business. We think that the results presented here force a more rigid interpretation of such experiments. Positive results, those which demonstrate interactions between cells, can safely be taken at face value; negative results are not interpretable because they fail to distinguish between the alternate possibilities that interactions do not exist or that antidromic spikes do not reach the site of interaction.

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8. We thank Donald Kennedy for the original suggestion that we check the effectiveness of antidromic spikes in these cells. Supported by an NIH postdoctoral fellowship to B.M. and by NIH grant NS 09322-02 to A.S.

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Chromosomal RNA: Its Properties

Abstract. *We describe the properties of a special class of RNA associated with chromatin. We discuss why this RNA should be considered a distinct class of RNA and not an artifactual degradation product of either transfer or ribosomal RNA.*

We have described the preparation and properties of a class of RNA molecules associated with chromosomes, which we have termed chromosomal RNA or cRNA (*1–3*). It has been suggested that cRNA may be an artifact resulting from the degradation of transfer RNA (tRNA) (*4*), or that it may not exist at all (*5*, *6*). In this report we describe the known properties of cRNA and show that cRNA from Novikoff ascites in the rat is not detectably contaminated with tRNA, ribosomal RNA (rRNA), or their degradation products. We review the methods available for the isolation of cRNA and discuss whether cRNA should be considered a distinct class of RNA.

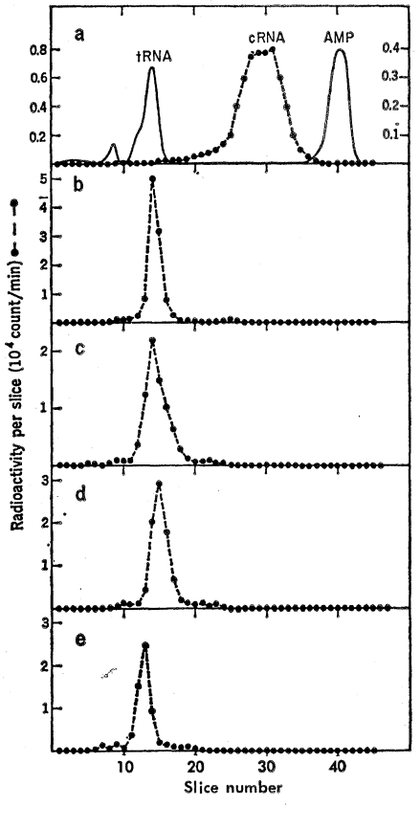

Fig. 1. Separation of tRNA, cRNA, and adenosine monophosphate (AMP) by polyacrylamide gel electrophoresis. Samples were applied in 20 μl of 50 percent glycerol (by volume) to a 6-cm column containing 14 percent acrylamide and 0.1 percent sodium dodecyl sulfate, and 5 ma per gel was applied for 2.5 hours. (a) The sample was [^{3}H]cRNA prepared from rat Novikoff ascites by a method similar to that of Dahmus and McConnell (*3*), yeast tRNA (Sigma), and AMP. Absorbance at 260 nm (scale at right) is given by the solid line. (b) The sample, [^{3}H]tRNA prepared from rat Novikoff ascites as described by Dahmus and McConnell (*3*) and stored at −18°C in 1 m*M* ethylenediaminetetraacetic acid, was applied directly to the gel without prior treatment. (c) The [^{3}H]tRNA in (b) was incubated in 0.01*M* tris(hydroxymethyl)-aminomethane (tris), *p*H 8, at 37°C for 2 hours before electrophoresis. (d) The preparation in (b) was incubated in 0.01*M* tris, *p*H 8, plus Pronase B (Calbiochem), 2 mg/ml, at 37°C for 2 hours before electrophoresis. The Pronase had been first incubated in 0.01*M* tris, *p*H 8, for 90 minutes at 37°C at a concentration of 20 mg/ml. (e) The sample was prepared as in (d) except that Pronase C (Calbiochem), 2 mg/ml, was used in place of Pronase B. The Pronase was first incubated as described in (d).

Three principal properties identify cRNA. First, it elutes as a symmetrical peak from diethylaminoethyl (DEAE) Sephadex in 7*M* urea at approximately 0.55*M* NaCl (*2*, *3*, *7–13*) and from DEAE cellulose at 0.38*M* NaCl (*11*, *14*, *15*). Second, cRNA hybridizes to DNA to a much larger extent than does either tRNA or rRNA (*2*, *3*, *8*, *14*). We have yet to isolate cRNA from any tissue which hybridizes to less than 2 percent of homologous DNA (*2*, *3*, *8–11*, *16*). Further, cRNA from pea buds (*7*) and rat ascites (*8*) hybridizes to the middle-repetitive sequences of homologous DNA. Third, cRNA isolated from a wide variety of organs and organisms contains from 7 to 10 percent dihydropyrimidine (*3*, *8–12*, *15*, *19*, *20*).

Both cRNA and tRNA elute from DEAE Sephadex at 0.55*M* NaCl in 7*M* urea. The contamination of cRNA by tRNA is minimized by using isolated chromatin as the starting material. Even if purified tRNA or isolated ribosomal subunits are added to isolated chromatin and the chromatin is processed for cRNA by the method of Dahmus and McConnell (*3*), less than 1 percent of the cRNA peak recovered on DEAE Sephadex is either tRNA or rRNA (*21*). The following observations also demonstrate that cRNA is not related to either tRNA or rRNA.

1) Ascites cRNA can be separated from tRNA on DEAE cellulose (*11*, *14*, *15*), Sephadex G100 (*8*), or by disc gel electrophoresis (Fig. 1a).

2) If tRNA is subjected to the same Pronase treatment that is used in the isolation of cRNA, no degradation of tRNA to fragments the size of cRNA is detected (Fig. 1, b to e). This result is not in agreement with one report (*4*). The discrepancy is probably due to the quality of Pronase used, that is, to residual ribonuclease activity present in those Pronase samples which do degrade tRNA.

3) Ascites cRNA hybridizes to an estimated 4.9 percent of rat DNA, whereas tRNA hybridizes to about 0.09 percent of rat DNA under the same conditions (Fig. 2, a and b). The relation between base sequences of ascites cRNA and tRNA has been tested by reciprocal competition experiments. At the level of sensitivity of these experiments, no relation could be found between these base sequences (Table 1). Furthermore, cRNA hybridizes exclusively to purified middle-repetitive DNA (*8*) and in a DNA-driven RNA hybridization reaction has a $Cot_{1/2}$

(product of concentration, in moles of nucleotides per liter and hybridization half-time in seconds) distinct from that of purified tRNA (*8*).

4) Dahmus and McConnell (*3*) reported that ascites tRNA compared to ascites cRNA contains five times more methylated bases and one third the amount of dihydropyrimidine.

5) Jacobson and Bonner (*14*) were unable to find universal nucleotide sequences in cRNA following ribonuclease digestion, whereas tRNA is known to contain such sequences (*22*). In addition they showed (*14*) that despite the high sequence diversity of ascites cRNA the 5′ ends are 90 percent cytosine and the 3′ ends are 99 percent guanine. This result suggests that cRNA is not a random mixture of RNA fragments, although the possibility that this merely reflects the specificity of nucleases cannot be excluded.

6) Additional evidence that ascites cRNA is a distinctive RNA class is derived from in vivo half-life studies (*10*). The decay kinetics of pulse-labeled cRNA follow a linear first-order profile; this suggests that cRNA is a single kinetic component (*10*). In addition, the half-life of cRNA (17 hours) is different from that of any other RNA species known in rat ascites (*10*).

7) Dahmus (*11*) found ascites cRNA to have no detectable amino acid acceptor activity. Purified tRNA, treated with Pronase and chromatographed on DEAE Sephadex by the procedure for the preparation of cRNA, exhibited the same acceptor activity as did untreated tRNA (*11*). This finding contrasts with that of Heyden and Zachau (*4*), who reported the loss of acceptor activity after Pronase treatment of pure tRNA, a result that suggests that their Pronase treatment resulted in RNA degradation.

One of the first methods developed for isolating cRNA was the CsCl (skin) method (*2*, *3*). In this method chromatin is dissolved in 4*M* CsCl and centrifuged overnight at 100,000*g*. The proteins aggregate and float to the top of the tube, forming a skin, while the majority of the DNA and the RNA is pelleted. The cRNA is found in the skin and can be released by protease treatment and purified away from other skin RNA's by chromatography on DEAE cellulose.

The CsCl skin method is not a satisfactory procedure for preparing cRNA because (i) other RNA species are apparently trapped adventitiously by the aggregated proteins (*5*, *6*, *21*), and (ii) treatment of the skin with protease can result in the degradation of RNA if large amounts of endogenous ribonuclease are present in the starting chromatin, or if the protease is contaminated with ribonuclease. Therefore, we wish to draw attention to three additional methods for the preparation of cRNA. In the procedure described by Huang and co-workers (*23*), chromatin is dissolved in 2.5*M* guanidinium chloride and the DNA is pelleted at 105,000*g* for 24 hours. The supernatant, containing proteins and various RNA's, is adjusted to 4*M* CsCl and 2*M* guanidinium chloride and subjected to buoyant density equilibrium centrifugation. Fractions containing cRNA are collected, and the cRNA is further purified by DEAE cellulose chromatography and disc gel electrophoresis. Other methods have been described by Jacobson and Bonner (*14*) and by Mayfield and Bonner (*10*).

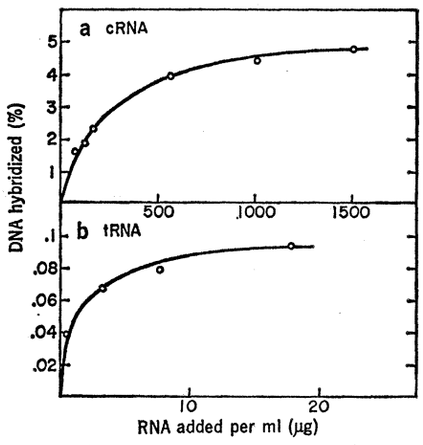

Fig. 2. Hybridization of cRNA and tRNA to homologous DNA. Samples of DNA, cRNA, and tRNA were prepared from rat Novikoff ascites as described by Dahmus and McConnell (*3*). The tRNA was labeled in vitro with [^{3}H]dimethyl sulfate to a specific activity of approximately 180,000 count/min per microgram by a method similar to that of Mayfield and Bonner (*10*), and cRNA was labeled in vivo with [^{32}P]orthophosphate to a specific activity of approximately 4000 count/min per microgram (*3*). Filters containing DNA were prepared by the method of Gillespie and Spiegelman (*17*); 10 μg (a) or 2 μg (b) of denatured DNA with trace amounts of [^{14}C]DNA were used per filter. The hybridization reaction was carried out in 5 × SSC (0.1*M* sodium chloride and 0.15*M* sodium citrate) and 50 percent formamide for 18 hours (a) or 24 hours (b) at 37°C (*18*). At the end of the reaction, unhybridized RNA was removed by incubating the filters in 2 × SSC at 37°C for 30 minutes with pancreatic ribonuclease A, 50 μg/ml, and ribonuclease T1, 50 units/ml (both, Worthington Chemical Co.).

Table 1. Hybridization competition between labeled tRNA and cRNA and unlabeled cRNA, rRNA, and tRNA. Preparation of cRNA, rRNA, and tRNA and of DNA filters was as described by Dahmus and McConnell (*3*). Reaction conditions were described in the legend to Fig. 2 and in (*3*).

Competing unlabeled RNA	Ratio of total RNA to labeled RNA	Labeled hybrid remaining (%)
	Labeled cRNA	
None	1	100*
Transfer	3.8	103
Ribosomal	3.8	103
Chromosomal	2.5	76
Chromosomal	3.8	50
	Labeled tRNA	
None	1	100†
Transfer	2.1	76
Transfer	5.5	45
Chromosomal	5.1	97

* This is 74 percent of saturation value. † This is 70 percent of saturation value.

The properties of rat ascites cRNA described in this report suggest that it is a distinct class of RNA which is not detectably contaminated with tRNA, rRNA, or their degradation products. The relation of rat ascites cRNA to other nuclear RNA's has been studied (*8*). Principal among the distinguishing features of rat ascites cRNA are its base sequence heterogeneity and its high content of dihydropyrimidine. The data indicate that cRNA preparations from other tissues also contain RNA with these unusual properties, but there is insufficient evidence to eliminate the possibility of contamination by tRNA or degraded rRNA.

Much of the controversy surrounding the existence of cRNA stems from a misunderstanding of its definition. Artman and Roth define cRNA as "the RNA associated with the proteins which float in 4*M* CsCl" (*5*). A similar definition of cRNA is used operationally by Szeszák and Pihl (*6*). The RNA that associates with the CsCl skin is more properly termed "skin RNA," and includes, in addition to cRNA as defined earlier in this report, a heterogeneous collection of RNA molecules that are adventitiously trapped by the proteins that float to the surface of CsCl (*5*). Skin RNA must be further processed on DEAE cellulose to remove these adventitiously trapped RNA's and permit recovery (at least in the case of preparations from rat ascites cells) of purified cRNA. The methods described by Huang and co-workers (*23*) and Jacobson and Bonner (*14*) eliminate the problems inherent in the CsCl skin

method and appear to be the methods of choice for the isolation of purified cRNA.

Much work remains to be done on the characterization of cRNA. We hope that this report will help others to extend these studies and will stimulate further investigations of the cellular function of this RNA.

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21. To isolated rat ascites chromatin (*3*) were added [^{3}H]tRNA (3×10^6 count/min) and [^{32}P]ribosomal subunits (5×10^7 count/min). Recovered in the CsCl skin (*3*) were 1.3×10^2 count/min from ^{3}H and 4×10^6 count/min from ^{32}P, and in the cRNA peak on DEAE Sephadex (*3*) were 96 and 8 count/min from ^{3}H and ^{32}P, respectively, values representing less than 1 percent of the cRNA when the input tRNA and rRNA is normalized to the mass of these species present in ascites cells.
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Indole-3-Acetic Acid in Human Cerebrospinal Fluid: Identification and Quantification by Mass Fragmentography

Abstract. *Indole-3-acetic acid has been identified in human cerebrospinal fluid by the gas chromatographic–mass spectrometric technique called mass fragmentography. A specific and sensitive method for quantitative determination of indole-3-acetic acid down to 2 nanograms per milliliter of cerebrospinal fluid has been developed. Samples of cerebrospinal fluid from 24 patients with depression contained 6.1 ± 3.1 (range 2.6 to 15.8) nanograms of indole-3-acetic acid per milliliter.*

5-Hydroxytryptamine (5-HT) is catabolized in the central nervous system (CNS) by monoamine oxidase to 5-hydroxyindole-3-acetic acid (5-HIAA). Several investigators (*1*) have found low concentrations of apparent 5-HIAA in cerebrospinal fluid (CSF) of depressed patients compared to controls. The level of 5-HIAA in CSF has been used as an index of brain 5-HT metabolism (*2*).

The formation of 5-HT is a minor pathway in the biotransformation of tryptophan in animals and man. The

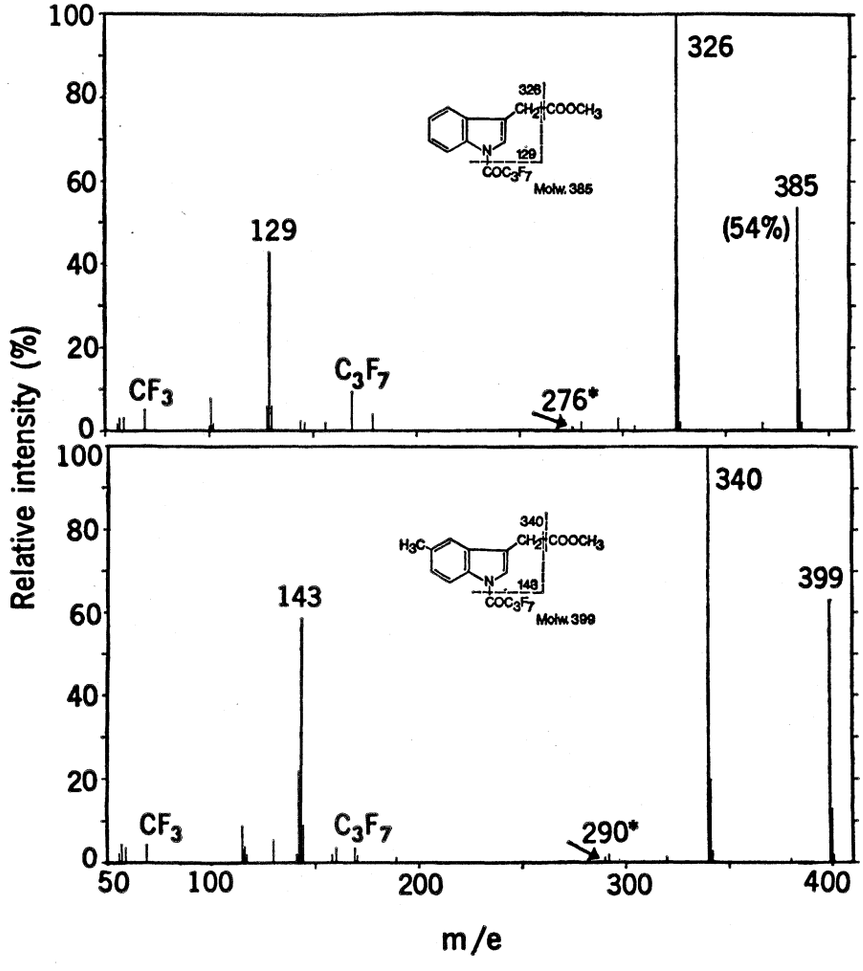

Fig. 1. Mass spectra and proposed fragmentation patterns for the heptafluorobutyryl methyl ester derivatives of reference IAA (top) and 5-MeIAA (bottom). Asterisks indicate metastable peaks. An LKB 9000 gas chromatograph–mass spectrometer with a silanized glass column (1 m by 3 mm, inside diameter), packed with 3 percent XE-60 on Gas Chrom P was used. Column, flash heater, and ion source temperatures were 130°, 210°, and 270°C, respectively. The ionizing potential was selected to 40 ev, as this was found to give the highest yield of *m/e* 326 from the IAA derivative (shown by mass fragmentography). The trap current was 60 μa; *Molw.*, molecular weight; *m/e*, mass-to-charge ratio.