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# Probing the program of gene expression utilized in early development

EDDY M. DE ROBERTIS

Medical Research Council, Laboratory of Molecular Biology Hills Road, Cambridge CB2 2QH, U.K.

## Estudio del programa de la expresión génica utilizado en el desarrollo temprano

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Somatic cell nuclei injected into amphibian oocytes enlarge, exchange protein with the surrounding cytoplasm, and actively synthesize RNA for weeks. The message activity of the RNAs synthesized within the oocytes can be detected as new proteins made a few days after the injection of somatic nuclei. The oocyte cytoplasm seems to *reprogram* the injected nuclei, allowing the expression only of those genes wich are normally active in oocytes (De Robertis and Gurdon, 1977). Genes which are unexpressed in somatic cells can be activated by injecting *Xenopus laevis* somatic nuclei into oocytes of *Pleurodeles waltlii* (Urodela). The genes that were activated are normally expressed in *Xenopus oocytes* but not in somatic cells. Conversely, genes which are normally expressed in somatic cells but not in oocytes become inactive after injection into oocytes. We conclude that genes which become inactive during cell differentiation can be reactivated, in the absence of cell division, by normal components of oocyte cytoplasm. These components could turn out to be examples of the "determinants" of egg cytoplasm responsible for nuclear activity in early development.

### INTRODUCTION

I will review here recent work in which nuclear transplantation was used to probe gene expresion in amphibian oocytes.

The classical nuclear transplantation experiments of Gurdon showed that when a single somatic nucleus is injected into an enucleated amphibian egg, normal development can be obtained. Two days after transplantation a tadpole containing nerve, blood, muscle and other differentiated tissues is obtained. This experiment, in addition to showing that genes are not irreversibly lost during cell differentiation, also implies that the somatic nucleus is *reprogrammed* to the gene expression pattern of the various differentiated tissues. However, this experiment does not tell us *when* these nuclei are reprogrammed. This is because by the time the first indications of differentiated cell function are apparent many cell divisions have occurred. It is not possible to distinguish whether the agents responsible for gene reprogramming are present in unfertilized eggs, or if they arise in later development (for example, as a result of cell movements and interactions during gastrulation). By injecting somatic nuclei into oocytes (nuclei injected into oocytes unlike eggs, do not divide nor replicate their DNA) we were able to show that oocytes contain conditions or components able to reprogram specific genes. This finding is of some em-

bryological interest, since it is likely that these gene-controlling substances are important in early development.

### 1. *Somatic nuclei injected into Xenopus oocytes are transcribed*

Somatic nuclei are isolated by gentle procedures which allow them to survive after injection. A suspension of 200 nuclei is injected into each oocyte. The nuclei remain morphologically healthy, and undergo a substantial enlargement (10 to 100 fold) during the first few days after injection (1).

The injected nuclei tend to resemble morphologically the oocyte's nucleus (also known as germinal vesicle, or GV). They disperse their chromatin and bind the histological stain light green, in the same way as the GV does. Occasionally structures resembling prophase chromosomes are observed in the injected nuclei (the oocyte's nucleus is in late lampbrush state, i.e. meiotic prophase (2). When nuclei are injected into oocytes induced to mature by hormone stimulation, the chromosomes will condense if the oocyte nucleus is at the metaphase stage (3).

The injected nuclei exchange proteins with the surrounding cytoplasm. When $^{3}$H-leucine labelled HeLa nuclei are injected into oocytes, 85% of the radioactivity is lost from nuclei which have enlarged for 3 days in oocytes (4). By injecting *Xenopus* cultured cell nuclei labelled with $^{3}$H-arginine (which labels non-histone proteins, since histones do not contain trytophan), we were able to show that while most of the non-histone proteins are lost, there is no detectable loss of histones from the transplanted nuclei (2), Similar results were obtained by Diberardino and Hoffer (5) who analyzed protein exchange in *Rana pipiens* nuclear transplant embryos.

During the same time, the injected nuclei accumulate histones (tested by injecting $^{125}$I-histones) and non-histone proteins $^{3}$H-tryptophan-labeled) from the surrounding cytoplasm (4). Unfortunately it has not been possible, as yet, to reisolate the microinjected nuclei in order to analyze which proteins are taken up from the oocyte. (This difficulty is due to the presence of large number of yolk platelets which cosediment with the nuclei and to the heterogeneous degree of swelling of individual nuclei). However, it is likely that at least some of these proteins will belong to the class of oocyte nuclear proteins which are able to accumulate selectively in the germinal vesicle after microinjection, as described by Bonner (6).

The injected nuclei synthesize substantial amounts of RNA, as shown by autoradiographic experiments (4). RNA synthesis takes place for prolonged periods in culture (in one case up to 28 days), and increases as the nuclei enlarge).

When *Xenopus* somatic nuclei are injected into *Xenopus* oocytes, the nucleolus is very prominent and active in RNA synthesis (2, 4). In more distant combinations of nucleus and cytoplasm (for example HeLa - *Xenopus),* the nucleolus decreases in size and is inactive in RNA synthesis. This suggests that oocyte cytoplasm exerts some degree of control on RNA synthesis by the injected nuclei.

Since oocytes are very efficient in protein synthesis, it seemed possible that the message activity of the RNAs synthesized by the somatic nuclei could be detected by the synthesis of new proteins. This was greatly facilitated by a technological break-through: in 1975 Patrick O'Farrell published a two-dimensional electrophoresis system (2D-gels), which allowed 100 times more resolution in the separation of proteins than previously available methods (8). When the proteins synthesized by oocytes injected with somatic nuclei were analyzed, new polypeptides were detectable. Various experiments established that these proteins were indeed coded for by the injected nuclei (1, 9), and that they arise from mRNA synthesized within the oocytes during the first few days after injection (1, 9). We therefore had a way of analyzing the effect of the oocyte cytoplasm on gene expression by the somatic nuclei.

### 2. *Gene expression by microinjected somatic nuclei is reprogrammed*

When HeLa nuclei were injected into Xenopus oocytes, only a selected group of HeLa proteins was expressed. Only a few new protein spots were detected although many more could have been

detected as different from *Xenopus* proteins (9) (Table IA).

When HeLa nuclei were injected into oocytes of another amphibian species (the newt *Pleurodeles waltlii*) a similar (although not identical) set of proteins was expressed preferentially (9) (Table IB). When Hela nuclei infected with adenovirus were injected, expression of the viral genes seemed to be switched off relative to that of HeLa genes (9) (Table II). Similarly, when nuclei of a mouse myeloma cell line that secretes inmunoglobulins were injected, the

TABLE I

Gene Expresssion by HeLa nuclei in amphibian oocytes

A. Injected into *Xenopus* oocytes

| *Cell type analyzed* | *Number of newly synthesized proteins detectable by 2D gels* | |
|---|---|---|
| | *HeLa proteins* | *Unknown origin* |
| HeLa cells | 25 | 0 |
| | ↓ | |
| *Xenopus* oocytes injected with HeLa nuclei | 3 | 2 |

B. Injected into *Pleurodeles* oocytes

| *Cell type analyzed* | *Number of newly synthesized proteins detectable by 2D gels* | |
|---|---|---|
| | *HeLa proteins* | *Unknown origin* |
| HeLa cells | 20 | 0 |
| | ↓ | |
| *Pleurodeles* oocytes injected with HeLa nuclei | 3 | 1 |

Oocytes were injected with HeLa nuclei, cultured for 3 days, and then labeled with $^{14}C$ amino acids. Proteins were analyzed by 2-dimensional gels. Only major HeLa proteins which electrophorese differently from oocyte proteins were scored. For details see De Robertis *et al.*, 1977.

TABLE II

Gene Expression by HeLa nuclei infected with Adenovirus-5 and injected into Xenopus oocytes

| *Cell type analyzed* | *Number of newly synthesized proteins detectable by 2-D gels* | | |
|---|---|---|---|
| | *HeLa proteins* | *Adenovirus proteins* | *Unknown origin* |
| Uninfected HeLa cells | 25 | 0 | 0 |
| Adeno-infected HeLa cells | 25 | 7 | 0 |
| Adeno-infected HeLa nuclei in *Xenopus* oocytes | ↓ 3 | ↓ 0 | 2 |

For details see De Robertis *et al.* (1977).

expression of the IgG genes was minimal relative to that of some other mouse proteine (unpublished observations). Etkin (1976) injected urodele liver nuclei into oocytes of a related species (he used Amblystoma texanum and Ambystoma mexicanum) and found that some isoenzymes are expressed by the injected nuclei, but liver-specific enzymes are not (10). Experiments of this type (turning off of some genes), although indicative of some degree of selectivity in the gene expression process, could also have arisen from damage to the nuclei during the experimental manipulations. Therefore, an experimental design in which turning on of genes can be demonstrated was more desirable.

We were able to show that the oocyte cytoplasm can activate the expression of oocyte-active genes that were previously inactive in the somatic nuclei. The oocyte cytoplasm seems to *reprogram* the injected nuclei, so as to conform to the oocyte pattern of gene expression. These experiments, described in detail by De Robertis and Gurdon (11), involved the injection of *Xenopus laevis* somatic nuclei obtained from a cloned cell line of *Xenopus* kidney cells. These cells do not express several proteins that are normally present in *Xenopus* oocytes; but they do synthesize many proteins expressed in both types of cells, as well as other proteins that are present only in cultures cells, but not in oocytes. The *Xenopus* cultured cell nuclei were injected into oocytes of a newt, *Pleurodeles waltlii,* which provides a different protein background. The proteins induced by the *Xenopus* somatic nuclei were analyzed by 2D electrophoresis. The results from this experiment, summarized in Table III, showed that:

a) several *Xenopus* oocyte-specific proteins are expressed— that is, proteins normally synthesized by *Xenopus* oocytes but *not* by the cultured cells used as nuclear donors;
b) several proteins normally synthesized in both types of cells are also expressed;
c) none of the cultured cell-specific proteins is detectable.

We conclude from these experiments that amphibian oocytes contain components which are able to *reprogram* gene expression by the injected nuclei, in the absence of cell division; this involves a turning on of oocyte-active genes that were previously inactive in the somatic nuclei.

As shown in Tabla III, only some *Xenopus* proteins (3 out of 16 oocyte specific proteins) were activated by the *Pleurodeles* oocyte cytoplasm. This may be due to the fact that *Pleurodeles,* being an urodele, is only distantly related to *Xenopus* from an evolutionary standpoint. That the proteins of both species are indeed very far apart is not only shown by the different 2D gel patterns (11) but also by immunological analysis. A rabbit antiserum directed against

TABLA III

Gene expression by Xenopus nuclei in Pleurodeles oocytes

| | *Number of newly synthesized proteins detectable by 2-D gels* | | | |
|---|---|---|---|---|
| *Cell type analyzed* | *Oocyte-specific* | *Cultured cell-specific* | *General (both cell types)* | *Unknown origin* |
| *Xenopus* oocytes | 16 | 0 | 20 | 0 |
| *Xenopus* kidney cultured cells | 0 | 8 | ↓ | 0 |
| *Xenopus* cultured cells in *Pleurodeles* oocytes | ↓ 3 | ↓ 0 | 3 | 2 |

Only major *Xenopus* proteins distinct from *Pleurodeles* oocyte proteins were scored.
For details see De Robertis and Gurdon (1977).

total *Xenopus* oocyte proteins crossreacted only 15% with *Pleurodeles* oocyte proteins. It is therefore possible that the *Xenopus* nuclei might be able to recognize some, but not all, of the cytoplasmic signals that regulate gene expression in *Pleurodeles* oocytes. In the future, it could be desirable to perform experiments with more related amphibian species, for example *Xenopus laevis* and *Xenopus borealis* (or *mullerii)* which can interbreed, giving unfertile hybrid frogs.

### 3. *The injection of somatic nuclei and early development*

The most important implication of the experiments reviewed here is that the oocyte cytoplasm contains conditions or molecules that can determine a particular spectrum of protein-coding genes to be active and others to be inactive. It is of interest that this reprogramming occurs in the absence of cell division and mitosis, since it has been proposed that mitosis and cell division are prerequisites for any major change in the differentiated state of eukaryotic cells (12).

These gene-controlling substances could turn out to be examples of "determinants" of egg cytoplasm. In mosaic eggs, "determinants" are found associated with certain regions of the egg ("cytoplasmic localization"). When development starts, determinants are distributeed unequally betweem daughter cells and are thought to be responsible for the first steps of cell differentiation (for a recent review see ref. 13). The oocyte substances that reprogram genes can be thought of from this point of view. Alternatively, it could also be possible that the somatic nuclei are treated by the oocyte in the way the egg normally processes the sperm nucleus after fertilization. Morphologically, the male pronucleus also enlarges enormously and disperses its chromatin in the way micro-injected nuclei do. In some species (reviewed by Davidson, 13), the set of genes expressed in early development is similar to those expressed during oogenesis. The possibility therefore exists that the somatic nuclei are being reprogrammed in the same way as the sperm nucleus during early development.

It is clear from our results (11) that oocytes express as proteins a specific subset of genes. This is therefore very much against those models of development which propose that the oocyte is a totally undifferentiated cell in which all genes are expressed and that development is only a gradual switching off of certain genes. In fact, the oocyte is a highly specialized cell that not only expresses a defined set of genes, but can also impose this program of gene expression on other nuclei.

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