Somatic nuclei in amphibian oocytes: evidence for selective gene expression

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SUMMARY

Previous work has shown that multiple HeLa nuclei injected into Xenopus oocytes remain transcriptionally active for many days and that the expression of HeLa genes in oocytes can be detected by 2-D gel electrophoresis.

We show here that of 25 proteins which have the electrophoretic properties of HeLa gene products, only 3 are expressed in injected oocytes. To test that these proteins are products of HeLa genes, and not products of activated oocyte genes, we have injected HeLa nuclei into enucleated oocytes. Three days later, several HeLa proteins were synthesized.

The turning off of most HeLa genes in injected oocytes is apparently not at the translational level. This is indicated by the fact that adenovirus mRNA is efficiently translated when injected into Xenopus oocytes. When adenovirus-infected HeLa cell nuclei are injected into oocytes the adenovirus genes are not expressed, although some HeLa genes are expressed by the same nuclei.

The same HeLa genes as are expressed or switched off in injected Xenopus oocytes are also preferentially expressed or switched off in injected oocytes of a Urodele amphibian, Pleurodeles. This suggests that conditions or molecules may exist in oocytes which selectively impose on injected nuclei a new programme of gene expression.

INTRODUCTION

Amphibian oocytes injected with nuclei have a potential value for the analysis of gene control. This has become evident from the recent findings that gene expression by injected somatic nuclei can be recognized by the direct analysis of newly synthesized proteins (Gurdon, De Robertis & Partington, 1976a) or by enzyme activity assays (Etkin, 1976). The behaviour of somatic nuclei introduced into amphibian oocytes can be summarized as follows. During the first few days after injection the nuclei enlarge considerably and exchange their proteins with those of the oocyte cytoplasm (Gurdon, Partington & De Robertis, 1976b). The rate of RNA synthesis increases as the somatic nuclei enlarge. The message activity of the RNAs synthesized by the somatic nuclei within the oocytes can be detected coupled to translation. We reported previously that at least one new

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protein could be detected a few days after the injection of HeLa nuclei. The appearance of this new protein is not due to message carried over with the nuclei, as shown by injecting RNA isolated from our nuclear preparations. The appearance of the new protein is eliminated by the injection of α-amanitin under conditions in which transcription by type B RNA polymerases alone is inhibited (Gurdon et al 1976a).

In the experiments reported here we have investigated the selectivity of gene expression by nuclei injected into oocytes. We wish to know eventually whether oocytes contain molecules which regulate gene activity. We show here that most of the identifiable genes expressed in HeLa cells are turned off when HeLa nuclei are injected into Xenopus oocytes, and that the same spectrum of genes is inactive in HeLa nuclei injected into oocytes of an unrelated amphibian species. The injection of purified mRNA indicates that the switching off of gene activity does not occur at the translational level. We also provide further evidence, complementary to our previous results, showing that the new proteins observed are coded for by the injected nuclei and not by the oocyte’s nucleus. Enucleated oocytes were used for this purpose.

MATERIALS AND METHODS

(a) Chemical materials

Most of the chemicals used were ‘Analar’ grade from B.D.H. Chemicals Ltd. Urea (‘ultra pure grade’) was from Schwarz Mann. Proteins used for molecular weight calibrations were from Sigma. The radioactive isotopes were supplied by the Radiochemical Centre, Amersham, Proteinase K by Merck, Deoxyribonuclease I by Worthington Biochemical Co., tissue culture media by Gibco-Biocult, and Ampholines by L.K.B.

(b) Biological materials

Oocytes of Xenopus laevis and Pleurodeles waltlii were injected and cultured as previously described (Gurdon, 1976). After culture at 25 °C for varying amounts of time, oocytes were labelled and frozen. Samples for protein analysis were labelled for 6 h in 2 μl/oocyte of medium containing 250 μCi/ml of L-[U-14C]amino acid mixture (57 mCi/mAtom). In every experiment a few oocytes were fixed and sectioned serially for cytological examination.

HeLa cells were grown as monolayers in Ham’s F12 medium supplemented with 10 % foetal calf serum and as suspension cultures in Eagle’s minimal essential medium with spinner salts and 5 % foetal calf serum. Cultures were checked periodically for PPLO contamination.

Nuclei can be prepared for injection by several methods (Gurdon, 1976), but in all the experiments reported in this paper the lysolecithin-bovine serum albumin (LL-BSA) method of Gurdon (1976) was used. Successful results can also be obtained with cells ruptured by sucking them into a micropipette of smaller diameter (the method used to transplant nuclei into eggs). Nuclei
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Prepared with detergents such as Triton X-100 or Nonidet P-40 become pycnotic after injection into oocytes. About 200 HeLa nuclei were injected into each oocyte.

(c) Extraction of the samples for protein analysis

Groups of five oocytes were homogenized in 50 µl of 10 mM Tris-HCl, pH 7.4, 50 mM-NaCl, 5 mM-MgCl₂, 25 µg/ml deoxyribonuclease I and 25 µg/ml ribonuclease A. After standing for 10 min on ice the yolk platelets were removed by centrifugation at 1000 g for 5 min. The supernatant was removed, and the upper lipid layer discarded. After lyophilizing, the supernatant was resuspended in 5 µl/per oocyte of 9.5 M urea, 2% w/v Nonidet P-40, 2% ampholines pH range 5-7, 5% 2-β-mercapto-ethanol, filtered through siliconized glass wool and stored at -20 °C. The presence of salt in the homogenization medium is essential, otherwise several proteins bind to the yolk platelets.

(d) Two-dimensional gel electrophoresis (isoelectric focusing/SDS electrophoresis)

The oocyte proteins were separated in the two-dimensional electrophoresis (2-D) system described by O'Farrell (1975) modified as follows. The first dimension, isoelectric focusing in the presence of urea and Nonidet P-40, resolves proteins in a pH range from 5 to 7. The 4% polyacrylamide gels, 12 cm long, were polymerized inside 1 ml pipettes and the samples run at 400 V for 18 h in a standard disc-gel electrophoresis apparatus. 40000 cpm of the sample were loaded on each gel. In our labelling conditions, this usually corresponds to about 0.5 oocyte.

After focusing, each disc gel was equilibrated in 6 ml of SDS sample buffer (0.06 M Tris-HCl pH 6.8, 2% SDS, 5% β mercaptoethanol, 10% glycerol) for 20 min and stored frozen.

The second dimension was run in 15% polyacrylamide slab gels with the discontinuous buffer system described by Laemmli (1970), using an acrylamide to bisacrylamide ratio of 200:1 (Knowland, 1974).

After electrophoresis, the gels were fixed in 15% trichloroacetic acid 40% methanol for at least 30 min and submitted to fluorography (Bonner & Laskey, 1974). The dried gels were exposed for 1 week on Kodak RP/Royal X-O-mat or X-Omatic H film, prefogged to 0.2 O.D. units as described by Laskey & Mills (1975).

In order to refer to the different proteins in the two-dimensional map, each spot can be located by the use of two parameters. The first is its apparent molecular weight × 10⁻³, and the second its apparent iso-electric point expressed in pH units. For example, the major protein synthesized by HeLa nuclei in oocytes has a molecular weight of 66000 and an isoelectric point of 6.04 and is therefore named 66/6.04. The molecular weight calibrations were performed using β-galactosidase, bovine serum albumin, catalase, aldolase, chymotrypsigen and encephalomyocarditis virus-coded proteins as standards, in the same
slab gel in which oocyte proteins were electrophoresed. The pH determinations were performed on parallel isoelectric focusing gels which were sectioned and equilibrated for 1 h in water before pH measurement, and are less accurate than the molecular weight determinations (O'Farrell, 1975).

(e) Extraction of cytoplasmic RNA from adenovirus 5-infected cells

HeLa cells were infected with adenovirus 5 at a multiplicity of 100 p.f.u./cell. The cells were harvested and fractionated into nuclei and cytoplasm 13 h after infection by the method of Kumar & Lindberg (1972). The cytoplasmic fraction was then digested for 2 h at 37 °C with 500 µg/ml of Proteinase K in 50 mM Tris-HCl pH 7.5, 10 mM EDTA, 0.5% SDS (modified from Weigers & Hiltz, 1972, and Gross-Bellard, Oudet & Chambon 1973). The sample was then diluted into buffer containing 10 mM Tris-HCl pH 7.8, 1 mM EDTA, 0.5% SDS and extracted with 1 vol. of 1:1 phenol chloroform (Perry, La Torre, Kelley & Greenberg, 1972). The aqueous phase was re-extracted with phenol:chloroform and the first phenol:chloroform phase with 0.1 M Tris-HCl pH 9.0, 0.5% SDS (Brawerman, Mendicki & Lee, 1972). The aqueous phases were pooled and precipitated overnight with 2.5 vols. of ethanol at −20 °C, after addition of NaCl to a final concentration of 0.4 M.

RESULTS

(A) HeLa gene expression in frog oocytes

In order to detect the synthesis of HeLa proteins after injection of nuclei into oocytes, we have used two-dimensional electrophoresis (2-D electrophoresis). By this method of analysis uninjected *Xenopus* oocytes (Fig. 1A) and HeLa cells (Fig. 1B) show very different patterns of radioactive proteins. This is shown more clearly in Fig. 1C, in which HeLa and *Xenopus* proteins were mixed and coelectrophoresed. At least 25 major HeLa proteins are easily distinguishable from those of *Xenopus* oocytes (arrowed in Fig. 1C). Five major proteins co-incide in both species (indicated with arrows in Fig. 1B). One of them has been identified as actin by peptide analysis (Longthorne & De Robertis, unpublished results) and is the major labelled protein in both types of cells (indicated with an arrow in Fig. 1A).

We will now consider the proteins synthesized by oocytes injected with somatic nuclei. When oocytes are injected with HeLa nuclei and labelled with [14C]amino acids for 6 h during the first day after injection (Fig. 2A, B), no new spots are apparent by comparison with uninjected oocytes (Fig. 1A). If oocytes injected with HeLa nuclei are cultured for 3 days and then labelled in [14C]amino acids for 6 h, at least five new labelled proteins are seen. Of these the three indicated with arrows in Fig. 2D are in the same position as HeLa proteins (in gel positions 66/6.04, 68/5.92 and 66/5.97 using the nomenclature of apparent molecular weight × 10⁻³/isoelectric point). In the case of the two other new
Fig. 1. Fluorographs of 2-D gels of proteins synthesized by uninjected *Xenopus laevis* oocytes and by intact HeLa cells. Proteins were labelled with [¹⁴C]amino acids. (A) Oocyte proteins; the arrow indicates the position of actin. (B) HeLa cells, the arrows indicate proteins that superimpose with those of *Xenopus*. (C) Coelectrophoresis of labelled proteins from *Xenopus* oocytes (40000 cpm) and HeLa cells (40000 cpm). The arrows indicate the position of HeLa proteins that do not superimpose with *Xenopus* proteins.
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Isoelectric focusing (pH units)

Fig. 2. Fluorographs of proteins synthesized by Xenopus oocytes injected with HeLa nuclei. Two selected regions of the 2-D gels are shown enlarged. (A) and (B), Oocytes labelled 2–8 h after injection; (C) and (D), labelled from 72–78 h. The new proteins induced by HeLa nuclei are indicated with arrows in (C) and (D). Some of the oocyte proteins are lettered from O to X to help comparison. Oocyte spots which show variation of intensity from one gel to another are indicated by α and spots near the edges of the enlarged areas as β. Oocytes have a faint radioactive background in region 68/5-9 (in general less than is seen in (B), see Figure 1 A) in the same position as one of the proteins made by intact HeLa cells. Nevertheless, a significant increase in radioactivity in this region a few days after injection of HeLa nuclei was observed in seven independent experiments.

spots (54/6-28 and 55/6-33, Fig. 2C), we were unable to find any equivalent proteins in extracts of intact HeLa cells, and they could have been coded either by the HeLa or oocyte genomes.

Protein 66/6-04 is the most abundant of the new proteins, and its appearance is very reproducible; it has been observed in all nine independent experiments performed using oocytes from seven different frogs. Some of the minor new spots were not present in all of the 2-D analyses, but this could be due to insufficient exposure of the gels. Nevertheless, each of the five new proteins was found in four or more independent experiments. There are several reasons why we believe that the appearance of these proteins depends on the transcription of new RNA from the nuclei previously injected into oocytes (Gurdon et al. 1976a). Since the appearance of these spots is sensitive to low concentrations of α-amanitin (Gurdon et al. 1976a), the RNA is probably synthesized by a
Type B RNA polymerase (Chambon, 1975). HeLa proteins are detected only when the oocytes are labelled a few days after nuclear injection (and not during the initial day); this is presumably because in the course of this period the new mRNA which is synthesized by the transplanted nuclei accumulates in the oocytes (De Robertis et al. 1977).

We can now examine the question of whether all the genes that are expressed in intact HeLa cells are also active in HeLa nuclei injected into Xenopus oocytes. As shown in Fig. 1C, at least 25 major HeLa proteins can be readily distinguished from Xenopus proteins by 2-D gels. Of these only three were detected in injected Xenopus oocytes, although all 25 would have been detected if synthesized at the same rate as protein 66/6-04. It therefore appears that the expression of HeLa genes in oocytes is selective; only a restricted group of proteins is expressed, while many genes active in HeLa cells become inactive (or are expressed at rates below the sensitivity of our methods of detection).

(B) Somatic nuclei in enucleated oocytes

In this section we will examine the question of whether the new proteins induced by HeLa nuclei in Xenopus oocytes are indeed coded for by the injected nuclei or not. We have previously reported that the major protein induced by HeLa nuclei in oocytes has CNBr peptides of similar isoelectric points to those of protein 66/6-04 synthesized by intact HeLa cells (Gurdon et al. 1976a). Similar results (not shown) were obtained when the peptides were analysed in SDS gels. Although the CNBr peptides indicate that there are great sequence similarities between the polypeptide 66/6-04 extracted from injected oocytes and from HeLa cells, it is nevertheless conceivable that this protein is not of HeLa origin. For example, when we compared by similar techniques the peptides from the actin spot of uninjected oocytes with the actin peptides from HeLa cells, they were found to be similar, a finding that is not surprising since it is known that the sequence of actin is highly conserved through evolution (Fine & Bray, 1971). Therefore, it could be argued that the protein 66/6-04 is not coded by the HeLa nuclei and could be the product of a non-expressed oocyte gene whose transcription is induced by some factor present in the injected material.

We have been able to eliminate this argument using Xenopus oocytes enucleated by the method of Ford & Gurdon (1977). After the wound left by the manual enucleation had closed, the oocytes were injected with HeLa nuclei, cultured for 3 days, labelled for 6 h with [14C]amino acids and analysed by 2-D electrophoresis. The different steps involved in this experiment are shown in Fig. 3. It was found that protein 66/6-04 is also synthesized in the absence of the oocyte nucleus. We conclude that protein 66/6-04 does not result from the activation of some previously unexpressed gene of the host nucleus, and that we are detecting true activity of HeLa genes.
Fig. 3. Schematic representation of the steps involved in the enucleation experiment described in the text. The follicle cells that surround the oocytes are removed manually before enucleation, in order to allow the wound left by the removal of the oocyte nucleus to close (Ford & Gurdon, 1977). Oocytes injected with HeLa nuclei synthesize spot 66/6-04 (arrowed), although in less amount that non-enucleated oocytes.

(C) The translation of message for ‘unexpressed genes’

As shown in section A, many of the genes active in intact HeLa cells become inactive (or much less active) in nuclei-injected oocytes. This ‘turning off’ of some genes by the oocyte cytoplasm could occur at a transcriptional,
post-transcriptional (processing of the newly made RNA into active message) or translational level. It seems unlikely that the oocytes would display translational selectivity since they can translate efficiently many different types of injected mRNAs (Gurdon, Lane, Woodland & Marbaix, 1971; Lane & Knowland, 1975; Vassart et al. 1975; Lanclos & Hamilton, 1975; Yip, Hew, & Hsu 1975). However, this question of whether there is selective translation of certain mRNAs in oocytes injected with somatic nuclei can be tested directly by injecting mRNA coding for unexpressed genes.

We have performed this experiment using adenovirus-infected HeLa nuclei, since it is possible to isolate adenovirus late mRNA in sufficient amounts for protein synthesis studies. The genes coding for the adenovirus late proteins are an example of genes that are not expressed in oocytes. In the experiment shown in Fig. 4 HeLa cells were infected at high multiplicity with adenovirus Type 5 (100 p.f.u. cell) and the nuclei prepared and injected 6 or 12 h after infection. In the latter situation, the majority of the mRNA that is synthesized in infected HeLa cells has been shown to be adenovirus late mRNA (Lindberg, Persson & Philipson, 1972). Most of the proteins synthesized in HeLa cells during late infection are virion proteins and the ‘infected cell-specific polypeptides’ (Russel & Skehel, 1972). All these proteins are very well resolved in 2-D gels (Fig. 4B) and are readily distinguished from *Xenopus* endogenous proteins. Nevertheless, none of these seven adenovirus-induced proteins was detected when oocytes injected with 6- or 12-h infected HeLa nuclei were examined (Fig. 4A). This was not due to non-specific damage of the nuclei as indicated by cytological examination and by the fact that four of the HeLa-induced proteins were detected in the same experiment (arrowed in Fig. 4A).

In order to test whether the non-expression of the adenovirus genes in oocytes was due to translational or pretranslational control, we injected adenovirus mRNA. Cytoplasmic RNA was isolated from HeLa cells (see Methods) infected 12 h previously with adenovirus, and injected into oocytes. As shown in Fig. 5, adenovirus mRNA is translated by oocytes. We were able to detect synthesis of two virion proteins (hexon and fibre in gel positions 110/6-10 and 60/6-4) and of ‘infected cell specific polypeptides’ 1 and 2 (in gel positions 93/6-08 and 79/5-96). The proteins synthesized in oocytes comigrated in 2-D gels with the late adenovirus proteins synthesized by infected HeLa cells (Fig. 5, E and F).

We conclude from these experiments that the observed non-expression of some genes of somatic nuclei in oocytes is not due to an inability of the oocytes to translate certain messages (at least in the case of the adenovirus late genes). Therefore the turning off represents a decreased availability of active mRNAs due to a decreased rate of synthesis or processing of the gene transcripts.
Fig. 4. Adenovirus genes are not expressed in oocytes. (A), Proteins synthesized in Xenopus oocytes injected 3 days previously with HeLa nuclei which had been isolated 12 h after infection at high multiplicity with adenovirus type 5. The four arrows indicate new proteins, which are the same as those induced by uninfected HeLa nuclei (see Fig. 2). No adenovirus proteins are detectable. (B), Proteins synthesized by intact HeLa cells infected with adenovirus. The cultures were labelled with [14C]amino acids between 12 and 16 h after infection at high multiplicity. Proteins induced by viral infection are lettered from a to j (a similar gel of uninfected HeLa cells is shown in Fig. 1B). The viral proteins and 'infected cell specific polypeptides' of adenovirus type 5 have been studied by Russell & Skehel (1972) using one-dimensional SDS electrophoresis. Their apparent molecular weight determinations are in very good agreement with ours. This enables us to tentatively identify the adeno-induced spots in 2-D gels in the following way:
(Fig. 4. continued)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Mol. wt. $\times 10^{-3}$</th>
<th>M. Russell &amp; J. Skehel (1972)</th>
<th>Protein</th>
<th>Fig. 4(B)</th>
<th>2-D gel position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexon</td>
<td>115</td>
<td></td>
<td>a</td>
<td></td>
<td>110/6.10</td>
</tr>
<tr>
<td>ICSP-1</td>
<td>95</td>
<td></td>
<td>b</td>
<td></td>
<td>93/6.08</td>
</tr>
<tr>
<td>ICSP-2</td>
<td>79</td>
<td></td>
<td>c</td>
<td></td>
<td>79/5.96</td>
</tr>
<tr>
<td>Penton base</td>
<td>70</td>
<td></td>
<td>d</td>
<td></td>
<td>69/7.00</td>
</tr>
<tr>
<td>ICSP-3</td>
<td>64</td>
<td></td>
<td>e</td>
<td>63/6.3</td>
<td></td>
</tr>
<tr>
<td>Fibre</td>
<td>62</td>
<td></td>
<td>f</td>
<td></td>
<td>60/6.4</td>
</tr>
<tr>
<td>ICSP-4</td>
<td>50</td>
<td></td>
<td>g, h</td>
<td>48/6.23; 48/6.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>i, j</td>
<td>36/6.24; 36/6.17</td>
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</tbody>
</table>

- Isoelectric focusing

Fig. 5. Translation of adenovirus mRNAs in *Xenopus laevis* oocytes. Enlarged areas of 2-D gel fluorographs. The arrows indicate the relative positions of adenovirus proteins (hexon, ICSP-1, ICSP-2 and fibre, in order of decreasing molecular weight). (A, B) Mock-injected oocytes. (C, D) Oocytes injected with cytoplasmic RNA which had been extracted from HeLa cells infected with adenovirus 12 h previously (see Methods). (E, F) Coelectrophoresis of oocytes injected with adenovirus mRNA (40000 cpm of the same sample used in (C) and (D)) together with labelled adenovirus proteins (10000 c.p.m. of the late adenovirus-infected HeLa cell sample used in Fig. 4B). The adeno proteins translated in oocytes have the same 2-D mobility as in infected cells. For illustration purposes, the fluorograms were exposed to film for different amounts of time. This explains why the oocyte endogenous spots are less intense in some samples (e.g. compare samples (B) and (F)).
(D) HeLa gene expression in newt (Pleurodeles waltlii) oocytes

As shown in section A, most of the HeLa genes recognizable by 2-D gels are not expressed in Xenopus oocytes containing HeLa nuclei. An important question is whether this is a truly selective phenomenon or not. For example, the active HeLa genes could be those recognized as similar to the genes normally expressed by Xenopus oocytes, and the 'turned off genes' could be recognized as those of a kind not expressed by oocytes. Alternatively, the inhibition of most HeLa genes might have no biological significance, being just a consequence of the abnormal human-frog cell combination. Although it is difficult to provide a definitive answer to this question (see Discussion), the probable biological significance (hence selectivity) of the expression or non-expression of HeLa genes would be greatly increased if we found that the same HeLa genes are turned on and off in oocytes of another species unrelated to Xenopus.

We used oocytes of a newt, Pleurodeles waltlii, which have a 2-D distribution of proteins different from HeLa cells. Pleurodeles oocytes are also very different from Xenopus oocytes when compared by 2-D gels; this is not surprising since Pleurodeles (being an urodele) is considered to be only distantly related to frogs (see Nieuwkoop & Sutasurya, 1976). We therefore injected HeLa nuclei into Pleurodeles oocytes. At least three new proteins are detected when the injected oocytes are cultured for 3 days and then labelled with radioactive aminoacids. These proteins are not detected when oocytes are labelled immediately after injection (Fig. 6A). The most conspicuous of these new proteins coelectrophoresed with the HeLa spot 66/6-04 (Fig 6B) which is also the major protein induced in Xenopus oocytes. An increased amount of radioactivity was also found in region 68/5-92 (arrowed in Fig. 6B), which is in the position of another of the HeLa proteins expressed in Xenopus oocytes, but this protein is only partially resolved from an endogenous Pleurodeles spot (the spot labelled 'N' in Fig. 6). The second new spot corresponds to one of the major HeLa proteins that coelectrophorese with Xenopus proteins (actin and proteins 55/5-40 and 71/5-48) also coelectrophorese with Pleurodeles proteins. The third new spot was in position 67/6-13 (Fig. 6B) and we were unable to find any coincidence between it and a HeLa protein. We did not detect the synthesis of about 20 major HeLa proteins which are different from Pleurodeles proteins in 2-D gels, although they would have been detected.

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Fig. 6. Proteins synthesized in Pleurodeles waltlii oocytes. Fluorographs of 2-D gels. Some of the Pleurodeles proteins are lettered from K to N in order to help comparison. (A) Oocytes labelled from 0 to 6 h after injection with HeLa nuclei. (B) Oocytes labelled 72-78 h after injection with HeLa nuclei; arrows indicate new proteins. (C) Coelectrophoresis of proteins from uninjected Pleurodeles oocytes with proteins from labelled HeLa cells; the arrows indicate HeLa proteins.
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A

0-6 h

B

72-78 h

C

Mixture

K L M N

66/6-04

66/6-04

M L K N

66/6-04
if synthesized at a rate similar to protein 66/6-04. Most of these non-expressed genes are the same ones that are not expressed in *Xenopus* oocytes.

We conclude from this experiment that the mechanisms that determine the expression of some HeLa genes and the turning off of others are similar in *Pleurodeles* and *Xenopus* oocytes. In both species the same HeLa gene (66/6-04) is expressed preferentially and the same HeLa genes are turned off.

**DISCUSSION**

Gene expression by HeLa nuclei in oocytes appears to be restricted to a selected group of genes. A few new proteins can be detected by 2-D gels a few days after injection. These new proteins are dependent upon new transcription within the oocytes (Gurdon *et al.* 1976a), and are indeed coded for by the injected nuclei (and not by the host nucleus) as shown by enucleation experiments and by peptide analysis of protein 66/6-04. If all the HeLa genes were transcribed and translated in oocytes at the same rate as protein 66/6-04 at least 25 HeLa spots should have been detected, yet only three were detected. It is possible, however, that other proteins are induced by the nuclei (2-D gels do not resolve proteins with an isoelectric point above 7), or that some of them are synthesized at rates below the sensitivity of our methods. In any case, it is clear that some polypeptides are synthesized in much more abundance than others. Most of the HeLa genes therefore behave as if they were turned off in *Xenopus* oocytes. The cytoplasmic signals which mediate this inactivation are not species specific since a similar pattern of HeLa gene expression is also observed in newt (*Pleurodeles*) oocytes, which are only distantly related to frogs.

The level at which the expression of some genes is turned off has been explored further using adenovirus-infected HeLa nuclei. After injection into oocytes the expression of the adenovirus genome is undetectable even though some HeLa genes are expressed by the same nuclei. By injecting adenovirus late mRNA we showed that *Xenopus* oocytes can synthesize efficiently adenovirus proteins (and also that once synthesized these proteins are not degraded by the oocyte). We can therefore conclude that the turning off occurs at a step previous to protein synthesis, at the level of the synthesis or processing of the genetic message.

When nuclei are introduced experimentally into a foreign cytoplasm they tend to assume the nuclear synthetic activity and nuclear morphology characteristic of the host cell (for reviews see Gurdon & Woodland 1968; Gurdon 1974 and Harris, 1974). For example, when chicken red blood cells are fused to HeLa cells, the erythrocyte nuclei reassert RNA synthesis and their chromatin disperses (see Harris, 1974), or when frog brain nuclei (which normally do not divide) are injected into *Xenopus* eggs¹ they become active DNA replication

¹ A frog egg differs from an oocyte in that it has undergone a hormone-induced maturation process. The germinal vesicle breaks down and the oocyte is released from the ovary. In contrast to oocytes, eggs are very active in DNA synthesis (after sperm entry or experimental activation) and largely inactive in RNA synthesis (for a review, see Smith & Ecker, 1970).
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(Graham, Arms & Gurdon, 1966). Since the cytoplasm is able to modify nuclear activity, it seems possible that the oocyte cytoplasm could reprogram gene expression of specific protein-coding genes in the injected somatic nuclei. Oocyte regulatory molecules could enter the injected nuclei, stimulating the transcription of those genes which would be recognized as similar to the oocyte-active genes (or turning off other genes). Although this hypothesis could explain the selectivity of gene expression displayed by the HeLa nuclei in oocytes, the experiments described in the previous section cannot establish whether the oocyte cytoplasm reprograms nuclear expression or not. This is because the three HeLa proteins detected in oocytes are also synthesized in intact cells (our analysis would not distinguish whether these genes are being expressed at a similar rate as in intact cells or at a much higher one), and it could be argued that the selective turning off of genes might not be biologically meaningful (being for example a consequence of the heterologous nucleus-cytoplasm combinations or of the experimental manipulations). This question of whether gene expression by the somatic nuclei is reprogrammed by the oocyte cytoplasm can be answered by demonstrating the experimental turning on of oocyte-active genes previously inactive in the somatic cells. This has been achieved recently (De Robertis & Gurdon, 1977) in experiments which involved the injection of *Xenopus* somatic nuclei into newt (*Pleurodeles*) oocytes, and which show that at least some genes are indeed reprogrammed.

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