THE TRANSLATION OF VIRAL RNAs IN FROG OOCYTES

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It is desirable to be able to translate viral RNA in frog oocytes for two reasons. First, viruses provide RNA in an exceptionally pure form, and as such constitute an unusually favourable source of mRNA for studying the control of protein synthesis in development. Second, viral functions can be studied independently of the limitations imposed by the normal infection cycle in their host cells. With the exception of viral coat proteins, very little is known about the proteins coded for by viral genes, because it is hard to be sure which of the new proteins synthesized in virus-infected cells are the products of virus-coded genes as opposed to virus-activated host cell genes. This difficulty can be overcome if the virus RNA can be translated in a cell-free protein-synthesizing system, but it is not yet clear that this can be achieved satisfactorily with any of the oncornaviruses.

The first viral function to be investigated by microinjection into frog eggs was the replication of polyoma DNA. Laskey and Gurdon (1) made use of radioactive and density labels to provide evidence for the replication of purified polyoma DNA injected into unfertilised eggs.

The successful translation of viral messenger RNA in injected frog cells was first achieved by Laskey, Gurdon and Crawford (2). For these experiments, purified virion RNA of a picornavirus, Encephalomyocarditis, was injected into oocytes of *Xenopus laevis* according to the methods described by Gurdon, Lane, Woodland and Marbaix (3) and shown by Lane, Marbaix and Gurdon (4) to translate mammalian haemoglobin mRNA correctly. The method of analysis which has proved exceptionally useful, both in economy of time and in the precision of results obtainable, is to label injected oocytes in ^{35}S-methionine, analyse labelled proteins by SDS-poly acrylamide gel electrophoresis, and then quantitate the results by autoradiography. If oocytes are incubated in the saline solution, which we have found very satisfactory (5) in the presence of ^{35}S-methionine at 250 μCi/ml, each oocyte will incorporate up to 10^6 dpm of radioactivity into proteins within a few hours. Oocytes have a small pool of methionine, by comparison with most other amino acids, and perhaps for this reason most of the label in the medium is incorporated into proteins within 18 hours, assuming that oocytes are incubated in 5 μl of medium per oocyte at 250 μCi/ml.

The results of analysing oocytes injected with EMC virion RNA have been reported by Laskey, Gurdon and Crawford (2). As judged by size, all of the virus-coded proteins which could be detected in EMC-infected ascites cells could be found in oocytes after

injection of EMC viral RNA. None were found in control saline-injected oocytes. Three of the newly synthesized proteins were removed from gels, digested with trypsin, and the methionine-labelled peptides analyzed by thin layer chromatography and electrophoresis. In this way they were shown to be indistinguishable from known virus-coded proteins. We conclude that the oocyte probably translates the entire EMC genome.

These experiments are of interest from two points of view. First, they demonstrate that the RNA of a virus which normally infects the respiratory system of mice can be translated successfully, and probably completely, in frog oocytes. This at least raises the possibility that other mammalian virus messages may be translated in frog oocytes. Second, the virion RNA of many picornaviruses is a polycistronic message. It is initially translated into a large polypeptide, which is subsequently cleaved in a series of steps down to about 10 stable proteins. Rather surprisingly this cleavage process appears to proceed completely normally in frog oocytes. We do not know whether this is because oocytes and perhaps all vertebrate cells contain the necessary cleavage enzymes, or because part of the initially translated virus polypeptide folds up into an enzyme which cleaves itself. The first possibility seems more likely because oocytes appear to carry out a number of secondary modifications to proteins which they never normally contain (review by Lane and Knowland (6), and by Gurdon (7)). In this case it is again likely that frog oocytes may prove of general value for translating heterologous messenger RNAs.

At the time of writing no fully documented reports of the translation of RNA from other viruses in frog oocytes have been published. We are aware of attempts to translate Avian myeloblastosis virus in injected oocytes by several laboratories. In collaboration with Salden and Bloemendal we have injected the virion RNA of Rauscher murine leukaemia virus, in 65s form, or as 37s RNA with the low molecular weight RNA obtained from the 65s RNA by heat denaturation. In none of these cases has a positive result been obtained, but it would be premature to claim that the injected RNAs are not translated. The virion RNAs of various bacteriophage viruses, such as f2 and R17 have been injected into oocytes. The results (3) and Knowland (unpublished) have failed to reveal the synthesis of any of the three expected proteins, though in each case a general stimulation of protein synthesis, by 4–5 times, was observed. The apparent inability of bacterial messages to be translated in frog cells is not surprising in view of the difference between pro- and eu-karyote ribosomes. We do not yet know whether the lack of success, so far, in translating oncornavirus RNAs is due to a deficiency of the oocyte, such as the need for special translational "factors" or a deficiency in the RNA such as a low proportion of biologically active molecules, or the need to assume or unfold a special secondary structure.

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