Synthesis of 5S and 4S RNA in Metaphase-Arrested HeLa Cells

Abstract. *The continued synthesis of both 5S and 4S RNA in metaphase-arrested HeLa cells is demonstrated; 5S RNA is apparently synthesized at approximately 74 percent of the interphase rate, while 4S RNA is synthesized at approximately one-third the rate. The ratio of uridine incorporation to RNA methylation is used to correct for the alteration in the specific activity of the pyrimidine pool during metaphase arrest.*

In animal cells RNA synthesis largely ceases during mitosis when the chromatin is condensed into chromosomes. This was established originally by autoradiographic studies (*1*). A direct examination of the RNA labeled in metaphase-arrested cells has shown that synthesis of most nuclear RNA is inhibited, but that mitochondrial RNA synthesis continues during metaphase arrest at a rate similar to that of interphase cells (*2*).

A small amount of slowly sedimenting material labeled with [^{3}H]uridine was observed in the cytoplasm of metaphase cells (*2*). We now show that this labeled material is comprised of 5*S* and 4*S* RNA, which are synthesized at significant rates during metaphase arrest.

A large, relatively pure population of metaphase-arrested HeLa cells was obtained as described (*2*). Cells growing in suspension culture were synchronized by double thymidine arrest (*3*). After release from the second thymidine block the cells were allowed to become attached to plastic tissue culture dishes, Colcemid was added, and the cultures were allowed to enter metaphase arrest. The metaphase-arrested cells were removed from the plates by gently pipetting across the surface and maintained in suspension culture at approximately the same cell concentration as a random interphase culture. The arrested cells were also labeled in suspension culture.

After labeling, mitotic and interphase cells were fractionated in the same manner. The "nuclear" fraction obtained from metaphase cells actually consists principally of the condensed chromosomes, while the metaphase mitochondrial fraction resembles that obtained from interphase cells which contains both mitochondria and rough endoplasmic reticulum. The supernatant from which the mitochondria have been removed is termed cytoplasmic fraction in the following experiments.

The labeled RNA in the cytoplasm of an approximately equal number of interphase and metaphase-arrested cells is compared in Fig. 1. The cells were labeled for 90 minutes; the RNA was extracted from the cytoplasmic fraction and analyzed on tandem acrylamide gels. Optical density tracings of the gels (not shown) indicated that there were equivalent amounts of total RNA in the two preparations. The 18*S* ribosomal RNA labeled by both [^{14}C]-uridine and [methyl-^{3}H]methionine was in the 3.5 percent portion of the gel. The 28*S* RNA had not yet appeared in

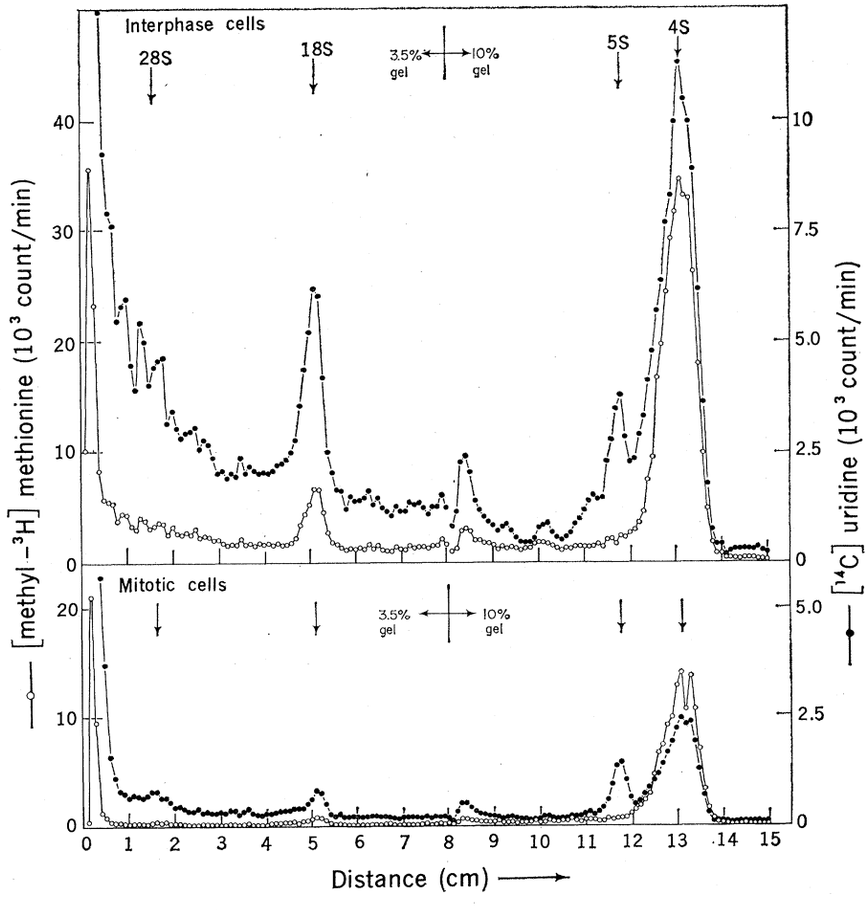


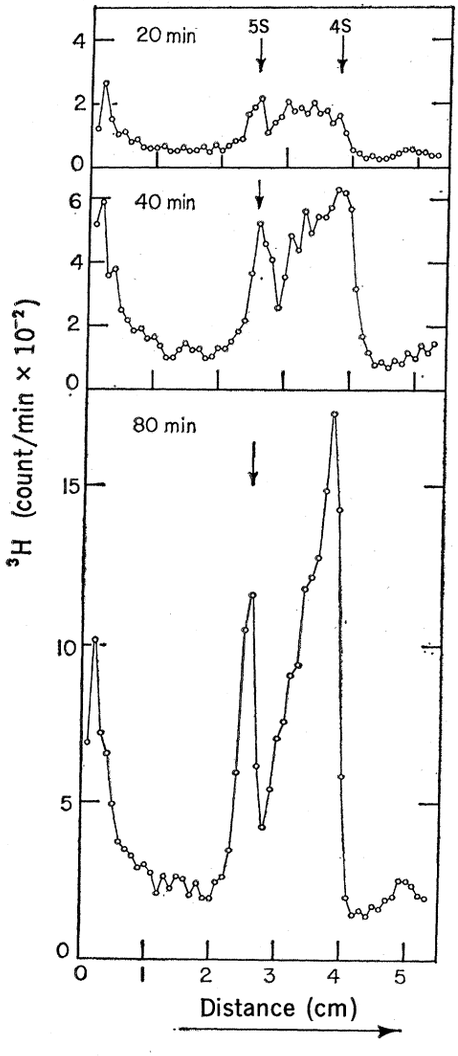
Fig. 1. Synthesis of 5*S* and 4*S* RNA in interphase and metaphase-arrested cells. HeLa cells (S-3) were grown in suspension (*8*). Cells were harvested at a concentration of about 4×10^5 cell/ml. Interphase cells: 1.2×10^8 cells were concentrated to 2×10^6/ml in medium with one-tenth the normal concentration of methionine supplemented with $2 \times 10^{-5}M$ adenosine and guanosine to reduce purine labeling (*9*), and with 7 percent dialyzed serum. Mitotic cells: 1.2×10^8 metaphase-arrested cells (mitotic index 92 percent) were collected from plates and placed in suspension (2×10^6/ml) in the same medium as interphase cells. To each culture [^{14}C]-uridine (0.3 μc/ml; 53.1 mc/mmole) and [methyl-^{3}H]methionine (20 μc/ml; 33 c/mmole) were added, and the cultures were incubated for 90 minutes at 37°C. Interphase cells and metaphase cells were fractionated by swelling in hypotonic RSB buffer (0.01*M* NaCl, 0.0015*M* $MgCl_2$, 0.1*M* tris, *p*H 7.4), and homogenized in a motor-driven Potter homogenizer (with a clearance of 0.004 to 0.006 inch). Immediately afterward, sucrose was added to a concentration of 0.25*M*, nuclei or chromosomes were deposited by centrifugation at 2000 rev/min for 2 minutes, and the supernatant was removed and centrifuged at 8000 rev/min for 10 minutes; the pellet contained the mitochondria. The mitochondria were washed twice with 0.25*M* sucrose RSB, and the washings were pooled and added to the supernatant, which is called here cytoplasmic fraction. The RNA was extracted with a mixture of phenol, chloroform, and sodium dodecyl sulfate (*9*) and analyzed by acrylamide gel electrophoresis. The gels consist of 8-cm, 3.5 percent acrylamide on top of 7-cm, 10 percent acrylamide. Electrophoresis was for 6 hours at 7 volt/cm; gels were fractionated and assayed for radioactivity (*9*).

Table 1. Ratio of [methyl-^{3}H]methionine and [^{14}C]uridine incorporated into 4*S* RNA from cytoplasmic and mitochondrial fractions in interphase and metaphase-arrested cells. The ratios for the cytoplasmic fraction were calculated from the data shown in Fig. 1. The mitochondrial fraction ratios were calculated from data obtained in the same experiment but not shown.

Cells	Ratio of [methyl-^{3}H]methionine to [^{14}C]uridine	
	Cytoplasmic fraction	Mitochondrial fraction
Interphase	2.87	2.40
Metaphase-arrested	4.64	4.02
Correction factor:	*R-metaphase/R-interphase*	
	1.61	1.66

the cytoplasm in appreciable amounts; therefore only a small peak is visible. Labeled 4*S* and 5*S* RNA were in the 10 percent portion; the 4*S* RNA is extensively methylated, but 5*S* RNA is not.

There is a small amount of radioactive 18*S* RNA from the cytoplasm of cells labeled during metaphase arrest (Fig. 1). Of the arrested cells 8 percent were still in interphase. The labeled 18*S* is probably made in these contaminating cells, as will be discussed below. In contrast to the small amount of radioactive 18*S* RNA, the metaphase-arrested cells have incorporated considerable label into 5*S* and 4*S* RNA. The data indicate that the amount of radioactivity in 5*S* RNA from the metaphase cells is 58 percent of that from the interphase population. It will be shown below that a significant correction must be made for specific activity of the pyrimidine pool in the metaphase cells and that the relative rate of synthesis of 5*S* RNA in metaphase cells is even higher than the rate of labeling indicates. The amount of uridine label in 4*S* RNA for the metaphase culture is 21 percent of that of the interphase population, whereas the



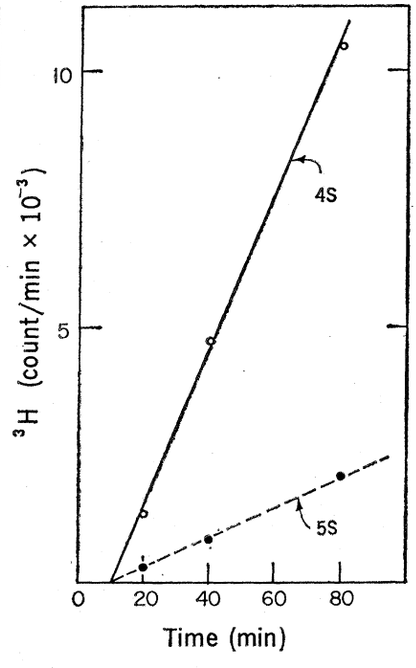


Fig. 2. Kinetics of labeling of 5*S* and 4*S* in metaphase-arrested cells. Metaphase-arrested cells (4×10^7) (mitotic index 93 percent) were concentrated to 2×10^6/ml and 10 μc/ml. [^{3}H]Uridine (22 c/mmole) was added. Portions of 1.2×10^7 cells were removed at 20, 40, and 80 minutes of incubation. Cytoplasmic fractions were prepared as described (Fig. 1), and the extracted RNA was subjected to electrophoresis (3 hours; 7 volt/cm) on 10 percent acrylamide gels. The total label in each RNA species was determined from the electrophoresis patterns.

Table 2. Incorporation of [^{14}C]uridine into 5*S* RNA in interphase (I) cells and metaphase-arrested (M-A) cells. Total radioactivity in 5*S* RNA was calculated from the data in Fig. 1 and from a gel electropherogram of the nuclear fraction, which is not shown. The corrected value of 5*S* RNA label in metaphase cells was obtained from the factor of 1.6 from Table 1 as explained in the text.

Cells	[^{14}C]Uridine (count/min)			
	Fraction		Total 5*S* label	Corrected 5*S* label
	Cytoplasmic	Nuclear		
M-A	5106	859	5965	9544
I	8817	4415	13,232	13,232

methyl label on this species is 34 percent of the amount in the interphase cells.

To determine rates of RNA synthesis from the labeling data, an estimation of the specific activity in the pyrimidine pool is necessary. In our experiments an indirect comparison of these specific activities was obtained by methylation of RNA to measure the alteration in rate of nucleotide labeling. Virtually all of the methyl groups in RNA are supplied exogenously and radioactivity of the methyl donor appears to reach an equilibrium fairly rapidly (*4*). Thus the methyl labeling of RNA can be used to compare relative rates of synthesis of methylated species in metaphase and interphase cells, provided that the degree of RNA methylation is the same in both cases. A correction factor for pyrimidine pools is obtained by comparing ratios of methyl to uridine for methylated RNA species in metaphase and interphase cells, as defined in Table 1. This correction can be applied to unmethylated RNA species such as 5*S* to determine relative synthesis rates from relative uridine incorporation in the two conditions.

Two principal species of methylated RNA are synthesized in mitotic cells. These are cytoplasmic 4*S* RNA and the 4*S* RNA synthesized in mitochondria. The ratio of methyl to uridine label for these two species is summarized in Table 1. Both species yield the same correction factor. If it is assumed that each of these RNA species is methylated to the same degree in the interphase and metaphase cells, then the ratios of methyl to uridine label indicate that the specific activity of the uridine pool is decreased during metaphase arrest, and uridine labeling of RNA in metaphase cells should be

multiplied by approximately 1.6 to permit a direct comparison with uridine labeling in interphase cells.

A possible significant error in this method of estimation would arise from a slow equilibration of the methyl donor pools with the exogenously added radioactive methylmethionine. While previous work has indicated that equilibration is rapid in interphase cells (*4*), the equilibration with exogenous radioactive methionine might be slower in mitotic cells. This would lead to an underestimation of the correction factor. Thus, if the methyl donor pool equilibrates more slowly in metaphase cells than in interphase cells the true rates of synthesis in the metaphase cells of 5*S* and 4*S* RNA would be even higher than obtained here.

An estimation of the relative rate of synthesis of 5*S* RNA in metaphase-arrested cells requires still another correction. In interphase cells, newly synthesized 5*S* RNA is first found free in the cytoplasmic fraction (*5*). After a considerable lag, the 5*S* RNA is incorporated into nucleoli and subsequently appears in mature ribosomes. Thus, in interphase cells radioactivity in cytoplasmic 5*S* RNA does not represent the total 5*S* RNA. No such process occurs in the mitotic cells since nucleoli are not found during metaphase. In Table 2 the amount of 5*S* RNA in interphase cells is corrected for the amount that has entered the nuclear fraction. The final estimate is that 5*S* RNA is synthesized in metaphase-arrested cells at approximately 74 percent the rate of interphase cells. This estimate is taken to indicate merely that a significant synthesis of 5*S* RNA occurs in metaphase-arrested cells.

A direct comparison of the methyl label in 4*S* RNA indicates that this species is apparently synthesized in metaphase-arrested cells at a rate approximately 34 percent of that found in interphase cells. Further evidence that the labeling of these RNA species is not due to contaminating interphase cells is obtained from the ratio of methyl to uridine in the small amount of 18*S* RNA of the metaphase preparation. The ratio of methyl to uridine of this RNA species is exactly the same as that found in the interphase culture, and this indicates that 18*S* RNA is derived from the 8 percent contaminating interphase cells. This is further supported by the fact that the amount of label in 18*S* RNA for the metaphase culture is 8 percent of the amount in the interphase culture. Thus, the pyrimidine pools from the contaminating interphase cells appear to be unaltered. If these contaminating cells continue to synthesize 5*S* and 4*S* RNA, their contribution to these species should constitute approximately 8 percent of the amounts of 5*S* and 4*S* RNA labeling in the interphase culture. This would result in small corrections downward for the relative rates of synthesis shown in Table 2.

The labeling of 5*S* and 4*S* RNA in metaphase-arrested cells proceeds at a linear rate for at least 90 minutes (Fig. 2). Thus, the incorporation seen here is not a transient phenomenon occurring only at the beginning of metaphase. The formation of cytoplasmic 4*S* RNA appears to be normal; the first product is a molecule of slightly higher molecular weight which is subsequently transformed into the mature form of 4*S* RNA (*6*).

Identical results have been obtained with Chinese hamster cells. The phenomenon, therefore, appears to be characteristic of cells of both human and rodent origin. The results suggest that some regions of the condensed chromosomes are transcribed. However, the possibility exists that some of the RNA labeled during metaphase may arise from nonchromosome-associated templates (*7*).

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References and Notes

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Periodical Cicada: Mechanism of Sound Production

Abstract. *The species* Magicicada septendecim *and* Magicicada cassini *of the 17-year cicada produce sound by sequentially buckling a series of stiff ribs embedded in a flexible tymbal. Each such collapse excites a damped oscillation in a resonant cavity. By this means the cavity (an abdominal air sac) is excited 10 to 12 times per muscle contraction, which permits a normal muscle to perform a task requiring very rapid repetitive activity.*

The periodical cicadas (*Magicicada*) are among the noisiest of insects (*1*, *2*). A single male cicada can produce a sustained call lasting several seconds, and repeat this call many times per day without apparent fatigue. The sound is produced by a rapidly repeated series of damped oscillations produced in the abdominal air sac by activity of the tymbal organs (Fig. 1A). In *M. cassini*, direct tap excitation of an air sac produces a brief oscillation which is damped out in about 2 msec (Fig. 2A). To maintain sustained sound, the air sac must be excited several hundred times per second. Pringle (*3*) has described the sound-producing mechanism in several species of Ceylonese cicadas; in these a "clicker" mechanism is popped in and out at rates up to 480 per second by a specialized muscle with highly unusual excitation-contraction properties.

In June 1970, I took advantage of the local emergence of brood X of the 17-year cicada to examine the mechanism of sound production in these species (*4*). Preliminary work was done on *M. septendecim*, but the bulk of the experiments were done on *M. cassini*, as specimens of this species were much more tolerant of surgical manipulation in the laboratory. In both species the tymbal organ of the male consists of 12 stiff ribs connected by a flexible sheet (Fig. 1, C and D). The tymbal muscle is connected close to the posterior end of this sheet by a flexible, tendon-like apodeme (Fig. 1B). Recordings of the mechanical behavior of the tymbal were obtained by slipping a fine, stainless steel wire hook through