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Cell Biology

Gene activation in somatic nuclei after injection into amphibian oocytes

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

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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 waltlii*. The genes that 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 that become inactive in the course of somatic cell differentiation rarely become active again. An experimental reversal of this stable inactivation would make it possible to investigate the molecular basis of gene control in development. One procedure that reverses 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 (1). But it is by no means clear that the conditions or molecules responsible for these new kinds 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 (2).

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 waltlii*, we have obtained evidence for the activation of previously inactive genes.

MATERIALS AND METHODS

Oocytes were obtained from a laboratory strain of *Pleurodeles waltlii* (7), referred to hereafter as *Pleurodeles*. Somatic cells of *Xenopus laevis* were from a cloned cell line derived from male *Xenopus* kidney (8) and were originally supplied by Anne Warner. These cells, which are aneuploid and of epithelioid morphology, were grown at 25° in Gibco-Biocult BHK 21 medium supplemented with 1% (vol/vol) nonessential amino acids (Gibco-Biocult) and 10% (vol/vol) fetal calf serum. *Xenopus* nuclei for injection were prepared by our lysolecithin/bovine serum albumin method (9). *Pleurodeles* oocytes were injected with about 200 nuclei per oocyte, cultured at 19°, and labeled with a ^{14}C-labeled amino acid mixture as described (6). In all cases the injected oocytes were labeled for 6 hr. The extraction of oocyte proteins and their analysis by two-dimensional electrophoresis (10) (isoelectric focusing/sodium dodecyl sulfate electrophoresis) has also been described (6). To refer to a particular protein in a two-dimensional gel, two parameters are used: its apparent molecular weight $\times 10^{-3}$ and its apparent isoelectric point expressed in pH units (3). For example, one of the *Xenopus* proteins that is activated in *Pleurodeles* oocytes has a molecular weight of 53,000 and a pI of 6.00 and is therefore referred to as 53/6.0. The methods used for the analysis of tryptic peptides are detailed in the legend to Fig. 5.

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

(*b*) *Cultured cell-specific proteins:* synthesized by cultured cells but not by oocytes. These include at least eight major proteins (Fig. 1*F*), 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. 1*D*).

These assignments represent a minimal estimate of the protein differences between oocytes and cultured cells, since only

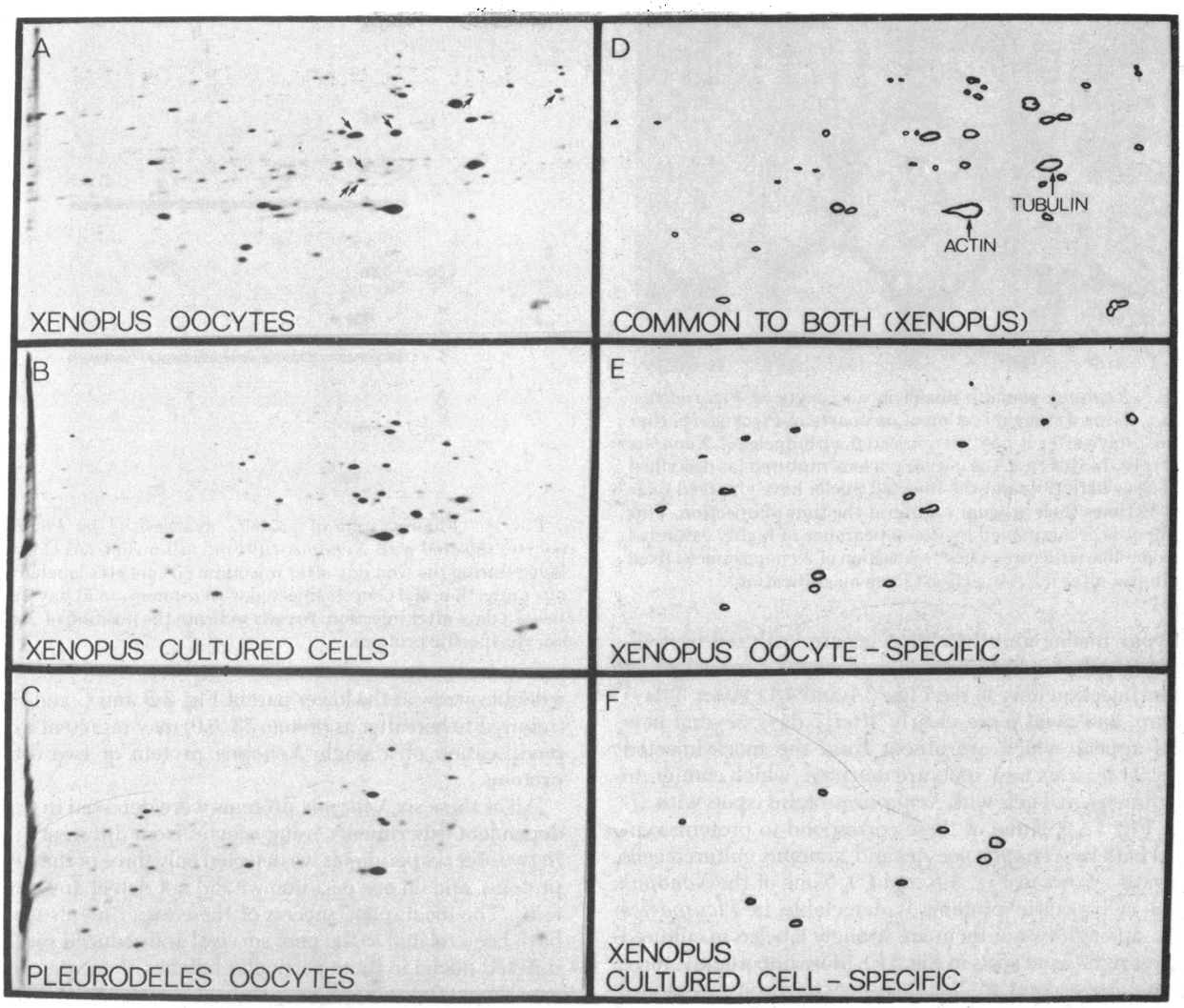

FIG. 1. Fluorographs of two-dimensional gels of [^{14}C]proteins synthesized by: (*A*) *Xenopus* oocytes; (*B*) *Xenopus* cultured cells; (*C*) *Pleurodeles* oocytes. Tracings of the three different classes of newly synthesized proteins that can be distinguished by comparing *Xenopus* oocytes and *Xenopus* cultured cells: (*D*) proteins present *both in oocytes and cultured cells;* (*E*) *oocyte-specific proteins* (present in oocytes and absent in cultured cells); (*F*) *cultured cell-specific proteins* (present in cultured cells and absent in oocytes). Comparison was made by superimposing films of two-dimensional gels on each other. The tracings in *D*, *E*, and *F* have been drawn at the same scale as the fluorographs in *A* and *B*. The positions of actin and tubulin are indicated in *D* (actin was identified by peptide analysis and tubulin by two-dimensional gels of purified brain tubulin; Longthorne and De Robertis, unpublished). The arrows in *A* indicate *Xenopus* proteins that are synthesized after injection of *Xenopus* somatic nuclei into *Pleurodeles* oocytes: upward arrows indicate *Xenopus* oocyte-specific proteins and downward arrows indicate proteins common to both *Xenopus* oocytes and *Xenopus* cultured cells. The assignment of proteins to the different classes shown in *D*, *E*, and *F* was made by comparing the exposed x-ray films of the fluorographs. Since the fainter spots do not reproduce well after photography, a few of the spots traced in *D* are difficult to see in the photographs shown in *A* and *B*.

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. 1*C*. Some of the major proteins (such as actin and tubulin, see Fig. 1*D*) 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

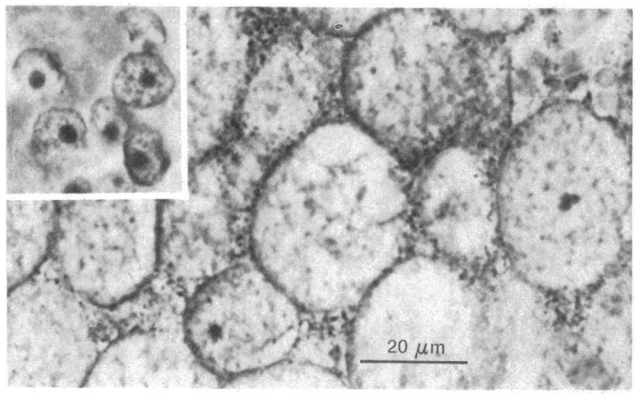

FIG. 2. *Xenopus* somatic nuclei in an oocyte of *Pleurodeles*. The figure shows a stained section of an oocyte of *Pleurodeles* that was fixed 7 days after it had been injected with nuclei of *Xenopus* cultured cells. In this case, the oocyte nucleus ruptured [as described in detail elsewhere (9)], and the injected nuclei have enlarged to as much as 30 times their original volume at the time of injection. This enlargement is accompanied by the appearance of highly extended chromosome-like structures. (*Inset*) A section of *Xenopus* nuclei fixed a few minutes after microinjection (same magnification).

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. 3*A* and 4*A*). 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. 1*A*). Three of these correspond to proteins synthesized both by *Xenopus* oocytes and *Xenopus* cultured cells (downward arrows in Fig. 3 *B* 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. 1*F*). 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. 3 *B* and *C* and 4*B*) therefore represent genes whose expression has been switched on by the oocyte cytoplasm. The spot indicated with

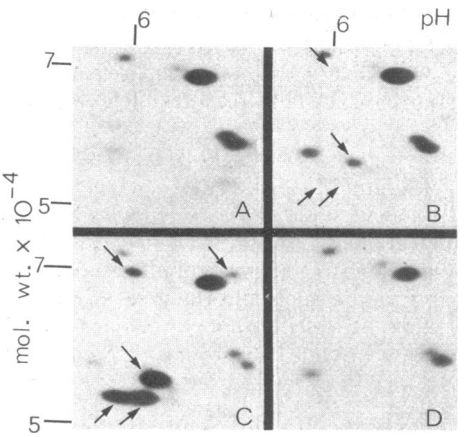

FIG. 3. Fluorographs of proteins synthesized by *Pleurodeles* oocytes injected with *Xenopus* cultured cell nuclei. (*A*) Oocytes labeled from 0 to 6 hr after injection; (*B*) oocytes labeled for 6 hr on the third day after injection; (*C*) oocytes labeled for 6 hr on the seventh day after injection; (*D*) same as *B*, but injected with α-amanitin at day 0. The upward arrows indicate the position of *Xenopus* "oocyte-specific" proteins, and the downward arrows *Xenopus* proteins, "common to both", as explained in the legend of Fig. 1.

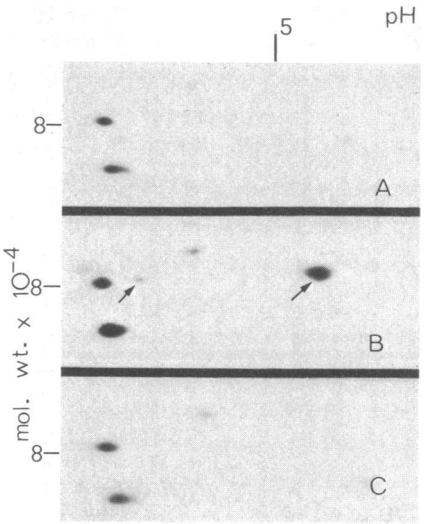

FIG. 4. Fluorographs of proteins synthesized by *Pleurodeles* oocytes injected with *Xenopus* cultured cell nuclei. (*A*) Oocytes labeled during the first day after injection; (*B*) oocytes labeled 7 days after injection; (*C*) oocytes injected with α-amanitin at day 0 and labeled 3 days after injection. Arrows indicate the position of *Xenopus* oocyte-specific proteins.

a double arrow at the lower part of Fig. 3 *B* and *C* and Fig. 1*A* (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 on 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 newt oocytes. All the new proteins were absent when the oocytes injected with nuclei received an injection of α-amanitin during day 0 (Figs. 3*D* and 4*C*). 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 RNAs (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. 1*A*). 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

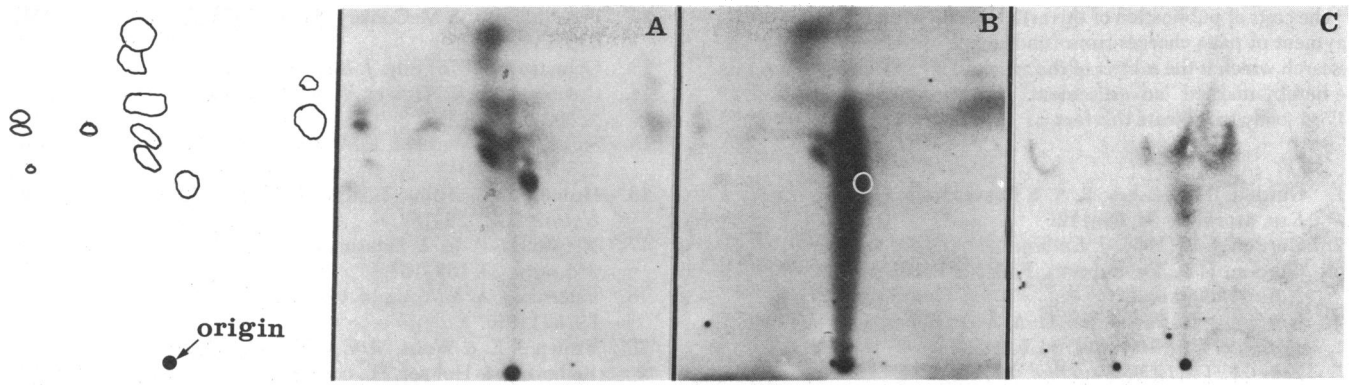

FIG. 5. Peptide analysis of protein 53/6.0. Region 53/6.0 was excised from autoradiographed two-dimensional gels of: (*A*) *Xenopus* oocytes; (*B*) *Pleurodeles* oocytes injected 7 days previously with *Xenopus* cultured cell nuclei; (*C*) *Xenopus* cultured cells. The position of the main peptides shared by samples *A* and *B* is indicated in the diagram to the left of *A*. The proteins were eluted from the gel fragments in 2 ml of 0.05% sodium dodecyl sulfate/0.01% 2-mercaptoethanol for 24 hr in a rotary shaker at 25°. The gel fragments were discarded and the supernatants lyophilized extensively. The proteins were iodinated by the chloramine T procedure (14) modified so that the reaction mixture contained 1% sodium dodecyl sulfate. One millicurie of ^{125}I (carrier-free, Amersham) was used for each sample. The reaction was stopped and then the samples were precipitated twice with 5% trichloroacetic acid in the presence of 1 mg of carrier human globin. The precipitate was extracted with ethanol/ether (1:1) and then ether, and digested with trypsin (15). The tyrosine-labeled peptides were separated in Silica gel GF 20 × 20 cm thin-layer plates (250 μm, Anachem) in two dimensions. Electrophoresis at pH 6.5 (10% vol/vol pyridine/0.3% vol/vol acetic acid) was carried out at 1500 V for 2 hr, followed by chromatography at right angles in butanol/acetic acid/water/pyridine (30:6:24:20, vol/vol) (16). The radioactive peptides were fluorographed on Kodak X-Omat-H film using an Ilford Fast Tungstate x-ray intensifier screen (Laskey and Mills, personal communication). In *B* a peptide has been encircled because it is obscured by nearby radioactivity smearing from the origin. This peptide is clearly evident in less heavily exposed films.

thin-layer chromatography plates. As shown in Fig. 5 *A* and *B*, the peptide patterns of protein 53/6.0 synthesized in uninjected *Xenopus* oocytes or in *Pleurodeles* oocytes injected with nuclei are very similar, each containing 12 peptides of identical mobilities. As shown in Fig. 5*C*, 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 × 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 eukaryotic 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 reprograming does occur when chick erythrocytes are fused to other cells, it must be a rare phenomenon, occurring at a frequency of less than 1×10^{-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).

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