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CYTOPLASMIC DISTRIBUTION OF A MOUSE EMBRYONAL CARCINOMA CELL SPECIFIC RNA, MS3 RNA

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SUMMARY

A cloned cDNA designated as MS3 sequence can detect an RNA species with a size of about 6 kb in undifferentiated embryonal carcinoma (EC) cell lines but not in several differentiated cell lines derived from EC cells. This paper deals with the cytoplasmic distribution of this RNA species, MS3 RNA. No MS3 RNA is observed in the cytoplasm of TDM1 (trophoblastoma) cells. In the cytoplasm of PCC3 and F9 (EC) cells, however, MS3 RNA is found in both polysomal fraction and postpolysomal fraction. About 20—55% MS3 RNA are included in polysome structure. This result implies that MS3 RNA is an actively translated message sequence and the protein specified by MS3 RNA will be a good marker for multipotent early embryonal cells.

INTRODUCTION

We have cloned a cDNA sequence comprising about 300 nucleotides. This cDNA sequence can detect an RNA species with a size of about 6 kb in the total cellular RNAs and the total poly $(A)^+$ RNAs of some mouse embryonal carcinoma (EC) cell lines such as F9, PCC3, PSA1, but not in several differentiated derivatives of EC cells examined so far, i. e., TDM1 (trophoblastoma cell), PYS—2 (parietal york sac-like cell), 3/A/1—D3 (embryonic fibroblast-like cell) and C17—S1—T384 (myoblast-like cell). Therefore we designate this cDNA sequence as multipotent EC cell specific sequence (MS3 sequence). MS3 sequence is moderately repeated in mouse genome, it can hybridize to about 1% phage plaques in a mouse genomic library. Three positive genomic clones were isolated and they all were shown to contain a long stretch of sequence expressed specifically in EC cells. Thus it seems that most,

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if not all, of the positive genomic clones have the sequence specifying the specific 6 kb RNA species (MS3 RNA). Although these characteristics of MS3 sequence are reminiscent of retroviruses, we can not find any homology between MS3 sequence and retrovirus particle A sequences (Brûlet, et al., 1982).

EC cells exhibit strong analogies with cells of the inner cell mass and early ectoderm in mouse embryo (Graham, 1977; Jacob, 1978; Martin, 1978). It would prove interesting if MS3 sequence were expressed also and only in those embryonic cells. The prerequisite for approaching this possibility is to find the protein encoded by MS3 RNA. In our previous work, however, the total cellular RNA and total poly $(A)^+$ RNA were employed for the RNA blot hybridization. Thus it is not clear whether MS3 RNA is an mRNA or rather a nuclear RNA or some cytoplasmic RNA that is packaged in ribonucleoprotein particles which are inactive in protein synthesis (Buckingham, et al., 1976). In order to solve this problem, the first step is to examine the distribution of MS3 RNA in cytoplasmic compartments. The preliminary results are reported in this paper.

MATERIALS AND METHODS

Cell: The following teratocarcinoma derived cell lines were cultured as mentioned in the articles describing the different lines; PCC3/A/1 (Jakob, et al., 1973), F9 (Bernstine, et al., 1973) are embryonal carcinoma lines. TDM1 (Nicolas, et al., 1976) is a trophoblastoma cell line.

Preparation of polysomal and postpolysomal fractions: Cells were collected at half saturation. The cytoplasmic fractions were prepared from these cell lines by the technique of Croce et al. (1981). The postmitochondrial supernatants were isolated and loaded onto preparative 7—47% sucrose density gradients containing 50mM $MgCl_2$, centrifuged, fractionated into postpolysomal and polysomal fractions as described by Ouellette et al. (1976). The polysomal structure was disassociated with 10 mM EDTA also by the technique of Ouellette et al. (1976).

Extraction of RNA: Total RNA was extracted from polysomal and postpolysomal fractions according to Auffray and Rougeon (1980).

1.5% formaldehyde agarose gel electrophoresis of RNA preparations and Northern blot: The total RNA prepared from polysomal and postpolysomal fractions was denatured, electrophoresed on 1.5% formaldehyde agarose gel and then blotted onto nitrocellulose filter as described by Rave et al. (1979).

^{32}P—Labelling of MS3 plasmid DNA: MS3 plasmid DNA was labelled by the nick translation reaction (Rigby, et al., 1977) to a specific activity of

$7.6\ 10^7$cpm/μg.

Hybridization of Northern blots with ^{32}P—labelled MS3 plasmid DNA: This was conducted under the conditions described by Thomas (1980).

RESULTS AND DISCUSSION

To our knowledge, MS3 sequence is the first cloned cDNA sequence specific for EC cells. The attractive promise that MS3 sequence may well be the first molecular marker for the undifferentiated early embryonic cells renders it desirable to search for the protein encoded by MS3 RNA. As a first step, it should be proven that MS3 RNA is a real mRNA being actively translated. To examine the cytoplasmic distribution of MS3 RNA is one of the approaches to this problem.

The mouse teratocarcinoma system was used in this study, in which TDM1 and F9, PCC3 (EC lines) were considered as models of differentiated and undifferentiated early mouse embryonic cells respectively (Jacob, 1978). A post-mitochondrial supernatant from these cell lines was loaded onto preparative 7—47% sucrose density gradients containing 50 m*M* $MgCl_2$, centrifuged and fractionated into postpolysomal and polysomal fractions as described by Ouellette et al. (1976). The total RNA prepared from each fraction was denatured, electrophoresed on 1.5% formaldehyde agarose gel and then blotted onto nitrocellulose filter as described by Rave et al. (1979). The RNA blots were hybridized to ^{32}P—labeled MS3 probe as described in Materials and Methods.

Table 1 Distribution of MS3 RNA in cytoplasmic compartments

cell line	percentage in	
	polysome region	postpolysome region
PCC3	55	45
F9	20	80

As expected, no RNA could be detected with MS3 probe in the cytoplasmic fraction of TDM1 cells (data not shown). In PCC3 and F9 cells, however, a considerable amount of MS3 RNA were found in the polysomal fractions (Fig. 1, lanes D and F). For a quantitative estimation, the autoradiographic films were scanned with a photodensitometer. The calculated data are shown in Table 1. About 20—55%MS3 RNA present in cytoplasm of EC cells are located in

polysome region. However, it was suggested that some microsomal material and membrane fragments would cosediment with polysome (Buckingham, et al., 1976). Total RNA in the comparable fractions of 10 m*M* EDTA containing gradients from F9 cells was therefore extracted, fractionated, blotted and hybridized to MS3 probe in the same way as for Mg^{2+}containing gradients. The autoradiogram (Fig. 1, lanes A and B) was scanned. It was observed that in the EDTA-containing gradients, nearly all of the MS3 RNA was distributed in the postpolysomal fraction and only less than 6 % MS3 RNA remained in the polysome region. It can thus be concluded that a great proportion of MS3 RNA detectable in polysome region is not cosediments with polysomes but is associated with an authentic polysome structure capable of disruption with EDTA. This result strongly suggests that MS3 RNA is an actively translated message sequence in undifferentiated EC cells. Although our preliminary attempts to isolate sufficient quantities of the RNA complementary to MS3 sequence for translation *in vitro* have not yet been successful, the results reported in this paper do imply that a protein encoded by MS3 gene should exist only in undifferentiated EC cells. This putative protein will be a good marker for multipotent early embryonic cells.

The moderately repetitive nature in mouse genome of MS3 sequence and the large size of its transcripts make it supposable that MS3 sequence might be some retrovirus or transposable element. In this respect, it is especially interesting to note that a major part of the transcripts of proretroviruses and eucaryotic transposable elements are preferentially included in postpolysomal fraction (Ryokov, et al., 1980) and may not be functional messages (Flavell, et al., 1980). The different behavior of MS3 RNA in respect of cytoplasmic distribution seems thus to be not in favor of this hypothesis.

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小鼠胚胎癌细胞特异 RNA, Ms_3 RNA 在细胞质中的分布情况

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摘 要

MS3顺序是一个克隆化的 cDNA顺序。它在未分化的小鼠胚胎癌细胞中能探查出一个分子量约为 6 kb 的 RNA，可是在已分化的由胚胎癌细胞衍生的其他细胞株中，它不能查到这RNA。由于小鼠胚胎癌细胞与小鼠胚胎的内细胞团及早期原始外胚层细胞十分相似，能否在这些胚胎细胞中查到MS3顺序的表达产物将是件使人感兴趣的事。研究这一问题的一条途径是寻找MS3 RNA所编码的蛋白质。为此需首先确定 MS3 RNA 是一种真正能被翻译的mRNA。因此本文研究MS3 RNA 在细胞质中的分布情况。首先用离心法除去细胞质中的线粒体等大颗粒，然后通过 7—47%蔗糖密度梯度离心，把其余部份分成多核糖体组份及次多核糖体组份，并从这些组份中提取出各自的总 RNA。为了探查这些RNA中MS3 RNA的含量，先把这些RNA进行1.5% 甲醛琼脂糖凝胶电泳，接着将凝胶中的RNA转移到硝酸纤维纸上，与经过缺口翻译标上^{32}p的MS3 质粒杂交。根据通过放射自显影显示的杂交信号，我们证明在代表已分化细胞的TDM1（滋养母细胞瘤）细胞的细胞质中不能查到MS3 RNA，但在代表未分化细胞的二种胚胎癌细胞株 PCC_3 和F_9细胞的细胞质中，MS3 RNA 不仅存在于多核糖体组份中也存在于次多核糖体组份中。通过对放射自显影底片的分光光度扫描，估计MS3 RNA 的大约20—55%分布在多核糖体结构中。若用EDTA分介多核糖体结构，则约94%以上的MS3 RNA 便出现在次多核糖体组份中。这些结果表明MS3 RNA确实是一种极积合成蛋白质的 mRNA，并提示由MS3 RNA 翻译成的蛋白质将是研究早期未分化胚胎细胞的一个理想的标记蛋白质。

许以盛等：小鼠胚胎癌细胞特异*RNA*，MS_3 *RNA*在细胞质中的分布情况

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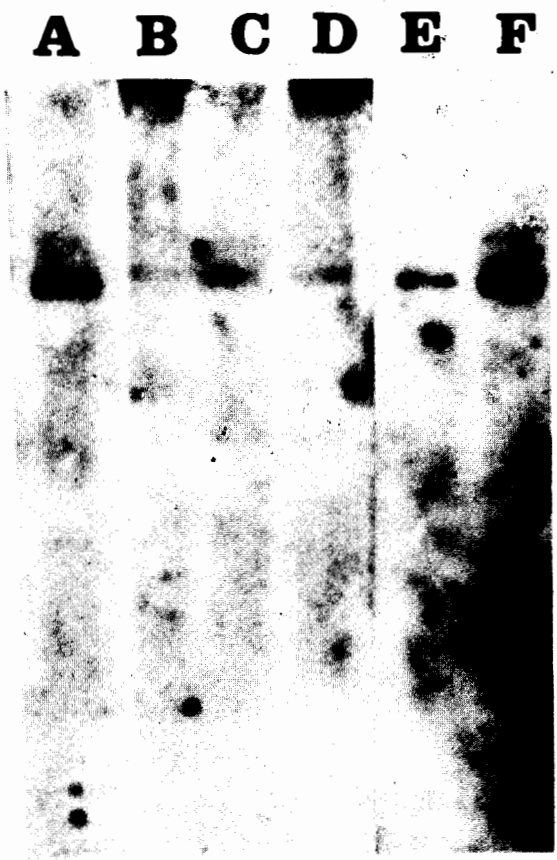

ig. 1 Presence of MS3 RNA in polysomal fraction. Postmitochondrial supernatants from PCC3, F9 cells were fractionated on sucrose gradients containing Mg^{2+} or EDTA to separate polysomal and postpolysomal ribonucleoprotein by the technique of Ouellette et al. (1976). The total RNA prepared from each fraction was denatured, electrophoresed on 1.5% formaldehyde agarose gel, blotted onto nitrocellulose and hybridized to ^{32}P-labelled MS3 probe (Specific Activity, 7.6×10^7 cpm/μg) by the technique of Rave et al. (1979). 10 μg RNA from each fraction was applied on the gel except the polysomal fraction of the EDTA-containing gradient of F9 cells, of which only 2 μg RNA were loaded on the gel. Postpolysomal and polysomal RNAs from EDTA-containing gradients are shown in lanes A and B, comparable fractions from Mg^{2+} containing gradients are shown in lanes C and D for F9 cells, in lanes E and F for PCC3 cells, respectively.

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图2.蛇毒胆碱酯酶的凝胶电泳图谱。聚丙烯酰胺凝胶7%，*Tris-HCl*缓冲液*pH*8.9，化学聚合，电流3毫安/管，电泳时间约3小时，酶显色用*Shafai*法。自左至右，样品分别为原蛇毒、经疏水层析后，经亲和层析。

图1.恒河猴血清蛋白电泳图象

Fig. 1. Cellulose acetate electrophoretic pattern.