Gene activation in somatic nuclei after injection into amphibian oocytes

(cell differentiation/animal development/Xenopus laevis/Pleurodeles newt/two-dimensional electrophoresis)

E. M. De Robertis and J. B. Gurdon

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England

Communicated by Max Perutz, March 28, 1977

ABSTRACT Genes that are unexpressed in somatic cells have been activated by injecting cultured cell nuclei of the frog Xenopus laevis into oocytes of the newt Pleurodeles waltl. The genes were activated are normally expressed in oocytes but not in cultured cells. Conversely, genes that are normally expressed in cultured cells but not in oocytes became inactive when cultured cell nuclei were injected into oocytes. These changes in gene activity were detected by two-dimensional gel electrophoresis of proteins synthesized by oocytes injected with nuclei. Controls, which included the use of α-amanitin, showed that these changes in protein synthesis are dependent on gene transcription. We conclude that genes that become inactive during cell differentiation can be reactivated, in the absence of cell division, by normal components of oocyte cytoplasm.

One of the principal characteristics of animal development is that genes become inactive in the course of somatic cell differentiation; these become active again. An experimental reversal of this stable inactivation would make it possible to investigate the molecular basis of gene control in development. We have found that the natural inactivation of genes entails the transplantation of single nuclei from specialized cells to enucleated amphibian eggs. For example, tadpoles with specialized cells of many types (such as blood, lens, and muscle) can be prepared by transplanting nuclei from keratinized adult skin cells. But it is by no means clear that the conditions or molecules responsible for this new kind of gene activity exist in egg cytoplasm; they might arise later in the course of development. For this reason we have explored the use of oocytes for these experiments, since, unlike eggs, they can be cultured without cell division for many days after the injection of nuclei.

Recent work from our laboratory has shown that somatic nuclei introduced into frog oocytes enlarge considerably, disperse their chromatin, exchange their proteins with those of the surrounding cytoplasm, and are very active in RNA synthesis for long periods of time (3, 4). The expression of individual genes by the injected nuclei can be detected by a coupled transcription-translation process (3, 5). More importantly, only a restricted set of genes is expressed by mammalian nuclei in oocytes, while many other genes are "turned off" at a pretranslational level (6). These results suggest the possibility that the oocyte cytoplasm might reprogram gene expression by the injected nuclei, in such a way that only those genes that are recognized as similar to the oocyte-active ones are expressed. In this paper we investigate the ability of oocyte cytoplasm to induce the experimental activation of genes which are inactive in somatic cells but which are normally expressed in oocytes. By transplanting somatic nuclei of Xenopus laevis into oocytes of the newt Pleurodeles waltl, we have obtained evidence for the activation of previously inactive genes.

RESULTS

Some Xenopus proteins are cell type specific

To demonstrate the activation of previously inactive genes in nuclei injected into oocytes, we require nuclei from somatic cells which do not express some of the proteins normally synthesized in oocytes of the same species. In this section we will show that this requirement is fulfilled by a Xenopus cultured cell line that was originally derived from Xenopus kidney (see Materials and Methods). Cultured cells were used because our method for preparing viable nuclei for oocyte injections (see Materials and Methods) is readily applicable to cell suspensions, but not to solid tissues.

The proteins synthesized by Xenopus oocytes and by Xenopus cultured cells were analyzed by two-dimensional electrophoresis, and the resulting fluorographs are shown in Fig. 1 A and B. By comparison of these gels three classes of proteins can be distinguished:

(a) Oocyte-specific proteins: synthesized by oocytes (Fig. 1A) but not by cultured cells (Fig. 1B). At least 16 major proteins can be identified as belonging to this class, and are shown schematically in Fig. 1E.

(b) Cultured cell-specific proteins: synthesized by cultured cells but not by oocytes. These include at least eight major proteins (Fig. 1F), two of which are synthesized in great abundance.

(c) Proteins synthesized both in oocytes and in cultured cells. These include the great majority of spots (Fig. 1D).

These assignments represent a minimal estimate of the protein differences between oocytes and cultured cells, since only
the major spots seen in two-dimensional gels were taken into account in this classification and differences in intensities were not scored; only those labeled proteins that were undetectable in one cell type are regarded as cell-type specific. The presence of many common proteins in the two very different Xenopus cell types is not surprising since two-dimensional gels will show mainly the most abundant proteins and many of these, such as actin, tubulin, and membrane proteins, are expected to be present in all cell types. Furthermore, two-dimensional gel analysis of cells undergoing differentiation, such as fusing myoblasts (11) or Friend leukemia cells (12), has shown that only a very limited number of proteins are characteristic of the differentiated state.

Having found a source of nuclei that do not express some oocyte-active genes, we now require an oocyte host with a two-dimensional distribution of proteins which is different enough from Xenopus oocytes for us to be able to detect the expression of Xenopus oocyte-active genes. This requirement is fulfilled by oocytes of a newt, Pleurodeles, which have the two-dimensional protein pattern shown in Fig. 1C. Some of the major proteins (such as actin and tubulin, see Fig. 1D) superimpose over Xenopus spots, but the great majority have different mobilities. At least 15 Xenopus oocyte-specific proteins can be clearly seen on a background of Pleurodeles oocyte proteins.

Xenopus oocyte-specific genes are activated in somatic nuclei injected into Pleurodeles oocytes

Xenopus cultured cell nuclei injected into Pleurodeles oocytes enlarge considerably during the first few days after injection and tend to resemble morphologically the oocyte's nucleus (Fig. 2), as described for other somatic nuclei (3, 9). To detect new gene activity, the newt oocytes are labeled for 6 hr with a $^{14}$C-labeled amino acid mixture 0.3, or 7 days after the injection
of *Xenopus* nuclei, and their proteins are analyzed by two-dimensional gels. No new proteins are detected during the first day after injection (day 0; see Figs. 3A and 4A). After 3 days in culture, and even more clearly after 7 days, several new proteins appear which are absent from the mock-injected controls. At least six new spots are detected, which comigrate in two-dimensional gels with *Xenopus* proteins (spots with arrows in Fig. 1A). Three of these correspond to proteins synthesized both by *Xenopus* oocytes and *Xenopus* cultured cells (downward arrows in Fig. 3B and C). None of the *Xenopus* cultured cell-specific proteins is detectable in *Pleurodeles* oocytes, although two of them are strongly labeled in cultured cells (most right-hand spots in Fig. 1F). More importantly, three new spots correspond to *Xenopus* oocyte-specific proteins, which are not synthesized by the cultured cells used as nuclear donors. These proteins (upward arrows in Figs. 3B and C and 4B) therefore represent genes whose expression has been switched on by the oocyte cytoplasm. The spot indicated with a double arrow at the lower part of Fig. 3B and C and Fig. 1A (referred to hereafter as protein 53/6.0) may represent a charge modification of a single *Xenopus* protein or two different proteins.

All of these six *Xenopus* proteins were detected in three independent experiments, using oocytes from different animals. In two other experiments we detected only three of the *Xenopus* proteins, and in one occasion we did not detect any new proteins. The incomplete success of these experiments may well have been related to the poor survival and reduced swelling of injected nuclei in these particular batches of oocytes. In some experiments two other new spots were also detected, which did not coelectrophorese with *Xenopus* proteins and which could have been coded for either by the *Xenopus* or the *Pleurodeles* genome.

We believe, for several reasons, that the activation of *Xenopus* oocyte-active genes results from the translation of mRNAs synthesized by the injected nuclei within the new oocytes. All the new proteins were absent when the oocytes injected with nuclei received an injection of α-amanitin during day 0 (Figs. 3D and 4C). The inhibitor was used at a concentration of 10 μg/ml, previously shown not to affect, in frog oocytes, either the synthesis of 28S, 18S, 5S, or 4S rRNAs (13) or the translation of endogenous and injected mRNAs (3). The new *Xenopus* proteins were not detected during the first day after injection, and became apparent only when labeled 3 or more days after injection. Presumably during this period sufficient mRNA is accumulated in the oocyte cytoplasm to be detected as the labeled proteins seen in two-dimensional gels.

The new spots are indeed *Xenopus* proteins. Their electrophoretic mobilities in two dimensions match exactly those of known *Xenopus* oocyte proteins (arrows in Fig. 1A). To confirm the *Xenopus* origin of the new spots, we have analyzed the tryptic peptides of protein 53/6.0. Region 53/6.0 was cut out from two-dimensional gels of: (a) *Xenopus* oocytes, (b) *Pleurodeles* oocytes injected 7 days previously, with *Xenopus* somatic nuclei, and (c) *Xenopus* cultured cells. The proteins were eluted from the gel fragments, iodinated by the chloramine-T method, and digested with trypsin. The resulting tyrosine-labeled peptides were chromatographed in silica-gel.
thin-layer chromatography plates. As shown in Fig. 5A and B, the peptide patterns of protein 53/6.0 synthesized in uninjectected Xenopus oocytes or in Pleurodeles oocytes injected with nuclei are very similar, each containing 12 peptides of identical mobilities. As shown in Fig. 5C, region 53/6.0 of Xenopus cultured cells shows a completely different peptide pattern (probably due to other proteins that run in nearby positions in two-dimensional gels); this is understandable, since protein 53/6.0 is expressed only in Xenopus oocytes (Fig. 1).

We conclude from these experiments that somatic nuclei injected into oocytes are able to express genes of the type normally active in oocytes. Of most interest, the expressed genes include some of those which were previously inactive in the somatic cells.

**DISCUSSION**

The results presented in this paper indicate that the oocyte cytoplasm is able to reprogram gene expression by Xenopus somatic nuclei. We have seen that Xenopus oocyte-specific genes, which are not expressed in the cultured cells used as nuclear donors, can be activated by injecting nuclei into oocytes of a different amphibian species. Only proteins of the type normally synthesized by Xenopus oocytes were detected, and none of the eight readily recognizable cultured cell-specific proteins was expressed after nuclear injection. However, only some of the Xenopus oocyte-specific proteins that are distinguishable from Pleurodeles were expressed in detectable amounts. Three Xenopus oocyte-specific proteins were activated by the Pleurodeles cytoplasm, although 15 spots of this type should have been detected if all had been expressed at the same rate as protein 53/6.0. Similarly, only a small proportion of Xenopus proteins common to both oocytes and cultured cells were expressed in Pleurodeles oocytes containing Xenopus nuclei. The paucity of Xenopus proteins expressed in Pleurodeles oocytes may be due to the fact that Pleurodeles, being an Urodele, is only distantly related to Xenopus (17). The Xenopus nuclei might be able to recognize some, but not all, of the cytoplasmic signals that regulate gene expression in Pleurodeles oocytes.

The experimental activation of protein-coding genes by a foreign cytoplasm has also been demonstrated in other systems. Certain hybrid clones of rat hepatoma X nonliver mouse cells can express albumin and some other mouse liver proteins (18, 19). Similarly, the transplantation of a single skin cell nucleus into an enucleated amphibian egg (not oocyte) gives rise to a normal tadpole containing functional muscle, blood, and other differentiated tissues (1). However, in both these cases no expression of new genes is detected until many cell divisions have taken place (to give a cloned hybrid cell line or a young tadpole). This is an important difference between these experiments and those described in this paper, since nuclei injected into oocytes never divide or undergo mitosis (2, 9). This is of interest, since it has been proposed that mitosis and cell division are prerequisites for any major change in gene activity in eu-karyotic cells (for a collection of reviews, see ref. 20). A reactivation of genes also takes place in chick red blood cell nuclei after fusion to cultured cells (for reviews see refs. 21 and 22), but the activated genes are among those that were previously active in early erythropoiesis and do not therefore belong to a different program of gene expression. If gene reprogramming does occur when chick erythrocytes are fused to other cells, it must be a rare phenomenon, occurring at a frequency of less than $1 \times 10^{-7}$ (23).

An important implication of our results is that the oocyte cytoplasm contains conditions (and therefore presumably molecules) that can determine a particular spectrum of protein-coding genes to be active and others to be inactive, and that nuclear injection provides an assay for their activity. This shows that oocytes contain specific gene-controlling substances. The possibility exists that these gene-controlling substances will turn out to be examples of the "determinants" of egg cytoplasm, commonly considered responsible for the first steps of cell differentiation (24, 25).

We thank Dr. G. A. Partington for growing the cell cultures used in these experiments and for helpful discussions during this work; R. A. Laskey, A. Wyllie, and D. Melton for advice; and R. Longthorne, J. Lang, and J. Price for technical assistance. E. DeR. is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.
The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.