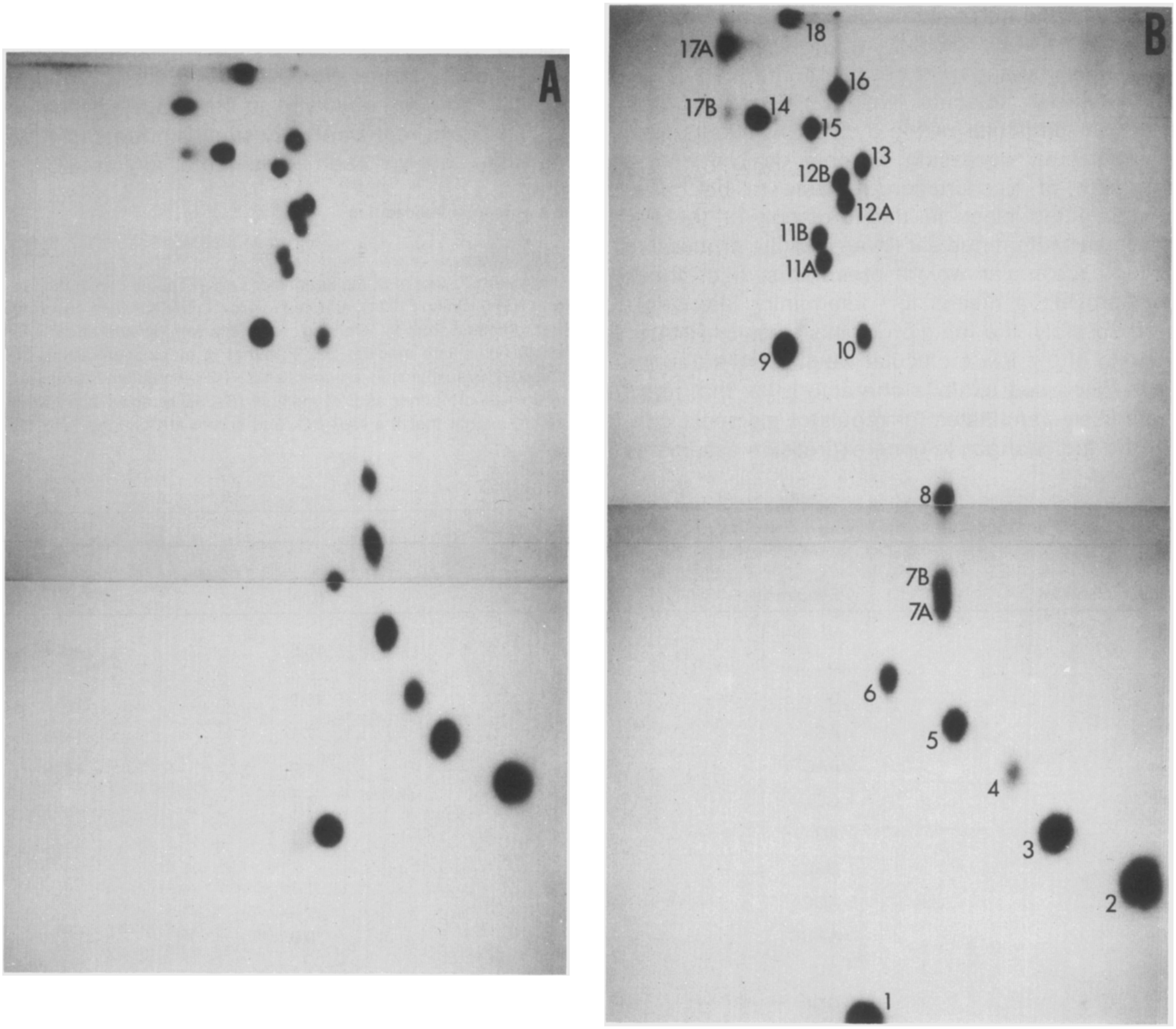

Figure 3. Autoradiographs of Two-Dimensional Fractionations of Pancreatic RNAase Digests of (A) f′ RNA, (B) 5S RNA, (C) g′ RNA, and (D) 5.8S RNA

(A) and (B) *above*; (C) and (D) *opposite.*

Electrophoresis was carried out as described in Figure 2. The f′, 5S, and 5.8S RNAs were fractionated as described in Figure 2, and the g′ RNA corresponds to the band indicated in Figure 1.

final volume is brought to 1100 ml. The concentration of inorganic phosphate in D20-P is about 1 mM, but varies somewhat depending on the source of the yeast extract and the lactalbumin hydrolysate. All experiments were performed on mid-log phase cells (3–7 × 10^6/ml) in spinner flasks.

RNA Extraction and Purification

Cells were collected by centrifugation for 5 min at 1000 × g at room temperature and resuspended in a 0.05 vol of 50 mM sodium acetate, 1 mM EDTA (pH 5) at 0°C. An equal volume of 50 mM sodium acetate, 1 mM EDTA, 1% SDS was added. The cell suspension was vortexed, combined with an equal volume of water-saturated phenol, and extracted at 55°C for 10 min. The phases were separated by centrifugation for 20 min at 15,000 × g at 0°C. To the aqueous layer were added a 0.1 vol of 2 M sodium acetate (pH 5.0) and 2 vol of ethanol. After precipitation overnight at -20°C, the RNA was fractionated by polyacrylamide gel electrophoresis and recovered from the gels as previously described (Rubin, 1975).

RNA Sequence Analysis

RNAase digestions and the subsequent fractionation and analysis of the oligonucleotides were performed as described by Barrell (1971).

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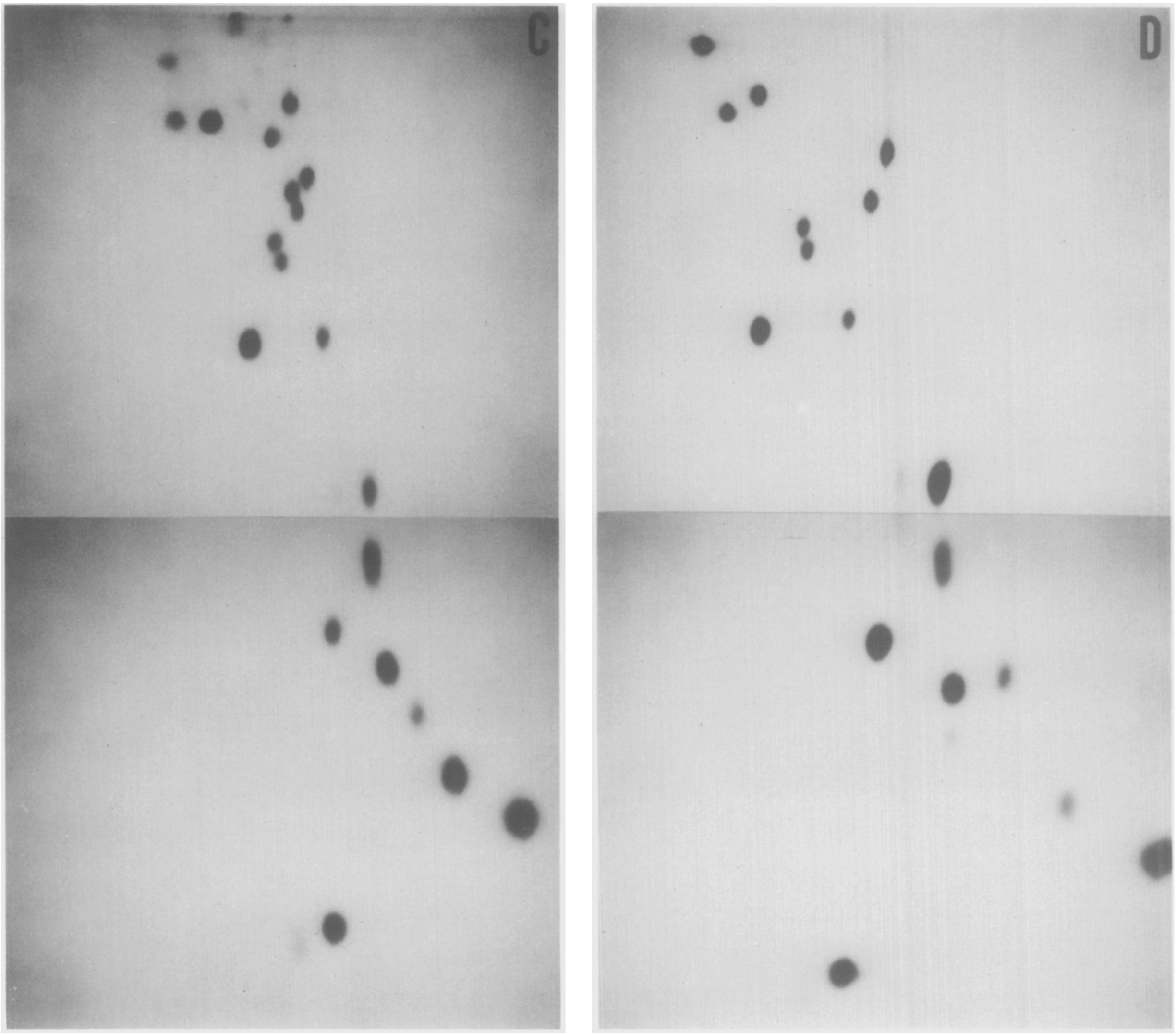

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