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Experimental Models for An In Vitro Embryonic Genosystem

—— Embryonic Liver scRNA Mediated Transcription ——

Tamito Noto

Department of Oral Anatomy, Kagoshima University Dental School,
Kagoshima 890, Japan

Abstract

A series of experiments to test the availability of theoretical models on gene differentiation[1),2),3),4)] are in progress. In these experiments, transcriptionally active 4-7 Kb EcoRI DNA fragments of the chick genomic origin[5),6)] were inserted to the pT 7-1 vector (Genescribe, USB) after being hybridized in vitro with-300 bp scRNAs from the chick embryonic liver. It was concluded that the embryonic scRNAs should have some role to influence in vitro transcriptional activity of the experimental genosystem. Most interestingly, the scRNAs had also a significant effect on the transcriptional activity of a plasmid (pBR 322) in the eucaryotic transcription system (BRL).

Key words

gene differentiation, experimental models, uncloned chick EcoRI DNA fragments, embryonic liver scRNAs

1. Introduction

In one or two years since the second theoretical model on gene differentiation[4)] was proposed, favourable evidences suggesting important roles of the RNA in embryonic gene expressions have been accumulated. Most recently, the small RNAs in herpes virus saimiri were emphasized to play an essential role for viral oncogenesis, though their functional mechanisms were uncertain yet[7)]. Furthermore, a possible mechanism of transduction of the proto-oncogene c-fps was demonstrated suggesting intermediating role of RNA molecules by "copy-choice" during reverse transcription[8)]. Three of recent papers should be also cited as favourable evidences to give an important theoretical suggestion about RNA-mediating control of gene expression ; 7S RNA from embryonic chick cardiac muscle cells[9)] ; retrotransposon in Ty elements[10)] ; a processed fragment Hn RNA as activator of ribosomal gene transcription[11)].

By the way, the author has no intention to insist that DNA-binding RNAs only have roles

能登民人
鹿児島大学歯学部口腔解剖学講座

to control embryonic gene expressions. It is a well known and established fact that DNA binding proteins (regulator proteins) play an important role to regulate gene expressions as the Brown's school[12),13)] or others[14)] are speculating excellently.

Gene expressions by regulator proteins exert a controlling mechanism probably available to several genes such as adult genes and some of terminal or prevalently functioning genes. It seems however that embryonic cells have another controlling mechanism by regulator RNAs whose momery had acquired at the ancient and original stages of biogenesis, and that because of the extremely high turnover in an interaction between gene DNAs and DNA-binding RNAs (hybridizor RNAs) it is difficult at present to detect sufficiently how the phenomenon had actually occurred.

Now in this short paper, two of the simpler genosystems were demonstrated as experimental models in the hope of adding an evidence to the author's theoretical models.

2. Materials and methods

Most of experimental procedures (isolation of the chick EcoRI DNA fragments and of the embryonic liver scRNAs from the 16-day-old chick embryo ; RNA-DNA hybridization ; reverse transcription ; electrophoresis etc.) were the same as described in the previous reports[5),6)]. Ligation of both the RNA-DNA hybrid and DNA-DNA integrate fragments to the pT 7-1 vector (Genescribe, USB) and in vitro reaction of the recombinants were performed under the condition indicated by the manufacturer. In RNA-pBR 322 plasmid hybrid experiments, the eucaryotic transcription system (BRL) was used by adding 10 μCi ^{32}P-UTP per 50 μl of the reaction medium according to the manufacturer's indication.

3. Results and Discussion

It was found in the previous experiment that the embryonic liver scRNAs showed the most effective influence on the transcriptional activity of EcoRI DNA fragments among the scRNAs from various chick embryonic organs[6)]. In order to simplify the experimental model, the present experiment was mainly focused on the liver scRNAs.

The hybrid and integrate DNA fragments were hybridized or reverse transcribed with the embryonic liver scRNAs, respectively, and were inserted to the pT 7-1 vector. Reactions in vitro of the recombinants revealed that as shown in fig. 1 ; (1) hybrid fragments synthesized the RNAs ranging approximately from 100 to 500 bp just in the same pattern as that in the control, and ; (2) on the contrary, the integrate fragments failed to synthesize the higher molecular RNAs. Although the controlling mechanisms of disappearance of the RNAs is uncertain at present, negative regulation of in vitro transcription was undoubtedly functioned in this genosystem by the reverse transcribed liver scRNAs, but not by merely hybridizing scRNAs. As seen in fig. 2, pBR 322 plasmid also showed a similar negative regulation of RNA synthesis by the same reverse transcribed liver scRNAs (integrate). However, in the case of the plasmid on the contrary, scRNA-plasmid hybrids showed a prominent novel synthesis of RNAs approximately at sizes of 600-800 bp (hybrid, in fig. 2). Thus, it can be concluded at least that the embryonic liver scRNAs show either negative or positive in vitro transcription according to genetic informations of the host DNAs or to reacting situations if the scRNAs are hybridized merely or are reverse transcribed.

On the other hand, a preparatory experiment demonstrated that the embryonic liver scRNAs mainly consisted of an extermely faint 230 bp band, a clearly detectable (able to isolate either) single band and heterogeneous components at around 70-120 bp through the 4-16 % gradient polyarylamide slab gel electrophoresis. In another preparatory experiment, both S1 nuclease and exonuclease VII digests of the liver scRNA-pBR 322 plasmid hybrids proved an existence of an undigested 300 ± 50 bp component by the 1.2 % agarose gel electrophoresis.

Based on these observations, quantitative detection of the genetic significance of the embryonic liver scRNAs is under investigation.

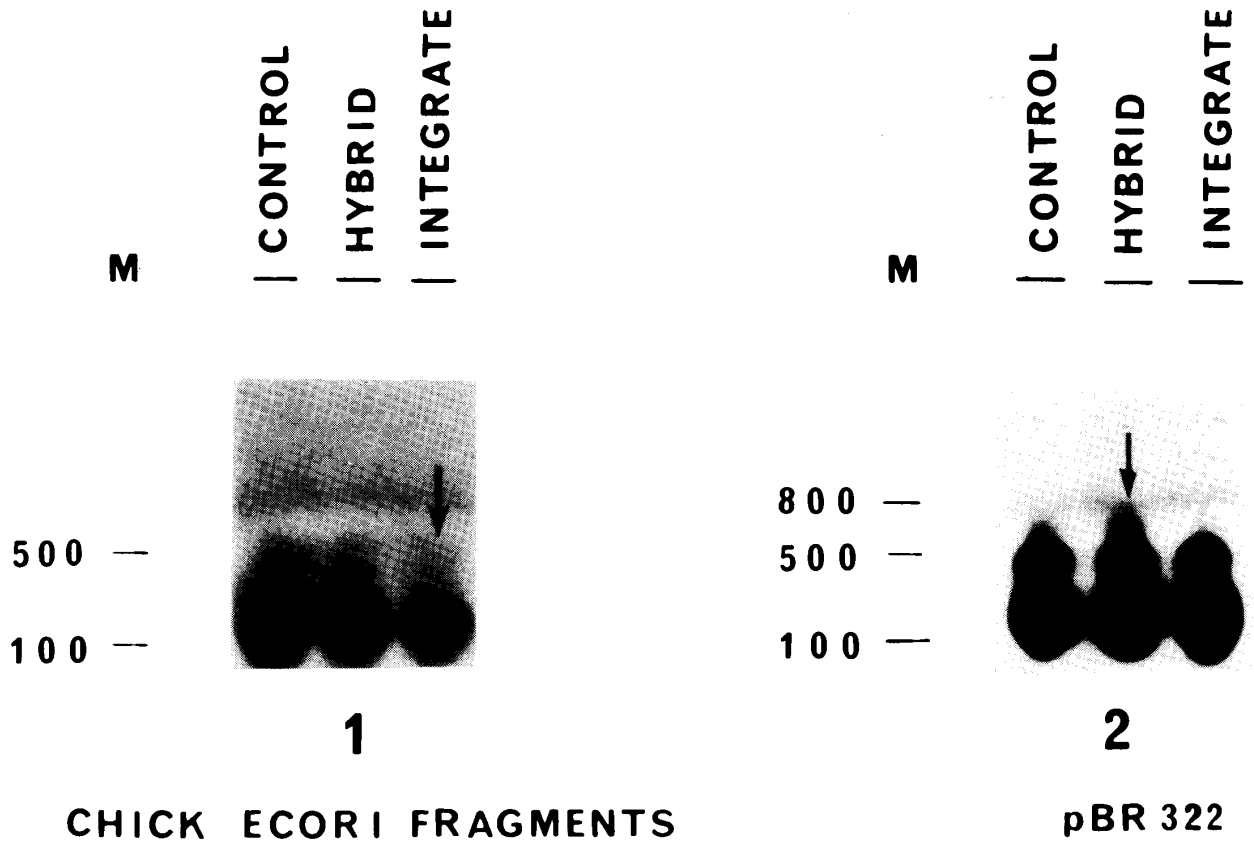

Fig. 1 A typical pattern of in vitro RNA synthesis in one of experimental embryonic genosystems. According to an ordinary procedure (genescribe, USB), 4-7 Kb DNA fragments were ligated into the pT 7-1 vector after being hybridized (Hybrid) or reverse transcribed (Integrate) with -300 bp scRNAs from the liver of 16-day-old chick embryos.
The recombinants were linearized with Hind III before performing in vitro reaction with pT 7 RNA polymerase. The linearization was essential for eliminating completely the back ground RNA synthesis by the vector used. A disappearance of higher molecular RNA component is clearly shown in the integrate fragments (arrow). Molecular markers (bp) were determined by the ϕX174 RF DNA Hae III fragments.

Fig. 2 RNA synthesis in another experimental embryonic genosystem. pBR 322 plasmid was hybridized (Hybrid) or reverse transcribed (Integrate) with the same liver scRNAs as those in fig. 1. Synthetic reactions were carried out using the eucaryotic transcription system (BRL, USA). A prominent novel RNA component is observable in the scRNA-pBR 322 plasmid hybrid (arrow).

Foot Note

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