Changes in somatic cell nuclei inserted into growing and maturing amphibian oocytes

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When somatic cell nuclei are injected into enucleated unfertilized eggs they very soon cease RNA synthesis (Gurdon & Brown, 1965; Gurdon, 1967a), and commence DNA synthesis (Graham, Arms & Gurdon, 1966). By means of such experiments it has been shown (i) that components of living egg cytoplasm can substantially change the nature of nuclear activity, (ii) that nuclear activity is altered in such a way as to conform to that of the host egg cell, and (iii) that these effects are obtained with the nuclei of adult as well as embryonic cells. The experiments to be reported here were designed to find out whether the same generalizations apply to nuclei inserted into cells other than eggs, and whether they are true of changes in nuclear activity other than the repression of RNA synthesis and the induction of DNA synthesis. For this purpose growing and maturing amphibian oocytes have been used. Growing oocytes are characterized by the very active synthesis of RNA (Brown & Littna, 1964), and by the absence of nuclear DNA synthesis whether [3H]thymidine is supplied intraperitoneally (Izawa, Allfrey & Mirsky, 1963a), or is injected directly into living oocytes (Gurdon, 1967b). Maturing oocytes are in the process of completing meiosis and contain condensed chromosomes inactive in both RNA and DNA synthesis. If cytoplasmic components are of general importance in controlling nuclear activity, it would be predicted that nuclei inactive in RNA synthesis, or unaccustomed to division, should display these activities following insertion, respectively, into growing or maturing oocytes. The results reported here show that this expectation is realized, and permit some general conclusions to be drawn on the widespread importance of the cytoplasmic control of nuclear activity during cell differentiation.

MATERIALS AND METHODS

All experiments were carried out on Xenopus laevis. Developmental stages of embryos are described by the stage numbers of Nieuwkoop & Faber (1956). The results reported were obtained on oocytes taken at all seasons of the year. Each main result is based on the use of at least fifty oocytes taken from at least four different frogs.
Selection of oocytes. Oocytes were taken from females within 2 h of decapitation and, in most experiments, from females which had been induced to ovulate within the last 4 days by administration of a hormone (Pregnyl, Organon Laboratories) having the effect of a pituitary LH. This was done to ensure that oocytes were synthetically active at the time of injection, though it was later found that previous hormone stimulation was not necessary to achieve this in respect of RNA synthesis.

Oocytes described as ‘growing’ consisted of those attached to the ovary, that is surrounded by follicle cells, but not those with a pigment-free area at the animal pole. The oocytes used ranged from those of half the maximum diameter to those of maximum size. Oocytes described as ‘completing meiosis’ or ‘maturing’ were present in the ovary and surrounded by follicle cells, but had a white pigment-free area at the animal pole (fig. 1C, H, of Gurdon, 1967b). Dissection of over fifty oocytes has shown that this pigment-free area is consistently related to the absence of an intact germinal vesicle. Oocytes in this state were obtained from females which had received a hormone injection about 8 h previously. Ovarian oocytes with the pigment-free area at the animal pole have just undergone rupture of the germinal vesicle and their chromosomes, which are condensed, are proceeding towards or have reached the first meiotic metaphase (Humphries, 1956; Watson & Callan, 1963, for newts; J. Brachet, personal communication, for Xenopus). Soon after this such oocytes are ovulated.

Injection and culture of oocytes. Just before injection, small pieces of ovarian tissue containing 4 to 6 full-sized oocytes and several smaller ones were removed from the sacrificed female and placed on a dry microscope slide. Each oocyte was then injected with nuclei or label, and the whole piece of ovary placed in the culture medium. Owing to their covering of follicle cells, oocytes do not become desiccated during the few minutes required for injection. The culture medium used was a modified Barth’s medium (Barth & Barth, 1959) of the following composition: 88·0 mM-NaCl, 1·0 mM-KCl, 0·33 mM-Ca(NO₃)₂, 0·41 mM-CaCl₂, 0·82 mM-MgSO₄, 2·4 mM-NaHCO₃, 10 mg/l. of penicillin and streptomycin, and 2·0 mM Tris-HCl bringing the pH of the whole medium to 7·6. Injected Xenopus oocytes remain synthetically active for up to 3 days at 19 °C in this medium, as judged by the normal pattern of incorporation of [³H]uridine into nuclei and nucleoli of oocytes already cultured for 2 days. A complex medium which supports the growth of Xenopus cells in culture (Balls & Ruben, 1966)—that is, Leibovitz L-15 (Flow Laboratories, Irvine, Scotland), 35 mM Tris-HCl, pH 7·4, and foetal bovine serum albumin, mixed in the ratio 5:4:1 respectively, and including antibiotics—was found to be slightly less satisfactory for injected oocytes than the modified Barth’s medium (R. A. Laskey, unpublished).

Enucleation of oocytes. Oocytes were enucleated by making a small slit at the animal pole and squeezing out the germinal vesicle when it appeared in the
Nuclear transplantation into oocytes

Preparation of nuclei for injection. Adult brain nuclei were injected as an impure suspension of isolated nuclei prepared as described by Graham et al. (1966) in 0.25 M sucrose, 2 mM-MgCl₂, and 0.01 mg/ml each of penicillin and streptomycin. Blastula and gastrula nuclei were obtained by cutting open an embryo and inserting a micropipette into ectoderm tissue. The tissue sucked into the pipette contains free nuclei surrounded by cytoplasm which is very little diluted by the modified Barth's saline medium in which the opened embryo lies.

Labelling of oocytes and autoradiography. Label was introduced either by mixing with nuclei immediately before injection or by a second injection at any desired time after the first containing nuclei. [³H]nucleosides at 20–30 c/mm (Radiochemical Centre, Amersham) were made up in modified Barth's medium. About 30 μl of fluid and 5 μM of nucleoside were injected into each oocyte.

Oocytes were fixed in Perenyi's fluid for 12–24 h, washed out in 70% alcohol, embedded in paraplast wax (Shandon, London), and sectioned at 7 μ. Sections were stained in Mayer's haemalum and light green. Following examination, slides were covered with Ilford K2 dipping emulsion diluted with an equal volume of water. Slides were usually exposed to emulsion for 2 weeks.

RESULTS

Nuclei injected into growing oocytes

Identification of injected nuclei

In most experiments oocytes were not enucleated, since the resident oocyte nucleus (germinal vesicle) served conveniently as a reference with which to compare the behaviour of the injected nuclei. The identity of injected nuclei was established in preliminary experiments by the use of blastula nuclei whose DNA had been previously labelled for several hours with [³H]thymidine. Subsequent autoradiography (Plate 2A) showed that the injected nuclei could be reliably distinguished from the resident oocyte nucleus by the following morphological characteristics. Their relatively small size and the absence of multiple large nucleoli (Plate 1A–E) distinguishes them from the germinal vesicle or from peripheral lobes of the latter, which happen to have been sectioned tangentially (Plate 2C). Their distinct nuclear membrane distinguishes injected nuclei from spheres of yolk-free cytoplasm sometimes observed in injected oocytes after a few days culture in vitro (Plate 2B).

Morphological changes

Among the characteristics which distinguish the nucleus of an oocyte from that of other cell types are its enormous volume, its diffuse chromatin, and its possession of multiple large nucleoli. In order to determine whether oocyte cytoplasm can induce these characteristics in nuclei not normally possessing
them, mid blastula, late gastrula, and adult brain nuclei were injected into oocytes. Nuclei from all these sources were found to undergo a very pronounced enlargement in oocyte cytoplasm. Although a sample of nuclei from any one source (e.g. blastula or adult brain) differ considerably in the volume they reach in a single oocyte, the curves for volume increase with time are characteristic for the state of differentiation of the cell from which the nuclei are taken. As cells differentiate there is a steep decline in the rate at which their nuclei swell after injection into an oocyte (Text-fig. 1a). This difference is not accounted for by a delay in the commencement of swelling of nuclei from differentiated cells, since the curves for volume increase in nuclei of different cell-types can be exactly superimposed after an appropriate alteration of the scale (Text-fig. 1b). This finding shows that at any given time after injection blastula nuclei are swelling at about twice the rate of gastrula nuclei, and at about 20 times the rate of adult brain nuclei. The most highly swollen blastula nuclei sometimes reach a volume of $110000 \mu^3$ after 3 days residence in a cultured oocyte (Plate 1A–E). While this constitutes a 250-fold increase in volume, enlarged blastula nuclei are still much smaller than the oocyte nucleus. In the same time adult brain nuclei may reach a volume of $4000 \mu^3$ (Plate 1H–K), which constitutes a volume increase of about 40 times. Since the condition of injected oocytes deteriorates after 2–3 days in culture, it is not known whether the enlargement

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**PLATE 1**

Sections of oocytes containing injected somatic cell nuclei, stained with Mayer’s haemalum and light green.

_A–G and M. Nuclei from stage 7/8 mid-late blastulae_

A. Unenlarged nuclei 2 h after injection; no nucleoli.
B. Twenty-four hours after injection; enlarged nucleoli.
C and D. Forty-eight h after injection, showing different degrees of chromatin dispersion.
E. Forty-eight hours after injection; a much enlarged nucleus with multiple small granules.
F. Whole blastula cell 24 h after injection into an oocyte; no nuclear enlargement has taken place. Compare with fig. b.
G. Two blastula nuclei 5 h after injection into the germinal vesicle of an oocyte; nucleoli are enlarged. Yolk granules sometimes enter an injected germinal vesicle.
M. Part of an enlarged blastula nucleus 48 h after injection; three nucleolus-like inclusions can be seen in this section.

_H–K. Nuclei from adult frog brain_

H. Unenlarged nuclei 3 h after injection.
I. Thirty-six hours after injection; nuclei somewhat enlarged and chromatin partly dispersed; nucleoli not yet apparent.
J, K. Forty-eight hours after injection; enlarged nuclei with dispersed chromatin and very large nucleoli.
L. Much enlarged nucleus from brain of recently metamorphosed frog; oocyte fixed 72 h after injection.
of injected nuclei would continue beyond this time, though there is at present no reason to believe that it would not.

It seemed possible that the 20-fold slower enlargement of brain nuclei compared to blastula nuclei could be related to the fact that embryonic nuclei are surrounded by cytoplasm during preparation, a form of protection not afforded to adult nuclei prepared in a different way (see Methods). This possibility was tested by preparing blastula nuclei in exactly the same way as was used for adult brain nuclei. Isolated blastula nuclei of this kind enlarged at about the same rate as blastula nuclei prepared by the usual means, and very much faster than brain nuclei. The rate of nuclear enlargement is not therefore related to the method of nuclear preparation but to some property of the injected nuclei.

An oocyte nucleus differs from a blastula or brain nucleus not only in size, but also by its possession of disperse chromatin and by its capacity to stain strongly with an acidic dye like light green. As injected blastula nuclei swell they come to stain strongly with light green, in marked contrast to nuclei of the same kind before and immediately after injection into oocytes. The chromatin becomes dispersed throughout the enlarging nuclei as can be seen by the even distribution of grains over swollen nuclei whose DNA was labelled with \(^{3}H\)thymidine before injection (Plate 2 A). The appearance of diffuse chromatin and acidophilic nucleoplasm coincides with the increase in nuclear volume, and both events take place more slowly in brain nuclei than in blastula nuclei (Plate 1, A–E, H–L).

**Plate 2**

A. Autoradiograph of two blastula nuclei 48 h after injection into an oocyte; the DNA of the nuclei was labelled with \(^{3}H\)thymidine before injection. The swollen nucleus is labelled less heavily than the unswollen one. Magnification of A–F same as B.

B. Localized region of yolk-free cytoplasm sometimes seen in injected oocytes after culture *in vitro* for several days.

C. Sections through lobes of an oocyte germinal vesicle containing large nucleoli.

D. Autoradiograph of two blastula nuclei 24 h after injection into an oocyte; \(^{3}H\)uridine was injected at the same time as the nuclei. The swollen nucleus is more heavily labelled per area than the unswollen one, in contrast to fig. A.

E. Autoradiograph of two adult brain nuclei, injected into an oocyte at the same time as \(^{3}H\)uridine, and fixed 48 h after injection.

F. Autoradiograph of germinal vesicle nucleoli and nucleoplasm from the same oocyte as received the blastula nuclei shown in fig. D. The nucleoli are more heavily labelled and the nucleoplasm less heavily than the nuclei injected into the same oocyte.

G–J. Sections of maturing oocytes (ruptured germinal vesicle) 1½ h after injection of adult brain nuclei.

G. Low-power view of asters with condensed chromosomes (chr) apparently in metaphase on the equator of a spindle.

H–J. Condensed chromosomes at metaphase or anaphase, associated with multipolar spindles and asters.
In *Xenopus* an oocyte nucleus possesses about 1500 very large nucleoli (Perkowska, Macgregor & Birnstiel, 1968), while blastula nuclei do not possess true nucleoli (Hay & Gurdon, 1967), and adult brain nuclei have only one or two nucleoli, neither of which exceeds 1 μ in diameter. Blastula and brain nuclei which have swollen after 2–3 days residence in an oocyte sometimes contain more than two but never more than ten large inclusions resembling nucleoli. Thus after incubation for 3 days injected nuclei clearly do not acquire the many hundreds or thousands of nucleoli typical of oocyte nuclei. This is not unexpected since the nuclei of the rather large host oocytes used do not themselves acquire more nucleoli at this stage of growth (Perkowska *et al.* 1968). During nuclear swelling the size of each nucleolus increases enormously, and nucleoli frequently reach 3 μ in diameter in enlarged blastula and brain nuclei (Plate 1 J, K, M). In addition to large nucleoli, oocyte nuclei contain several hundred small granules in the vicinity of their chromosomes; these granules are not normally observed in the nuclei of blastula or brain cells but large numbers of them are frequently observed in enlarged blastula nuclei (Plate 1 E).

The morphological changes observed in nuclei injected into an oocyte are

![Graph](image_url)
Nuclear transplantation into oocytes

known to be due to some property of cytoplasm and not to some influence of the nucleus resident in the host cell. This has been proved by injecting nuclei into manually enucleated oocytes (see Methods). Under these conditions blastula and brain nuclei increase their volume in the usual way. The same conclusion can be drawn from experiments in which whole cells are injected into oocytes. A blastula cell of this kind remains intact inside an oocyte indefinitely and its nucleus shows no response to the new cytoplasmic environment of the cell (Plate IF); the intact cell membrane has apparently prevented any interaction between the injected nucleus and the oocyte cytoplasm, a contact which is therefore essential for a change in nuclear activity. Though some donor cell cytoplasm is always introduced to oocytes at the same time as nuclei, it is unlikely to have any effect on nuclear behaviour, since, as mentioned above, the same response is observed in blastula nuclei prepared in the usual way with much associated cytoplasm and in blastula nuclei isolated from most of their cytoplasm by centrifugation through sucrose. Thus the response of nuclei to oocyte cytoplasm is independent of the purity of the nuclear preparation, but is entirely dependent on the rupture of the donor cell membrane; the changes that take place in injected nuclei must therefore be caused by oocyte cytoplasm.

It has been possible to show that the changes in nuclear activity observed are not a pathological response to the cytoplasm of oocytes cultured in vitro. This was done by injecting blastula nuclei into oocytes as usual, and then inserting the oocytes in the abdominal cavity of a living male frog. When the oocytes were withdrawn two days later, the injected blastula nuclei had undergone the same enlargement and chromatin dispersion as they do in oocytes cultured in vitro.

It sometimes happens that nuclei are deposited inside the oocyte nucleus rather than in the oocyte cytoplasm. In such cases brain nuclei show a rapid enlargement of their nucleoli (Plate 1G) and within a few hours their nuclear membrane disappears and they fuse with the contents of the germinal vesicle.

Changes in nucleic acid synthesis

Amphibian oocytes are characterized by very active chromosomal RNA synthesis (Gall & Callan, 1962). The general features of nucleic acid synthesis in injected nuclei have been investigated by determining autoradiographically the extent to which such nuclei incorporate $[^3H]$nucleosides into RNA or DNA. $[^3H]$nucleosides were introduced into oocytes by injection at the same time as, or at any desired time after, the injection of nuclei. This procedure would not provide a valid test of nucleic acid synthesis unless it could be shown that some of the injected label is converted quite rapidly into nucleoside triphosphates. H. R. Woodland (unpublished) has shown that about 50% of the usual amount of $[^3H]$uridine injected into oocytes is phosphorylated into nucleoside triphosphates within 1 h. This is also true of $[^3H]$thymidine so long as oocytes are taken from frogs which have recently been induced to spawn (H. R. Woodland, unpublished). $[^3H]$uridine has been used as a specific label for RNA synthesis,
since autoradiography and biochemical analysis have shown that it is rapidly incorporated into RNA by medium and large Xenopus oocytes (Izawa, Allfrey & Mirsky, 1963b; Davidson & Mirsky, 1965).

Autoradiographic experiments do not permit an estimate of the specific activity of the nucleic acid precursor pool and therefore of the actual amount of nucleic acid synthesized. The incorporation of [3H]uridine into RNA by injected nuclei has therefore been compared to the level of incorporation by the germinal vesicle of the same oocyte. Since both kinds of nuclei are in the cytoplasm of the same cell, it is assumed that they draw on the same precursor pool. In some experiments [3H]uridine was inserted at the same time as nuclei, and RNA synthesis was observed for the whole of a 2- to 3-day period. In other experiments the label was introduced by a second injection at some time after the injection of nuclei, and RNA synthesis by the two kinds of nuclei was compared over different periods of the 2–3 days for which the cultured recipient oocytes remained synthetically active.

The principal result of these experiments is that RNA synthesis can be observed in all nuclei which have enlarged after injection into oocytes. This constitutes a change in synthetic activity for mid-blastula nuclei, which pass through frequent mitotic divisions and periods of DNA synthesis but which synthesize little if any RNA (Gurdon & Woodland, 1969). Blastula nuclei injected into oocytes at the same time as [3H]thymidine are observed to continue DNA synthesis for the first 3 h after injection (J. B. Gurdon & K. Arms, unpublished), though they never undergo division in growing oocytes. After this, DNA synthesis ceases and does not start again while the blastula nuclei remain in the oocyte. By injecting [3H]uridine at various times, it has been found that injected nuclei continue to synthesize RNA for as long as the oocyte nucleus itself remains synthetically active, that is, for up to 3 days. Oocyte cytoplasm has therefore suppressed the sequential division and DNA synthesis typical of mid-late blastula nuclei, and has induced them to enter a period of continuous RNA synthesis never normally observed in nuclei at this stage of development.

It is not certain whether brain nuclei alter their pattern of RNA synthesis after injection into oocytes, but it seems likely that the rate of RNA synthesis may be increased. Brain nuclei do not show autoradiographically detectable RNA synthesis at any time during the first 24 h after injection into oocytes. This is also true when the same concentration of label and the same autoradiographic conditions are applied to brain nuclei in living adult frogs, to excised brain tissue, or to isolated brain nuclei incubated in vitro. However, when tested more than 24 h after injection into oocytes, many brain nuclei are swollen, and these show appreciable RNA synthesis (Plate 2E). The nucleoli of enlarged brain nuclei are more heavily labelled than the nucleoplasm, and this suggests that they are very active in the synthesis of ribosomal RNA, as also are the nucleoli of the oocyte germinal vesicle (Edström & Gall, 1963; Davidson & Mirsky, 1965).

Further information on the amount of RNA synthesized by nuclei injected
Nuclear transplantation into oocytes has been obtained by counting grains in autoradiographs after [3H]uridine labelling. As before, the resident oocyte nucleus was used as a reference by which to judge the activity of the injected nuclei. It provides a measure of the amount of label that has remained in a given oocyte (much can leak out), as well as an indication of the viability of the oocyte. Except for the first few hours after injection, blastula nuclei were always more heavily labelled.

Text-fig. 2. Progressive increase in rate of RNA synthesis with increase in nuclear volume. The rate of RNA synthesis by each nucleus was calculated as follows from autoradiographs of sectioned oocytes. The number of grains per unit area of injected nucleus was divided by the number of grains over the same area of germinal vesicle nucleoplasm (excluding nucleoli) in the same oocyte. This value was then multiplied by the volume of the injected nucleus in μ3 to give the figures recorded on the ordinate. This procedure corrects for variations in the amount of label injected and in the synthetic activity of different oocytes. If nuclei had synthesized RNA at a constant rate irrespective of volume the points should have been distributed along the horizontal (dashed) line. ○, Values for different nuclei in one oocyte which received an injection of [3H]uridine 24 h after injection of blastula nuclei; this oocyte was fixed 8 h later. ●, Values from different oocytes which were labelled with [3H]uridine from 5–8, 12–24, or 33–46 h after injection of blastula nuclei.

per area than the oocyte nucleoplasm, but less so than the oocyte nucleoli (cf. Plate 2D, F). To determine whether a relationship exists between the degree of enlargement of an injected nucleus and its rate of RNA synthesis, grains were counted over injected blastula nuclei which had undergone various degrees of enlargement. For this purpose nuclei were scored which had reached different sizes in the same oocyte as well as nuclei which had been cultured for short or long periods in different oocytes. The results (Text-fig. 2) clearly show that the more swollen nuclei are very much more active in RNA synthesis than the less
swollen nuclei. The paucity of grains over unenlarged nuclei is observed not only in unswollen nuclei a few hours after injection, but also in any nuclei which have remained little if at all swollen after 2–3 days in an oocyte. The magnitude of the enhanced RNA synthesis associated with swelling can often be appreciated directly in an oocyte which contains several nuclei swollen to a varying extent. In such cases it is frequently observed that the density of grains per area is greater over swollen nuclei than over much smaller nuclei in the same egg (Plate 2D). The validity of these results is satisfactorily confirmed by the observation that blastula nuclei whose DNA has been labelled with $[^3H]$thymidine before injection into oocytes display a progressive dilution of grains with increasing size (cf. Plate 2A).

The possibility that the labelled RNA present in injected blastula nuclei was not synthesized there but migrated there from the oocyte germinal vesicle or from the follicle cell nuclei is considered very improbable for two reasons: first, blastula nuclei acquire labelled RNA when injected into enucleated oocytes, and, secondly, they do so much more quickly than the follicle cell nuclei.

### Nuclei injected into maturing oocytes

If the entry of a nucleus into division is under cytoplasmic control, as other kinds of nuclear activity appear to be, nuclei from non-dividing cells would be expected to enter division after injection into maturing oocytes. To test this possibility isolated brain nuclei were used, since brain nuclei enter division very rarely and much fewer than 0.1% of the nuclei in adult frog brain are in mitosis at any time. A suspension of isolated brain nuclei was injected into ovarian oocytes with ruptured germinal vesicles; such oocytes were recognized and obtained as described under Methods. Each oocyte received 20–40 nuclei and was fixed for sectioning 1 h later. The results showed that the animal hemisphere of injected oocytes contained no intact nuclei, but instead had many spindles and asters to which were attached highly condensed chromosomes (Plate 2G–J). These must have been derived from the injected brain nuclei, since the many hundred chromosomes seen could not all have been derived from the oocyte nucleus, and sucrose-injected oocytes which served as controls did not contain large numbers of spindles and chromosomes. The spindles were often multipolar and perhaps for this reason the chromosomes were not evenly distributed. A few nuclei located at the vegetal pole of the egg remained in interphase; this part of the egg consists primarily of large yolk platelets and appears to be little if at all diluted by the contents of the newly ruptured germinal vesicle.

As might be expected of eggs containing so many asters and spindles, normal cleavage was not observed. Maturing oocytes fixed between 1½ and 3 h after the injection of nuclei all contained chromosomes in metaphase or anaphase and it is doubtful whether interphase nuclei are reconstituted in eggs containing large numbers of irregularly distributed chromosomes. It is not possible to say from these results whether the condensed chromosomes had entered meiosis or
mitosis. However, the main result of these experiments is clear: nuclei not normally accustomed to enter division can be rapidly induced to do so by insertion into oocytes undergoing nuclear maturation divisions. The rapidity of this response to oocyte cytoplasm is impressive since adult brain nuclei enter mitosis extremely rarely, even when cultured in conditions which lead to extensive outgrowth and mitosis by other cell types (Murray & Stout, 1947; Geiger, 1958).

These results can be compared with those obtained on sea urchins by Brachet (1922). He found that it is possible to fertilize oocytes which have not completed maturation but which are released at certain times of the year by Paracentrotus lividus. The resident nucleus of these oocytes was in metaphase of second meiosis, and the many sperm which entered became rapidly converted into condensed chromosomes attached to multipolar spindles, a situation very reminiscent of that just described for adult brain nuclei in maturing Xenopus oocytes. It is very relevant to the interpretation of the results with Xenopus brain nuclei that the fertilized sea-urchin oocytes contained chromosomes irregularly arranged on spindles and did not develop further. The lack of further development supported by Xenopus brain nuclei must therefore be attributed either to the immaturity of the recipient oocytes or to the presence of many nuclei in one cell, and not to some specialized state of adult brain nuclei.

DISCUSSION

The behaviour of somatic cell nuclei in oocytes has been observed in these experiments for no longer than 3 days, since injected oocytes do not appear to remain synthetically normal if cultured for longer than this period. The main reason for believing the behaviour of nuclei injected into oocytes to be biologically normal is that they are synthetically active in a manner which closely resembles that of the host cell nucleus.

The main outcome of these experiments is the demonstration that the nuclei of embryonic as well as differentiated cells quickly assume, in all respects investigated, the type of activity characteristic of growing and maturing oocytes into whose cytoplasm they have been inserted. This is what was previously found when nuclei undergo changes in activity following injection into unfertilized eggs (Gurdon & Brown, 1965; Graham et al. 1966).

Three general conclusions can be drawn from these experiments. First, they establish the widespread occurrence of cytoplasmic regulatory factors. The conditions which lead to the initiation of each main kind of nuclear activity are not therefore exclusively extracellular or intranuclear. Secondly, the nuclei of differentiated cells respond to oocyte cytoplasm in the same way, qualitatively, as do the nuclei of undifferentiated blastula cells. It is interesting, however, that the rate at which the change of function takes place differs consistently according to the state of differentiation of the cell from which nuclei are taken. This finding
appears to be most simply interpreted by supposing that as cells differentiate the DNA of their chromosomes becomes increasingly firmly, though by no means irreversibly, complexed with proteins or other molecules which prevent rapid changes of nuclear activity.

The third general conclusion to be drawn from these experiments concerns the nature of the process by which cytoplasmically induced changes in nuclear function take place. Attention has been drawn previously to an association that exists between nuclear swelling and changes in nucleic acid synthesis observed in nuclei transplanted to eggs (Gurdon, 1964; Graham et al. 1966). A similar relationship has been observed in the experiments reported here and in the case of blood cells fused with HeLa cells (Harris, 1967). Opposite changes of function are induced in the same nuclei according to whether they are injected into eggs or oocytes, and each kind of changed function is preceded by a pronounced swelling and dispersion of chromatin. This adds strong support to the view that nuclear swelling does not itself induce any one kind of nuclear change, but should rather be regarded as a process of derepression, the result of which is to make chromosomes more reactive to the particular cytoplasmic environment in which they happen to lie (Gurdon & Woodland, 1968). In support of this view is the fact that the structure and staining properties of chromatin are quite different according to whether swollen brain nuclei are synthesizing RNA in oocytes or DNA in eggs.

**SUMMARY**

1. Nuclei from early embryonic and adult brain tissue have been injected into growing oocytes and into oocytes undergoing maturation, in the frog *Xenopus laevis*. The injected oocytes were cultured *in vitro* for up to 3 days. During this period nuclei underwent pronounced changes in structure, as well as in synthetic activity judged by autoradiography.

2. During the 3 days for which injected nuclei remained inside cultured oocytes, they showed a progressive increase in nuclear and nucleolar volume, and in the degree of dispersion of their chromatin. Blastula nuclei may enlarge 250 times and adult brain nuclei 40 times. The rate at which injected nuclei swell is inversely related to the state of differentiation of the cells from which they are taken.

3. Soon after injection into oocytes, blastula nuclei cease DNA synthesis, and start to synthesize RNA at an increasing rate. Injected brain nuclei appear to increase their rate of RNA synthesis. Using the level of RNA synthesis supported by the nucleus of each recipient oocyte as a reference, a close relationship was found to exist between increasing nuclear volume and increasing rate of RNA synthesis.

4. Adult brain nuclei which have been injected into oocytes undergoing nuclear maturation divisions lose their nuclear membrane and are rapidly replaced by condensed chromosomes arranged on multipolar spindles.
5. The results show that in all respects examined, nuclei rapidly assume the activity characteristic of the host cell into which they have been inserted. Swelling does not itself cause a change in nuclear function, but appears to make the chromosomes more responsive to regulatory molecules or conditions present in the host cell cytoplasm.

ZUSAMMENFASSUNG

Veränderungen in Kernen von somatischen Zellen, die in wachsende und reifende Oocyten von Amphibien eingesetzt wurden


4. Injiziert man Kerne aus Gehirnzellen in Oocyten, die gerade die Reifeteilungen durchlaufen, so wird die Kernmembran unsichtbar, und man findet sehr bald kondensierte Chromosomen in multipolaren Spindeln.

5. Alle Ergebnisse zeigen in guter Übereinstimmung, daß transplantierte Zellkerne sehr schnell die Aktivitätseigenschaften der Zellen annehmen, in die sie überführt worden sind. Die Vergrößerung des Volumens selbst zeigt nicht unbedingt eine Veränderung in der Kernfunktion an; jedoch sind die Chromosomen unter diesen Bedingungen sensibler für Regulationseinflüsse.

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