HAEMOGLOBIN MESSENGER RNA

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Abstract

This is a short review on the isolation and translation in heterologous systems of a 9S RNA which carries the information of haemoglobin.

Rabbit haemoglobin (Hb) messenger RNA (mRNA) was the first eukaryotic genetic message to be isolated, quite a long time ago (1). This was mainly due to the fact that mammalian reticulocytes offer the favourable opportunity of being highly specialized cells whose protein synthesis is almost totally concerned with haemoglobin. Haemoglobin mRNA should amount to about 90 % of total polysomal mRNA. Furthermore, α and β globin chains have such lengths that their mRNAs must sediment at approximately 9S. Isolation of the latter by sucrose gradient centrifugation was therefore quite easy (Hb mRNA must sediment between the 4S region and the 18S ribosomal RNA region if one analyzes total reticulocyte polysomal RNA).

These criteria and others allowed us to isolate from rabbit reticulocyte polyribosomes a 9S RNA fraction presenting several properties the globin mRNA must possess (1) (2). The putative mRNA could also be detached as a specific 14S ribonucleoprotein when polyribosomes were dissociated by EDTA or CMC treatment (3). This 14S mRNP has been extensively studied in our laboratory (4) and its obtention now constitutes the first step in the preparation of 9S Hb mRNA.

But the definitive proof that the 9S RNA fraction was or contained Hb message could only be obtained if this RNA would direct Hb synthesis when added to an heterologous protein synthesizing system. A preliminary indication in this direction was obtained by SCHAPIRA and coworkers (5) but the first clearcut result was presented by LOCKARD and LINGREL in 1969 (6). They added mouse polysomal 9S RNA to a rabbit reticulocyte cell-free system and identified the mouse globin β chain in the synthesized products. Somewhat later, GURDON and coworkers showed that 9S rabbit reticulocyte RNA could be extensively translated into α and β globin chains when injected into frog oocytes (7). Translation could proceed for several days. The synthesized products were characterized by gel filtration, ion exchange chromatography (7) and finger printing (8). JONES and LINGREL also showed that the 9S RNA fraction isolated from mouse reticulocyte polyribosomes,

acted as the message for mouse α and β globin chains (9). The 9S mouse reticulocyte RNA has also been translated in the Krebs ascites cell-free system (10) and the 9S rabbit mRNA has been translated in the rat liver system (11). SCHERRER and coworkers reached quite similar conclusions using the duck reticulocyte system (12).

As a conclusion, it is clearly established that the 9S RNA fraction isolated from reticulocyte polyribosomes contains the messages for globin chains. This, of course, does not exclude the possibility that other RNAs be present in the 9S fraction.

A fractionation procedure using adsorption to cellulose (13) was applied to 9S reticulocyte mRNA, purified by sucrose gradient centrifugation. So far, we detected a clearcut heterogeneity in the preparation: 70 % to 80 % of the 9S RNA were adsorbed onto the cellulose in high ionic strength conditions whilst 20 % to 30 % were excluded. The excluded fraction was much less active than the retained one in directing globin synthesis in oocytes. Work is now in progress to further analyze these subfractions and determine their nature and function.

On the other hand, we focused our attention on nucleated erythroïd cells from spleens of anemic rabbits. This material constitutes a useful tool for the study of the selection mechanisms of the nuclear genetic information and of the transfer of this information from the nucleus to the cytoplasm. Avian reticulocytes have already been used for this purpose by another research group (14). In our laboratory we chose the spleen of anaemic rabbits as a source of nucleated erythroïd cells: this should allow us to compare the molecular characteristics of globin mRNA in young nucleated and old, anucleated erythroid cells.

So far, we have been able to prepare active globin 9S mRNA from the spleen of anaemic rabbits (15) and are now comparing its properties to those of the 9S mRNA from reticulocytes of the same animals.

Acknowledgements:

This work received support from the "Ministère de la Politique et de la Programmation Scientifique" and the "Fonds de la Recherche Fondamentale Collective". G. H. and G. M. are fellows of the belgian "Fonds National de la Recherche Scientifique" and F. G. and P. N. of the "Institut pour l'Encouragement de la Recherche Scientifique dans l'Agriculture".

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