Injected nuclei in frog oocytes: fate, enlargement, and chromatin dispersal

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SUMMARY

A method is described by which nuclei associated with some cytoplasm can be rapidly prepared from a suspension of cells. The method involves the use of lysolecithin and bovine serum albumin.

Oocytes of *Xenopus laevis* were injected with about 200 nuclei prepared from human HeLa cells by this method. Nuclei were deposited in oocyte cytoplasm, in the oocyte nucleus, or in the dispersed contents of a ruptured oocyte nucleus.

Injected HeLa nuclei enlarge up to several hundred times in volume in the course of a few days. Their enlargement is associated with chromatin dispersion, increased binding of an acidic dye, and with the reduction in size, and eventual disappearance, of nucleoli. The amount of HeLa nucleus enlargement is much greater when the oocyte nucleus is ruptured. The fate of injected nuclei was followed by the use of HeLa nuclei whose DNA had been previously labelled with [3H]thymidine. Labelled DNA does not pass from injected HeLa nuclei into the oocyte nucleus. Injected nuclei appear not to fuse with each other or with the oocyte nucleus.

Nuclei prepared by the above method look morphologically healthy in oocytes cultured *in vitro* for up to one month after nuclear injection. Nuclei prepared by other methods, such as those involving the use of detergents, undergo deterioration within a few days after injection into oocytes.

INTRODUCTION

The aim of these experiments is to find a way of introducing somatic cell nuclei into an oocyte so that they remain transcriptionally active for a long time. Cultured oocytes are very active in transcription, and an experimental system of this kind could eventually lead to the identification of cytoplasmic components which regulate gene activity.

It has been shown previously that small groups of nuclei can be injected into *Xenopus* oocytes, and that they appear viable 3 days later (Gurdon, 1968). A preliminary account has been published of related experiments in which proteins coded for by HeLa genes can be detected in oocytes containing HeLa nuclei (Gurdon, De Robertis & Partington, 1976a). The experiments described here extend the potential use of this experimental system in two ways. Firstly, a new method is described for rapidly preparing a suspension of nuclei in such a way that they remain morphologically healthy for a few weeks after injection.
into oocytes. Secondly, a detailed description is given of the fate of injected nuclei and of the morphological changes which they undergo, according to whether they are injected into the germinal vesicle (= nucleus) or cytoplasm of an oocyte.

The present investigation was undertaken with cultured mammalian cell nuclei, because the genetic differences between mammals and frogs are likely to be of value in distinguishing the genetic activity of injected nuclei from that of an oocyte. The methods described are, however, applicable to cell suspensions of other vertebrates.

**MATERIALS AND METHODS**

_Xenopus laevis_ were imported from the South African Snake Farm, P.O. Box 6, Fish Hoek, Cape Province, South Africa, or reared in the laboratory. Females providing oocytes had not been injected with pituitary gonadotropic hormone (to induce ovulation) within the last few months, but the results might not have been different if recently ovulated females had been used. Frogs were killed by injecting, into the dorsal lymph sac, 0.25 ml of veterinary Nembutal (pentobarbitone sodium, 60 mg per ml from Abbott Company, Kent) per 100 g body wt; this appears to be the most humane way of killing _Xenopus_. About 20 min after Nembutal injection, frogs were washed extensively on the ventral surface with Hibitane (chlorhexidine gluconate, 5%, from ICI), an anti-microbial agent used to eliminate a possible source of infection. Pieces of ovary were removed, at once separated into fragments containing about 50 oocytes, and kept until required for injection at 19 °C in our modified Barth solution. This solution (MBS-H) contains 88 mM-NaCl, 1 mM-KCl, 2.4 mM-NaHCO₃, 0.82 mM-MgSO₄, 0.33 mM-Ca(NO₃)₂, 0.41 mM-CaCl₂, 10 mM 'Hepes', 10 mg/l each of Benzylpenicillin and Streptomycin sulphate.

Clusters of four to six oocytes were injected as described by Gurdon (1974), and maintained at 19° or 25 °C in 15 ml dishes of filter-sterilized MBS-H. Oocytes were changed into fresh, sterile medium every 2–3 days. When required, oocytes were fixed for 2–16 h in Sanfelice's (1918) fixative: 1 ml of 1 % chromic acid is mixed immediately before use with 0.55 ml of a stock solution of 128 ml 40 % formaldehyde + 16 ml glacial acetic acid. Fixed oocytes were embedded in paraffin wax, sectioned at 10 μm, and stained in haemalum and light green, according to standard practice.

The cultured cells used for these experiments were collected from spinner cultures (HeLa strain G, myeloma MOPC 21) or trypsinized from the base of flat-bottomed, plastic bottles (HeLa, 3T6, _Xenopus_). Cells grown in bottles were provided with Ham's F12 medium supplemented with 10 % foetal calf serum (Ham, 1965); cells grown in spinner cultures were given minimum essential medium for suspension culture (Gibco Bio-cult, Scotland, medium BCL-111P). Cells were trypsinized for nuclear preparation at densities ranging from 1/3 confluence to near confluence.
RESULTS

Methods of preparing nuclei for injection

Many of the methods commonly used to prepare nuclei for transcription in vitro involve the use of non-ionic detergents. When HeLa, myeloma, or 3T6 cell nuclei were prepared by the use of Nonidet P40 or Triton X-100 (both from BDH) at low concentrations (method (b) below), the nuclei looked morphologically normal for a few hours after injection (Fig. 1A); but already by 24 h after injection they had crinkling nuclear membranes, and at 4 days most were no longer individually distinguishable, but had become pycnotic lumps of chromatin, resembling dead nuclei (Fig. 1C). Evidently detergent-treated nuclei are not satisfactory for long-term incubation in oocytes. Several other methods of preparing nuclei were tested by examining the morphological appearance of nuclei 4 days after injection into oocytes which had been incubated at 19 °C. Some of the procedures tested and results obtained are described below (methods a–d).

The most appropriate method of preparing suspensions of nuclei for injection into oocytes depends on the cell type used to provide nuclei. The best method is considered to be one which gives an even swelling of all nuclei after injection into oocytes (e.g. Fig. 1D); swelling is correlated with increasing transcriptional activity (Gurdon, Partington & De Robertis, 1976b). For HeLa nuclei, the following procedure (referred to as LL-BSA) has proved most satisfactory; it has been designed by a combination of methods previously used to isolate or transplant nuclei, or fuse cells (Howell & Lucy, 1969; Burgoyne, Waqar & Atkinson, 1970; Hennen, 1970; Koprowski & Croce, 1973).

**LL-BSA procedure for preparing nuclei for injection into oocytes:**

1. 10⁶ cells, collected from a suspension culture, or trypsinsized from a bottle (with excess serum subsequently added to inactivate the trypsin), are spun into a pellet (330 g for 30 sec).
2. The cells are suspended in 500 μl of 0·25 M sucrose, 75 mM-NaCl, 0·5 mM spermidine trihydrochloride, and 0·15 mM spermine tetrahydrochloride – a solution referred to as SuNaSp, at 18°–24 °C.
3. 10 μl of a 1 mg/ml H₂O solution of lysolecithin, i.e. lysophosphatidylcholine (from Sigma, but other brands would probably work as well) at 18°–24 °C is added.
4. The cell suspension is swirled by hand every 15 sec for a total of 90 sec, at 18°–24°C. This step causes lysis of the cells, yielding a suspension of cytoplasm-associated nuclei.
5. 1·0 ml of the SuNaSp medium (above) supplemented with 3 % BSA (a solution referred to as SuNaSpBSA) at 0 °C is added, and mixed into the nuclear suspension.
6. The suspension is centrifuged at 330 g for 30 sec, the supernatant poured off, and the tube drained and wiped dry (except for the pellet).

7. The nuclear pellet is resuspended in about 20 µl of SuNaSpBSA and placed on an ice bath.

8. About 2 µl of the nuclear suspension to which 10 µl of a 0-2 % solution of trypan blue in phosphate buffered saline has been added, is examined under the microscope to determine the percentage of cells lysed (having nuclei stained blue). This should be between 95 % and 99 %.

It is important in this procedure to add lysolecithin to a cell suspension at 18 °C or more, since it will precipitate and hence lose efficiency if added to cold solutions. At step 2 or 3 it is helpful to remove with a needle any visible dust particles or threads of material (probably DNA from lysed nuclei), since these may block the micro-injection pipette. To use the nuclear suspension for injection, it is placed as a drop on a siliconized glass slide kept on ice. 40 nl of the nuclear suspension can be injected into one oocyte, and this should contain at least 200 nuclei. After about 10 min, nuclei will settle to the bottom of the drop, and may need to be resuspended. Suspensions of nuclei may be kept on ice for at least an hour without adversely affecting their condition, judged morphologically several days after injection into oocytes.

When determining the best cell lysis procedure, the following methods other than LL-BSA were tested. (a) About 0-5 x 10⁶ cells were suspended in 500 µl of 0-01 % NaCl, 0-01 % Na citrate, swirled by hand at intervals over 1½ min, and then added to 500 µl of 0-5 m sucrose, 4 mM-MgCl₂, 1-2 % BSA. The lysed cells were pelleted and taken up in 0-25 M sucrose, 2 mM-MgCl₂, 1 mM-KH₂PO₄/K₂HPO₄ pH 6-8, 1-2 % BSA. (b) About 10⁶ cells were suspended in 500 µl 0-25 M sucrose, 2 mM-MgCl₂, 1 mM-KH₂PO₄/K₂HPO₄ pH 6-8, 0-6 % BSA; the suspension was made 0-025 % in respect of Triton X-100 (or Nonidet P40) and swirled by hand at intervals over 1½ min; an equal volume of 0-5 M sucrose, 4 mM-MgCl₂ and 1-2 % BSA was added. The lysed cells were pelleted and taken up in 0-25 M

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**Figure 1**

The survival of nuclei, prepared by different methods, after injection into oocytes.

(A) HeLa nuclei prepared by the Triton X-100 procedure (see p. 527, method b); oocytes fixed 5 min after injection.

(B) HeLa nuclei prepared by the LL-BSA procedure (p. 525); oocytes fixed 10 min after injection.

(C) HeLa nuclei prepared as for Fig. A; oocytes fixed after 4 days, at 19 °C.

(D) HeLa nuclei prepared as for Fig. B; oocytes fixed after 4 days at 19 °C.

(E) HeLa nuclei prepared by homogenization (p. 529, method d); oocytes fixed after 4 days.

(F) Myeloma nuclei prepared by the low salt citrate procedure (p. 527, method a); oocytes fixed after 4 days. The magnification for all figures is as shown at the bottom of the page.
Injected nuclei in frog oocytes. I

sucrose, 2 mM-MgCl₂, 1 mM-KH₂PO₄/K₂HPO₄, pH 6-8, 1.2 % BSA. (c) Cells were suspended in 0.025 M sucrose, 0.2 mM-MgCl₂, and 0.3 % BSA, and then sucked into a micro-pipette which was too small for the cells, so that over 90 % of the cells were ruptured - a technique used for nuclear transplantation. (d) Cells were suspended in 0.025 M sucrose, 0.2 mM-MgCl₂, 0.3 % BSA, and homogenized in a ball-piston homogenizer just tight enough to break over 95 % of cells with 10 strokes.

The suitability of different methods varies according to cell type. Thus, mouse myeloma cells (MOPC 21) and 3T6 cells (a mouse epithelial line) were successfully lysed by procedure (a) and their nuclei survived well after injection (Fig. 1 F) as well as by the LL-BSA procedure, the latter giving at least as good nuclear morphology as the former, 4 days after injection into oocytes. HeLa cells could not be lysed by procedure (a) even if left in the low salt medium for over 15 min. Procedure (b) always resulted in 'clean' (cytoplasm-free) nuclei, but such nuclei resulted in very poor nuclear morphology after injection, as already stated. Procedures (c) and (d), if carried out very gently, yielded 'crude' nuclei (covered by cytoplasm), of which some survived well after injection, but others became pycnotic and died (Fig. 1 E). In conclusion, the LL-BSA procedure gave far better results with HeLa cells than any of the other methods tried, and it gave at least as good results with myeloma and 3T6 cells as the low salt citrate method (a). Various cell types seem to require different amounts of LL to yield a 95 % lysed suspension after 1½ min; myeloma cells typically require about half as much as HeLa cells. It seems best to use as little LL treatment as possible, since too high a concentration or too long an exposure can lead to poor nuclear survival after injection. In the LL-BSA procedure, the substitution of SuNaSp by 0.25 M sucrose, 2 mM-MgCl₂ had little effect; the essential part of the method is the use of lysolecithin and its control by BSA.

Morphology and intracellular location of injected nuclei

All of the results to be described apply to HeLa cell nuclei, unless stated otherwise. Nuclei can be deposited in different regions of an oocyte. To ensure

Figure 2

HeLa nuclei, prepared by the LL-BSA procedure, and injected into the cytoplasm of oocytes. The figures show sections of oocytes, fixed after incubation for 4 days at 19 °C except in Fig. C.

(A) Whole oocyte showing GV and injected nuclei.
(B) Oocyte showing GV (top right) and label over injected nuclei.
(C) HeLa nuclei in an oocyte fixed 10 min after injection.
(D) Enlarged HeLa nuclei lacking nucleoli.
(E) Label over enlarged nuclei. B and E are autoradiographs of sectioned nuclei from cells prelabelled with [³H]TdR (p. 534). The magnification of Figs. C, D, and E are as shown at the bottom of the page.
that nuclei are deposited in the cytoplasm and not in the GV (germinal vesicle; see Fig. 2A), nuclei are injected into the unpigmented vegetal hemisphere of an oocyte, or just under the surface of the animal hemisphere. With practice it is possible to inject nuclei into the GV of an oocyte, even though the yolk and pigment make it impossible to see the part of the injection pipette which is inside the oocyte. The most successful procedure is to penetrate the oocyte as close as possible to the animal pole, and to aim the pipette tip at the centre of the oocyte, so that nuclei are dispersed half way between the animal and vegetal poles. At the same time the oocyte is squeezed equatorially with forceps. As a result of this procedure, the GV can be penetrated in up to 80% of all attempts.

Penetration of the GV results in one of two conditions. The GV may remain intact, some or all of the injected nuclei occupying the space within it (Fig. 3A, C). This is a relatively rare result, arising in not more than 10% of all GV injections. In some instances of this kind, injected nuclei may form a clump of deeply staining bodies (upper part of Fig. 3B). Much more commonly, the GV breaks down, and nuclei are deposited in the region in which its contents are released (Fig. 4A), where it is usual to see the crumpled remains of the GV membrane. The external appearance of the injected oocytes does not reveal whether nuclei have been deposited in the cytoplasm, in the GV, or in the region of the dispersed GV contents.

Nuclei prepared by the LL-BSA method described above always undergo a substantial enlargement in injected oocytes. But the amount of enlargement depends, to a great extent, on the location of injected nuclei. Cytoplasmic nuclei enlarge more when injected into the animal than into the vegetal hemisphere. Injected nuclei tend to accumulate around the GV. In the course of 4 days, cytoplasmic nuclei may enlarge to as much as 30 μm in diameter, a 10 times increase in volume (Fig. 2C, D). This is the greatest size to which HeLa nuclei usually enlarge within a few days in Xenopus oocyte cytoplasm (Table 1). However,
Table 1. Enlargement of injected nuclei in oocytes of different sizes

<table>
<thead>
<tr>
<th>Oocyte diameter (µm); stage (Dumont, 1972)</th>
<th>Oocytes with GV intact</th>
<th>Oocytes with GV dispersed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of injected</td>
<td>Max. diam. of injected</td>
</tr>
<tr>
<td></td>
<td>nuclei scored</td>
<td>nuclei (µm)</td>
</tr>
<tr>
<td>300–600; II and III</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>600–800; IV early</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>800–1000; IV late</td>
<td>40</td>
<td>29</td>
</tr>
<tr>
<td>1000–1200; V and VI</td>
<td>70</td>
<td>29</td>
</tr>
</tbody>
</table>

These results were obtained with HeLa nuclei injected into oocytes taken from one female, incubated at 19 °C, and fixed 4 days after injection. Measurements of the maximum diameter of injected nuclei were made by scoring the largest 10 injected nuclei in each oocyte (5 nuclei for oocyte stages II and III). The diameter of most HeLa nuclei immediately after injection is 10–12 µm (Fig. 5).

most nuclei do not reach this size when large numbers (such as 200) are injected. It is noticeable that when injected nuclei remain in close contact with each other in a dense cluster, it is the ones on the periphery which enlarge most; those near the centre of a dense cluster usually fail to enlarge at all, and may become pycnotic. These observations fit the interpretation that the enlargement of injected nuclei depends upon their response to, or uptake of, a normal component of oocytes; nuclei appear to compete for the swelling agent which is more concentrated in the animal than vegetal hemisphere.

Injected nuclei which remain in an intact GV usually enlarge until they have occupied all of it, presumably displacing the GV chromosomes and nucleoli. Nuclei in the GV may enlarge to more than 30 µm in diameter, but their

**Figure 4**

HeLa nuclei, prepared by the LL-BSA procedure, and injected into oocyte germinal vesicles which subsequently broke down releasing their contents around the nuclei. Oocytes were fixed after incubation for 4 days at 25 °C. All figures show sections of oocytes containing injected nuclei. The nuclei shown in Figs. B and F were from cells prelabelled with [3H]TdR (page 534).

(A) Whole oocyte with injected nuclei.

(B) Autoradiograph of injected nuclei.

(C) Greatly enlarged nuclei, with one unenlarged nucleus (presumably damaged) near top.

(D) A large group of enlarged nuclei.

(E) HeLa-nucleus derived ‘vesicle’ of 65 µm diameter.

(F) Autoradiograph of 45 µm and 27 µm HeLa nuclei showing dispersion of prelabelled DNA. In one of the two nuclei, labelled DNA is concentrated on the periphery, a condition often observed in [3H]TdR-prelabelled nuclei which have enlarged a lot.
maximum enlargement is not revealed since they are compressed within the space available (Fig. 3C, E). The most striking nuclear enlargement is seen in GV-dispersed oocytes, which usually contain several vesicles of 50 μm or more in diameter (Figs. 4C, D, E). As shown below, these vesicles are derived from injected nuclei. Assuming that the largest of these (100 μm) are derived from single HeLa nuclei (evidence below), this constitutes an enlargement of ca. 500 times. By no means all nuclei in GV-dispersed oocytes form such large vesicles; usually there is a range of nuclear sizes from unswollen nuclei (12 μm in diameter), with most at 30–40 μm, and the minority at sizes larger than 40 μm (see Table 1 and Fig. 4C–E). These results with nuclei in the dispersed GV plasm suggest that the enlargement of nuclei beyond 30 μm is dependent on their uptake of, or response to, material released from the ruptured GV.

Certain other morphological changes always take place in injected nuclei as they enlarge, independently of their intracellular location in oocytes. Newly injected HeLa nuclei differ sharply from the GV by not binding the histological stain light green. Within 1–2 days of incubation in an oocyte, most injected nuclei start to bind this stain strongly, and once they have enlarged, they do so as intensely as GV nucleoplasm. Although the molecular basis of light green (an acid dye) staining is not known, this observation implies that the properties of injected nuclei change and come to resemble increasingly the properties of the oocyte GV. Another change always observed in enlarging injected nuclei is the dispersion of their chromosome material. Comparison of Figs. 1B and 2C with 2D, 3E and 4E shows that enlarged nuclei contain a fine fibrilar nucleoplasm which resembles that of the GV. As shown below the DNA of [3H]TdR ([3H]thymidine) labelled nuclei is dispersed throughout the enlarged nuclei. Evidently nuclear enlargement is accompanied by chromatin dispersion, both increasing commensurately. The last cytological change worth noting in injected HeLa nuclei is the reduction in nucleolar size. Newly injected nuclei contain prominent nucleoli (Figs. 1B, 2C); not only do these fail to enlarge in proportion to nuclear volume, but they undergo an absolute decrease in size and often disappear (Figs. 1D and 2D). No increase in number of nucleoli is seen. While dispersion of chromatin is what would be expected of increasing genetic activity, the reduction in nucleolar size suggests a reduction or cessation of rRNA synthesis. The reduction in nucleolar size may be associated with a heterospecific combination of nucleus and cytoplasm, since it was not observed in injected Xenopus nuclei (Gurdon, 1968).

Injection of nuclei containing labelled DNA

The principal purpose of injecting prelabelled nuclei is to check their identification and fate in oocytes. This could not be done if labelled DNA from intact or broken nuclei were to enter the GV. To test this, HeLa cells were grown in medium containing [3H]TdR ([3H]methyl-thymidine, 1 μCi/ml; sp. act. 46 Ci/mMol) for 44 h before trypsinization and injection into oocytes; over 99 %
of the donor nuclei had been labelled. After incubation for 3 or 4 days, oocytes injected with nuclei were fixed, sectioned, and exposed for autoradiography for four weeks. This long autoradiographic exposure resulted in most injected nuclei being intensely labelled so that grain confluence and film saturation would have occurred, and grain counts over nuclei were almost certainly underestimates. Nevertheless, Table 2 shows that the GV was never labelled above the level of the cytoplasmic background. Assuming that the total volume of all cytoplasm-injected nuclei is 5% of the GV (see later), the transfer of 10% of the labelled DNA from injected nuclei to the GV would have resulted in sections of of the GV being labelled 200 times less intensely than the average injected nuclei. This should have been readily detectable, since very short (12 h) autoradiographic exposures of donor nuclei showed them to be labelled at least four times above the background. If much less than 10% of the injected labelled DNA had entered the GV, or if any of it had become evenly dispersed in the cytoplasm, the dilution involved would have been too great for it to have been seen in these experiments. The main point is that the GV is not labelled above background in such experiments, and [³H]TdR-prelabelling may therefore be legitimately used to identify injected nuclei.

Autoradiographs of sectioned oocytes containing [³H]TdR-prelabelled nuclei can now be used to answer two questions. First, are all objects believed to be HeLa nuclei labelled? Since 99-9% of the HeLa nuclei were labelled before injection, any unlabelled ‘nuclei’ seen in oocytes can be presumed to be fragments of the GV and not injected HeLa nuclei. Secondly, are all nuclei seen in oocytes labelled to the extent expected if each is derived from a single injected nucleus? If the difference in intensity of labelling between swollen nuclei in oocytes is much greater than the same difference between donor HeLa nuclei before injection, this would suggest that swollen nuclei may result from the fusion of injected nuclei.

The answer to the first question is clear. The autoradiographs shown in Figs. 2B, E, 3D, F, and 4B, F represent, respectively, nuclei in the cytoplasm, in the GV, and in the dispersed GV area. It can be seen that all of the objects so far described as nuclei are, in fact, labelled, whereas the GV (see Fig. 2B) is not. In GV-dispersed oocytes, huge spherical vesicles of up to 100 μm in diameter are sometimes seen, and even these are labelled to some extent. Occasionally large irregularly shaped (non-spherical) vesicles are seen in GV-dispersed oocytes, and these are unlabelled. In conclusion, all spherical objects in injected oocytes other than intact GVs are derived from injected nuclei. Irregularly shaped vesicles are rarely seen, and probably represent breakdown products of the GV.

The second question is harder to answer. There is considerable variation in the intensity of labelling of adjacent nuclei (Figs. 2E, 3D, and 4B). This is probably attributable in part to the very large variation in the extent to which donor nuclei were labelled (some having passed through more S phases than others), and in part to the variable enlargement of the injected nuclei (the amount of enlargement cannot be judged from 10 μm sections). As would be expected,
there is a general trend for the largest nuclei to be less heavily labelled than the smaller ones (Fig. 4F), and the variation in labelling between nuclei is about as great in highly enlarged nuclei (Fig. 4B, F) as in nuclei which have enlarged only moderately (Fig. 2B, E). There is no obvious reduction in the number of nuclei, as would be expected if fusion had taken place. There is therefore no reason to believe that nuclear fusion takes place. Probably each vesicle is an enlarged single HeLa nucleus.
HeLa nuclei, prelabelled with [³H]TdR (see page 534) were injected into oocytes, which were fixed after incubation for 4 days at 19 °C. They were then sectioned at 10 μm and subjected to autoradiography. Grains were counted at a magnification of ×1250 in 25 μm² areas.

Factors affecting the swelling of injected nuclei

Fig. 5 shows the time-course of swelling of nuclei during the first few days after injection into oocyte cytoplasm. After that, further swelling takes place gradually. In one experiment, injected oocytes remained viable for one month, and after this time, some of the nuclei in oocyte cytoplasm had enlarged to 40 μm in diameter. Nuclei in the region of a dispersed GV swell faster and to a greater eventual volume than nuclei in oocyte cytoplasm. Swelling is faster at 25 °C than at 19 °C.

The probability that the oocyte’s GV will be dispersed after injection of nuclei is much influenced by the temperature at which the oocytes are incubated. In one series of experiments involving 100 oocytes, the GV contents were dispersed in 56 % of the oocytes incubated at 19 °C, but in 77 % of those (from the same experiment) incubated at 25 °C. The GV also breaks down very readily at 30 °C, but most samples of *Xenopus* oocytes do not survive well at this temperature. Thus the immense swelling of injected nuclei can be induced in the majority of oocytes by combining the careful aiming of the injection pipette (p. 531) with incubation at 25 °C.

It may be useful, in future, to inject nuclei into oocytes of different sizes. The extent of lampbrush chromosome extension changes during oogenesis in Amphibian species so far examined (Callan, 1963). It may turn out that the effect of oocytes on HeLa gene expression will differ according to the stage of oogenesis. Table 1 shows that injected nuclei enlarge substantially in oocytes of the smallest conveniently injectable stage (300 μm upwards). In each size class of oocyte, the nuclei swell more in oocytes with dispersed GVs than in oocytes with intact GVs.

An attempt has been made to determine the total volume of all injected nuclei in an oocyte, in order to compare this to the volume of the oocyte’s own GV. Such a comparison will be useful when attempts are made to estimate the proportion of the total RNA synthesized by nucleus-injected oocytes which is likely to be specified by genes in the injected nuclei rather than by genes in the GV.
Table 3. Numbers and volumes of nuclei injected into oocytes

<table>
<thead>
<tr>
<th>Diameter of oocyte (μm)</th>
<th>Volume of oocyte GV* (as % of oocyte)</th>
<th>Number of nuclei in one oocyte†</th>
<th>Sum of volumes in all injected nuclei in one oocyte‡</th>
<th>As % of oocyte volume</th>
<th>As % of GV volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>GV intact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1125</td>
<td>3·7</td>
<td>220</td>
<td>0·444</td>
<td>0·06</td>
<td>1·61</td>
</tr>
<tr>
<td>1140</td>
<td>2·6</td>
<td>210</td>
<td>0·952</td>
<td>0·13</td>
<td>4·85</td>
</tr>
<tr>
<td>1160</td>
<td>3·8</td>
<td>160</td>
<td>0·750</td>
<td>0·09</td>
<td>2·41</td>
</tr>
<tr>
<td>GV dispersed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>875</td>
<td>3·3*</td>
<td>150</td>
<td>3·65</td>
<td>1·05</td>
<td>31·4</td>
</tr>
<tr>
<td>900</td>
<td></td>
<td>340</td>
<td>7·00</td>
<td>1·83</td>
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</tr>
<tr>
<td>935</td>
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</tr>
<tr>
<td>1010</td>
<td></td>
<td>300</td>
<td>5·68</td>
<td>1·05</td>
<td>31·6</td>
</tr>
<tr>
<td>1140</td>
<td></td>
<td>300</td>
<td>5·92</td>
<td>0·76</td>
<td>23·0</td>
</tr>
</tbody>
</table>

HeLa nuclei were injected into oocytes which were fixed 4 days later, after incubation at 19 °C (GV intact), or 25 °C (GV dispersed).

* Measured when GV was intact; assumed to be 3·3 % of oocyte volume when GV is dispersed.
† Estimated from serial sections.
‡ Estimated by measuring the maximum diameters of injected nuclei and summing these together to obtain the combined volume of all injected nuclei in one oocyte.

(Gurdon et al 1976b). Table 3 shows that about 200 cytoplasmic nuclei which enlarge only moderately will usually constitute 1–5 % of the volume of the GV. In GV dispersed oocytes, the injected nuclei enlarge enormously more, and usually reach a total volume of 20–50 % of that expected of the GV, when about 200 nuclei are injected.

Lastly, the agent responsible for nuclear enlargement has been further investigated. The results reported above have suggested that oocyte cytoplasm contains an agent which causes swelling of injected nuclei up to a limited level (ca. 30 μm), but that the GV contains more of the same or another agent which can cause enlargement up to 100 μm. Additional evidence that the initial swelling is independent of the GV or its contents has come from experiments in which HeLa nuclei were injected into oocytes enucleated as described by Ford & Gurdon (1977). Three days after injection, nuclei had swollen to 25 μm. Chromatin dispersion, disappearance of nucleoli, and light green staining all took place in nuclei injected in enucleated oocytes.

DISCUSSION

There is little previous work with which to compare the results reported here. Nuclei from early embryos and adult brain of Xenopus have been injected into oocytes (Gurdon, 1968) and underwent a substantial enlargement within 3 days. This establishes the point that the enlargement of HeLa nuclei is not a
pathological consequence of introducing mammalian nuclei into frog cells, which are then incubated at a temperature (19° or 25 °C) well below that at which HeLa cells will grow.

The method described here (LL-BSA) for preparing a suspension of nuclei for injection has a major advantage over other methods previously used for this type of work (Gurdon, 1968). A large number of nuclei (e.g. 200) can be conveniently injected in a small volume (35 nl); over 1000 nuclei can be injected into an oocyte if necessary. This method appears less damaging than any other to the nuclei being injected. Lastly, it is simply and rapidly performed on any kinds of cells which can be prepared as a single cell suspension. The nuclei prepared by this method are associated with a substantial amount of cytoplasm. It does not appear, however, that the amount of mRNA carried over with cytoplasm will prevent the genetic activity of injected nuclei being seen as newly synthesized proteins (Gurdon et al. 1976a).

The enlargement of injected HeLa nuclei in oocytes is not unexpected. It seems generally to be true that large cells have larger nuclei than small cells, and that nuclei increase their volume when transferred to the cytoplasm of larger cells. Thus, somatic cell nuclei transplanted to amphibian eggs undergo a very rapid enlargement (Gurdon, 1964; Graham, Arms & Gurdon, 1966), as also do red blood cell nuclei fused into cultured cells (Harris, 1967). Nevertheless, the amount of enlargement of HeLa nuclei in frog oocytes is remarkable; in some cases, it appears that these nuclei may enlarge by as much as 500 times, thereby becoming about 100 times larger than the cell from which they came. It seems possible that the chromosomes of enlarged HeLa nuclei in oocytes may take up a lambrush-like configuration. The reduction in size and eventual disappearance of nucleoli in injected HeLa nuclei does not apply to Xenopus nuclei (Gurdon, 1968, and unpublished observations).

Since nuclear enlargement appears to be associated with increasing genetic activity (Gurdon et al. 1976a), the agent which causes enlargement is of considerable interest. There is a strong indication from the morphological behaviour of nuclei in GV-dispersed oocytes that the agent responsible for nuclear swelling is concentrated in the oocyte’s GV, but is present to a lesser extent or in a different form in oocyte cytoplasm. Presumably this agent is released into the cytoplasm when oocytes undergo maturation into eggs, and the GV breaks down. It may then be responsible for the immensely rapid swelling of nuclei which enter the cytoplasm of eggs by fertilization or nuclear transplantation (Graham, 1966; Graham et al. 1966).

The eventual aim of this work has been partly fulfilled in the experiments reported here. A method has been developed by which large numbers of nuclei can be conveniently introduced into living oocytes in such a way that they remain morphologically healthy for many days, and in some cases for up to one month.
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