ENTRY OF RADIOACTIVITY FROM METHYL-LABELED METHIONINE INTO MITOCHONDRIAL AND CYTOPLASMIC RNA OF CULTURED HAMSTER CELLS

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1. Introduction

Following the work of Mandel and Borek [1] most analyses of post-transcriptional methylation of nucleic acid have employed methyl-labeled methionine as a specific precursor, via *S*-adenosylmethionine (SAM), of RNA or DNA methyl groups. For example, we as well as others have relied on this approach in studies comparing cytoplasmic and mitochondrial nucleic acids in cultured mammalian cells [2–6]. The validity of such studies depends on the assumption that, if separate SAM pools exist in the different sub-cellular compartments, they are labeled with comparable efficiency by exogenous methionine. This assumption has recently been questioned in a report on mitochondrial DNA [2], and there is evidence from a chick embryo system that compartmentalization of SAM can affect apparent methylation levels of cytoplasmic ribosomal RNA [7]. We now present experiments that validate the assumption of functionally equivalent SAM pools for cytoplasmic and mitochondrial RNA in a cell culture system.

2. Materials and methods

Growth and fractionation of hamster (BHK-21) cells, and isolation of mitochondrial and cytoplasmic RNA, were essentially as previously described [6]; further details are presented in the legends to the figure and tables. For isolation of methylated adenines, RNA samples were subjected to alkaline hydrolysis followed by electrophoresis at pH 3.5. The Ap regions (which contain both methylated and unmethylated Ap residues) were eluted, concentrated, and hydrolyzed with acid; under these conditions both mitochondrial and cytoplasmic tRNA yield N^6-methyladenine as the sole methylated adenine, and mitochondrial '13s' rRNA yield only N^6-dimethyladenine [3,6]. Adenine was then separated from N^6-methyladenine and N^6-dimethyladenine by paper chromatography (see fig. 1).

3. Results

Cells were labeled either during exponential growth, or in the presence of a low level of actinomycin; the latter procedure has been employed frequently (cf. [3–6]) to permit examination of mitochondrial rRNA in the absence of contaminating radioactivity from cytoplasmic rRNA.

Because of the small amount of mitochondrial RNA in the cells (approx. 2% of total) and the very low proportion of methylated vs. unmethylated adenine purine rings were determined as radioactivity incorporated from added adenosine. A preliminary experiment indicated that this precursor labeled mitochondrial and cytoplasmic RNA with equal efficiency (table 1).

To study the entry of radioactivity from methyl-labeled methionine into RNA, cells were labeled simultaneously with [8-^{14}C] adenosine and [methyl-^{3}H] methionine for periods of 18–20hr, and isotope contents of mitochondrial and cytoplasmic methylated adenines were compared. As illustrated in fig. 1, we were able clearly to resolve the ^{14}C in both N^6-methyladenine and N^6-dimethyladenine from the vast excess of counts in adenine. The resulting isotope ratios

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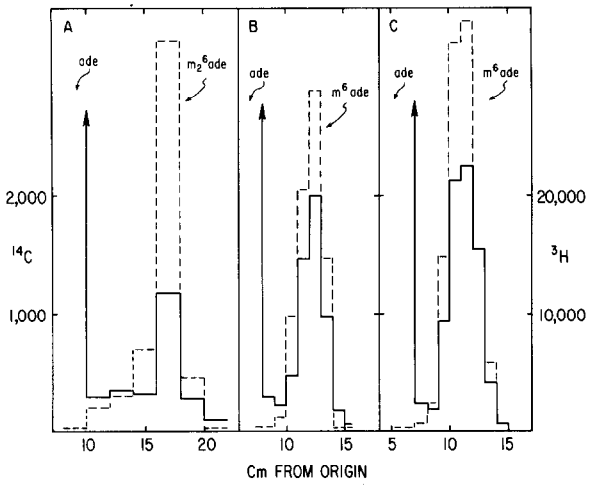

Fig. 1. Paper chromatography of adenine and methylated adenine residues from [^{3}H]methyl-, [^{14}C]adenine-labeled RNA. A 500 ml culture was labeled for 18 hr in the presence of actinomycin, 0.1μg/ml, with [methyl-^{3}H] methionine, 25μCi/3μg/ml and [8-^{14}C]adenosine, 0.25 μCi/7 nmoles/ml. Processing was as described for table 1. Transfer RNA was further purified by gel electrophoresis at elevated temperature [3]. Samples of adenine plus methylated adenine (see text) were subjected to paper chromatography ([6], fig. 9a) over a distance of 26 cm; only the regions from the leading edge of adenine through N^6-dimethyladenine have been plotted. Unlabeled adenine, N^6-methyladenine and N^6-dimethyladenine were added to each sample as markers; the center of the adenine spot was at 6.5 cm for A and B, 5.5 for C. Panel A: mitochondrial 13s RNA (cts per 30 min per 2 cm segment); Panel B: mitochondrial tRNA (cts per 30 min per cm); Panel C: cytoplasmic tRNA (counts per 20 min per cm). ^{14}C: (———); ^{3}H (– – – –).

Table 1
Labeling by [8-^{3}H] adenosine of adenine residues in mitochondrial and cytoplasmic RNA

	Specific activity, cpm/pmole adenine residue	
	Exponential	Actinomycin-treated
Mitochondrial		
4s	6.9	70
13s	7.0	71
17s	7.6	71
Cytoplasmic		
4s	7.7	73
18s	7.5	--
28s	6.7	--

Parallel 500 ml cultures were labeled for 18hr in the presence and absence of actinomycin, 0.1μg/ml, with [8-^{3}H] adenosine, 18μCi/20 nmoles/ml and 2μCi/40 nmoles/ml, respectively. Processing was as previously described [6] except that no carrier RNA was added to mitochondrial preparations. 'Low salt' gradients were employed to minimize extraneous RNA and DNA [6]. To determine specific radioactivities, 260 nm absorbancies of purified RNA samples were measured, and aliquots of the same samples were counted. The whole of each mitochondrial preparation, containing 10–20 μg of mitochondrion-specific RNA, was processed, yielding absorbancy readings of 0.2 – 0.4 in peak gradient fractions. Corrections were made for partial (75–80%) labeling of guanine residues and differences in mole percentages of adenine, cf. [8,9].

Table 2
Relative labeling by [^{14}C]adenosine and [^{3}H]methionine of methylated adenine residues of mitochondrial and cytoplasmic RNA

	Ratio of counts from methionine to counts from adenosine	
	Actinomycin-treated	Exponential
Mitochondrial		
tRNA m^6ade	14.6	22.0
13s RNA m_2^6ade	29.	--
Cytoplasmic		
tRNA m^6ade	15.3	21.8

The values represent averages of two runs such as presented in fig. 1. To label exponentially growing cells, parallel 250 ml cultures were incubated for 20 hr in the presence of either [methyl-^{3}H]methionine, 20μCi/7.5μg/ml or [8-^{14}C]adenosine, 0.20 μCi/20 nmoles/ml. Adenosine and guanosine were added to the methionine-labeled culture with the aim of suppressing spurious labeling of purine rings, cf. [6]; it was found subsequently that this was not necessary (cf. experiment of fig. 1). The ratios of molar specific radioactivities of the added methionine: adenosine were 12.9 for the actinomycin-treated cells and 21.5 for the exponential cells, as determined from nominal chemical concentrations of radioactive stocks and counting in the same system as used for the RNA samples from the respective experiments (efficiencies differed somewhat between the two experiments). Processing was as described for fig. 1.

([^{3}H] methyl: [^{14}C] adenine) for mitochondrial samples were not significantly different from those of corresponding cytoplasmic samples, taking into account the 2 methyl groups of N^6-dimethyladenine (table 2).

4. Discussion

Methylation of cytoplasmic tRNA occurs in the cytoplasm [10] and methylation of mitochondrial RNA presumably occurs in mitochondria. Thus, the above results show that labeling of SAM pools was effectively the same in the mitochondrial and cytoplasmic compartments. We infer that incorporation of radioactivity from methyl-labeled methionine provides reliable data on relative degrees of methylation of mitochondrial and cytoplasmic RNA in our system, and presumably in other similar systems as well.

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