第5卷 第2期 动 物 学 研 究 Vol. 5, No. 2
1984年 5月 ZOOLOGICAL RESEARCH May, 1984

POSSIBLE MECHANISMS UNDERLYING EXPRSSION OF MS3 RNA IN MOUSE EMBRYONAL CARCINOMA CELLS

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SUMMARY

The degrees of methylation of MS3 gene regions detectable with MS3 probe were found to be nearly the same in F9 genome and TDM1 genome. Nuclear transcripts of MS3 genes were detected not only in F9 cells but also in TDM1 cells. These results suggest that the failure of detection of MS3 RNA in the cytoplasm of differentiated cells may be mainly due to the defect of splicing the nuclear transcripts of MS3 genes or inability of transfer of the mature MS3 RNA from nucleus to cytoplasm.

INTRODUCTION

MS3 RNA is the only RNA species detectable in some embryonal carcinoma (EC) cells with a cloned cDNA probe (MS3 sequence), but MS3 RNA can not be found in the total cellular RNA and the total cellular poly (A+) RNA in some differentiated derivatives of EC cells. MS3 sequence is moderately repeated in mouse genome. About 1% of phage plaques in a mouse genomic library gave positive hybridization signals to MS3 probe. Three positive genomic clones were isolated and shown to contain EC specific sequences. Since none of these genomic sequences could detect any other RNA species than MS3 RNA in the poly(A+)

本文1982年5月21日收到，1982年9月14日收到修改稿。

fraction of an EC cell line, F9, it seems that the EC specific sequences in these genomic sequences all represent MS3 RNA genes (Brûlet, *et al.*, 1982). Thus several questions may arise: Are all or a large proportion of MS3 genes active genes? Or are only few MS3 genes transcribed? Why could no MS3 sequence specific RNA be found in both total cellular RNA and total cellular poly(A+) in differentiated cell lines?

One approach to these questions is to examine the degrees of methylation of MS3 gene regions in TDM1 genome and in F9 genome. As pointed out by Naveh-Many, *et al.* (1981), undermethylation is a general phenomenon in all actively transcribed genes. Another approach is to search for nuclear transcripts of MS3 genes in TDM1 cells and F9 cells. Since Hamer, *et al.* (1979) have suggested that nuclear precursor RNAs are rapidly degraded, the failure of detection of MS3 RNA in the cytoplasm of differentiated cells may not imply that MS3 genes are unable to be transcribed in differentiated cells.

Following these approaches, we have achieved some progress. The preliminary results are described in this paper.

MATERIALS AND METHODS

Cell, F9 is an embryonal carcinoma line cultured as described by Bernstine, *et al.*(1973); TDM1 is a trophoblastoma line cultured according to Nicolas, *et al.*(1976).

Preparation of high molecular weight DNAs: High molecular weight DNAs were extracted from TDM1 and F9 cells according to Blin, *et al.* (1979). Enzymatic digestions were made according to various companies' instructions.

Extraction of nuclear RNAs: Nuclear RNA was prepared from TDM1 and F9 cells by the technique of Lai, *et al.*(1978).

^{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 10^7 cpm/μg.

Gel electrophoresis, transfer to nitrocellulose sheets and hybridization: DNA was subjected to electrophoresis on 1.2% agarose gel in 20 m*M* sodium acetate, 40m*M* Tris-HCl, pH 8.4, 2m*M* EDTA. DNA fragments were blotted onto nitrocellulose sheets according to Southern (1975). Restriction fragments from λ DNA were used as size markers.

Nuclear RNAs were denatured, electrophoresed on 1.5% formaldehyde agarose gel and transferred onto nitrocellulose filters as described by Rave, *et*

al.(1979). Hybridization of the blots with ^{32}P-labelled MS3 plasmid DNA was conducted according to Thomas(1980).

RESULTS AND DISCUSSION

In order to gain some insights into the mechanisms underlying the specific expression of MS3 genes in undifferentiated EC cells, we have tried to examine first at which level its specific expression is regulated. We have shown previously that no MS3 sequence specific RNA species can be found in both total cellular RNA and total cellular poly(A+) RNA in the differentiated cell lines examined so far (Brûlet, *et al.*, 1982). The simplest interpretation of those results is that differentiated cells are unable to transcribe MS3 genes. If this were the case, there would exist a significant difference in the degrees of methylation of MS3 gene regions between TDM1 genome and F9 genome, because Naveh-Many, *et al.*(1981) have suggested that undermethylation is a general phenomenon in all actively transcribed genes. Therefore, high molecular weight DNAs were extracted from TDMI and F9 cells (Blin, *et al.*, 1976). They were digested with EcoR 1 plus the methylation-specific restriction enzyme, Hpa II, or the enzyme Msp 1, an isoschizomar of Hpa II. After 1.2% agarose gel electrophoresis, the digests were transferred onto nitrocellulose and hybridized to ^{32}P-labelled MS3 probe. The size distribution of each DNA population was determined by scanning the autoradiographic film. The number-average molecular weight of each population was determined by analysis of the size distribution scans by reference to molecular weight markers.As shown in Fig. 1 and Table 1, no significant difference is found between the average molecular weight of the Hpa II digests detectable with MS3 probe of F9 DNA and that of TDMI DNA.This result suggests that a large proportion of MS3 genes may be actively transcribed and they may be transcribed equally well in TDMI cells and in F9 cells, though it may be open to some other explanations.

In order to distinguish the possibility of MS3 genes being transcribed also in TDM1 cells, nuclear RNAs were examined. For the preparation of the nuclear RNA from F9 and TDM1 cells, the technique of Lai *et al.*(1978) was employed. The purity of these nuclear RNAs was checked by their digestion with RNase A and DNase 1 and the subsequent electrophoresis on formaldehyde agarose gel. No DNA contamination was detectable in these nuclear RNA preparations. The nuclear RNAs were denatured and subjected to formaldehyde agarose gel electrophoresis as described (Rave, *et al.*, 1979). After being blotted

onto nitrocellulose, they were hybridized to ^{32}P-labelled MS3 probe. As shown in Fig. 2, nuclear transcripts of MS3 genes were observed not only in F9 cells but also in TDM1 cells. The smear displayed on lanes A and B in Fig.2 may represent the degradation of MS3 nuclear transcripts, as Hamer and Leder(1979) have suggested that unspliced precursor RNAs are rapidly degraded. In the nuclear RNA prepared from the nuclei which had been added a little amount of cytoplasmic RNA, a discrete band of MS3 RNA against the background of a smear could be seen (data not shown). This fact argues for the degraded MS3 nuclear transcripts being the unspliced precursor RNAs of MS3 RNA rather than an artifact brought about during preparation of these nuclear RNAs. As no discrete band of MS3 RNA is distinguishable on F9 nuclear RNA blot (Fig. 2, lane A), the proportion of the mature MS3 RNA in the nuclear RNA may be very low. This implies that only a small proportion of MS3 nuclear transcripts is spliced into the mature RNA in the nucleus.

The two lines of evidence presented in this paper are thus in favor of the hypothesis that the expression of MS3 gene family may be mainly regulated at the post-transcriptional level, the failure of detection of MS3 RNA in differentiated cells may be chiefly due to splicing defect or inability of transfer of the mature MS3 RNA from nucleus to cytoplasm.

Table 1 Size analysis of Hpa II and Msp 1 digests of EcoRI digested TDMI and F9 DNAs

restriction enzyme	average molecular size, kb	
	F9 DNA	TDMI DNA
EcoR 1	14.5	14.5
EcoR 1 + Msp 1	1.55	1.35
EcoR 1 + Hpa II	5.2	5.9

Legend of Table 1

DNAs were digested with the restriction enzymes, fractionated by 1.2% agarose electrophoresis, blotted onto nitrocellulose and then hybridized to ^{32}P-labelled MS3 probe (Specific Activity, 10^8 cpm/μg) as described in Materials and Methods. The size distribution of each DNA population was determined by scanning the autoradiogram, The number average molecular weight of each po-

pulation was calculated by analysis of the size distribution scans by reference to the molecular weight markers of Hind III digested λ phage DNA.

ACKNOWLEDGEMENT

The authors are grateful to Professor Francois Jacob for his help and guidance in this work, which was conducted in his laboratory, Institut Pasteur, Paris.

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MS_3RNA 在小鼠胚胎癌细胞中特异表达的可能机理

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摘 要

MS_3 RNA 是小鼠胚胎癌细胞特异 RNA。为了阐明 MS_3 基因在未分化细胞中特异表达的机理，我们应该首先确定此特异表达是在哪一级水平上被调控的。Naveh-Many 等（1981）指出积极进行转录的基因一般是甲基化程度不足的。由于 MS_3 基因有一个中等重复的家族，因此如果 MS_3 基因的调控主要在转录水平上进行，我们应该发现 MS_3 基因的甲基化程度在分化细胞及未分化细胞之间有明显差别，于是用测定甲基化程度的一组酶 Hpa II 和 Msp 1 水介代表分化细胞的 TDM_1 及代表未分化细胞的 F_9 的高分子量 DNA，然后用 MS_3 cDNA 作探针分析 MS_3 基因区的甲基化程度。在可用 MS_3 cDNA 探查到的范围内，MS_3 基因区的甲基化程度在 TDM_1 及 F_9 细胞中未见明显不同。对这一结果可有四种不同解释：1. MS_3 基因家族中除少数成员外其余绝大部分成员在未分化细胞中是不转录的，在分化细胞中则全部不转录；2. MS_3 基因家族的绝大部分成员在未分化细胞以及在分化细胞中都是转录的；3. 虽然 MS_3 基因家族中的绝大部分成员都是不转录的，但个别成员在未分化细胞以及分化细胞中都是转录的；4. 由于 MS_3cDNA 只能探查 MS_3 基因的分端区域，可能此区域的甲基化程度与 MS_3 基因的转录水平无关。为了区别这些可能性，我们检查了 F_9 细胞及 TDM_1 细胞中的核 RNA，发现在这二种细胞核内都存在着数量几乎相等的大量 MS_3 基因转录物。因此我们推断，MS_3 基因家族中至少有一部分成员，很可能是大部分成员，不论在未分化细胞还是在分化细胞中都是转录的。在已分化细胞的细胞质中不能探查到 MS_3 RNA 的原因可能在于 MS_3 基因在转录后水平上的调控，即在已分化细胞的核内缺乏加工 MS_3 RNA 前体的能力或缺乏把成熟的 MS_3 RNA 从细胞核输送到细胞质中去的能力。

许以盛等：MS_3RNA在小鼠胚胎癌细胞中特异表达的可能机理

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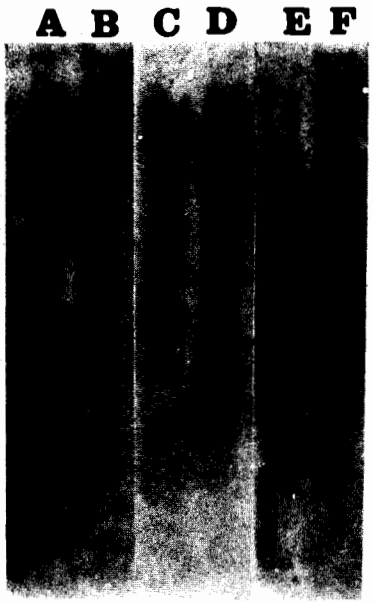

Fig. 1

The degrees of methylation of MS3 gene regions detectable with MS3 probe. DNAs were digested with the restriction enzymes, fractionated by 1.2% agarose electrophoresis, blotted onto nitrocellulose and then hybridized to ^{32}P-labelled MS3 probe (Specific Activity, 10^8 cpm/μg) as described in Materials and Methods. 10 μg DNA per slot were applied on the gel. Lane A, C, and E, TDM1 DNA digested with EcoR 1, EcoR 1 + Msp 1 and EcoR 1 + Hpa II, respectively; Lane B, D, and F, F9 DNA digested with EcoR 1, EcoR 1 + Msp 1, and EcoR 1 + Hpa II respectively. The arrows displayed on the right side indicate the size markers given by EcoR 1 digested λ DNA, which represent 21.4, 7.4, 5.4, 4.7 kb respectively.

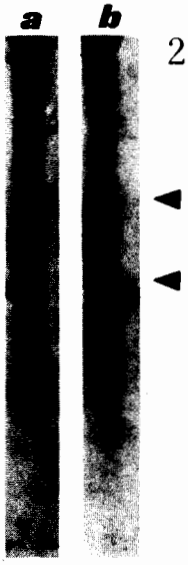

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Fig. 2

Detection of MS3 transcripts in nuclear RNAs of teratocarcinoma cells. Nuclear RNAs prepared as described (Lai, *et al*., 1978) were fractionated 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) as described (Rave, *et al*., 1979). 10 μg of each nuclear RNA were applied on the gel. Lane A, F9 nuclear RNA, Lane B, TDM1 nuclear RNA.