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Injected nuclei in frog oocytes: RNA synthesis and protein exchange

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SUMMARY

Nuclei from HeLa and other mammalian cells have been injected into *Xenopus* oocytes. The synthesis, uptake, and release of RNA and proteins by injected nuclei have been investigated by autoradiography.

Injected nuclei which undergo enlargement synthesize RNA continuously for up to 28 days. When oocytes are incubated in [^{3}H]uridine or [^{3}H]guanosine, injected nuclei are labelled nearly as strongly as the nucleoli, but much more strongly than the nucleoplasm of the oocyte's germinal vesicle.

Injected nuclei appear to increase their rate of RNA synthesis during incubation in oocytes. This apparent increase in the rate of RNA synthesis is correlated with nuclear enlargement, as well as with the loss of protein from injected nuclei and with their uptake of histone and non-histone proteins from oocyte cytoplasm. Injected HeLa nuclei lose most of the previously synthesized RNA from their nucleoplasm, but little if any of the RNA from their remaining nucleoli.

INTRODUCTION

The eventual aim of injecting nuclei into frog oocytes is to obtain an experimental system in which genes coding for identified products continue to be transcriptionally active for a long time. As explained before (Gurdon, De Robertis & Partington, 1976; Gurdon, 1976), a system of this kind might help the identification of molecules which regulate gene activity.

The accompanying paper (Gurdon, 1976) has established that mammalian nuclei, injected into oocytes, undergo a substantial enlargement which is accompanied by chromatin dispersion. In this paper we show that nuclei injected into oocytes synthesize RNA continuously for up to one month; as they enlarge, the rate of RNA synthesis appears to increase, and proteins are exchanged with the surrounding cytoplasm.

METHODS

The source and condition of female frogs used to provide oocytes as well as the preparation, storage, injection, incubation, fixation, sectioning and staining of oocytes containing nuclei have been described in the accompanying paper

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(Gurdon, 1976). Nuclei were prepared from a suspension of cells by the LL-BSA procedure already described (Gurdon, 1976).

Oocytes containing injected nuclei were usually labelled by incubating them in MBS-H medium (composition in Gurdon, 1976) to which [^{3}H]uridine (43 Ci/mmol), [^{3}H]guanosine (16 Ci/mmol) or [^{3}H]leucine (54 Ci/mmol) had been added to give a concentration of 1 mCi/ml. 2 μl of labelled medium were used per oocyte. The most favourable conditions for labelling oocytes have been investigated by Colman (1974). We have carried out further tests on oocytes and their enveloping follicle cells, and have established the following. Incubation at 25 °C gives about twice as much incorporation into RNA as incubation at 19 °C for the same time. Substantially more label ($(1\frac{1}{2}$-2 times) is incorporated into RNA during the second than during the first day after isolation, a finding most readily explained by presuming a reduction in the pool size of RNA precursors. After testing [^{3}H]nucleosides individually and in combination, we conclude that U + C or U + G give nearly as high incorporation as all four nucleosides together (A + C + U + G)[1]. Under the most favourable conditions specified above, one oocyte and its associated follicle cells can incorporate nearly 100000 d.p.m. into RNA in 24 h; only 5 % of this RNA is usually in the oocyte itself, the rest being in its follicle cells. The amount of [^{3}H]U and [^{3}H]G incorporated into RNA by oocytes and their follicle cells in 24 h at 25 °C is usually about 5 % of the total labelled nucleoside taken up. Oocytes were labelled with [^{3}H]GTP by injecting about 30 nl at 10 mCi/ml.

Enucleation of oocytes was carried out as described by Ford & Gurdon (1977). The effect of α-amanitin was tested by injecting it, in 35 nl, sometimes with nuclei, at 300 μg/ml. This dose should give an intracellular concentration of 10–20 μg/ml in oocytes, previously found by Tochini-Valentini & Crippa (1970), Roeder (1974), and Colman (1975) to inhibit non-ribosomal RNA synthesis in oocytes.

Cells of human HeLa (strain G) and mouse myeloma (MOPC 21) were grown in spinner cultures. HeLa cells required to be labelled were grown in plastic bottles. African green monkey cells (MA-134 and CV-1) were grown in plastic bottles. [^{3}H]thymidine or [^{3}H]uridine were added to the normal culture medium to give a concentration in the medium of 2 or 20 μCi/ml respectively. To label HeLa cells in bottles with [^{3}H]leucine, the standard medium was replaced by minimum essential medium minus leucine, but supplemented with 20 μCi/ml [^{3}H]leucine, 1/20th volume of 10 % foetal calf serum, and 1/20th volume of complete Dulbecco's medium. Some HeLa cell cultures were infected with adenovirus type 5.

Details of the preparation of adenovirus type 5 and of the infection of HeLa cells with adenovirus were carried out according to standard practice. In brief, confluent monolayers of HeLa cells were infected at a multiplicity of 100 p.f.u.

[1] *Abbreviations:* A, C, G, U, adenosine, cytidine, guanosine, uridine; TdR, thymidine; GTP, guanosine triphosphate; GV, germinal vesicle (= nucleus) of an oocyte.

per cell for 1 h at 37 °C, using virus which had been purified by two cycles of equilibrium density banding in CsCl. The beginning of the infection was timed from the addition of virus. Infected cells were cultured at 37 °C in Dulbecco modified Eagle's medium containing 5 % foetal calf serum. The synthesis of 'late proteins' was clearly seen 12 h after infection.

Histones prepared from calf thymus by the method of Van der Westhuyzen & Von Holt (1971) were iodinated by the chloramine T method of Greenwood, Hunter & Glover (1963).

To test RNase sensitivity, oocytes were frozen by immersion in 2-methylbutane (isopentane) previously cooled to about −100 °C in liquid nitrogen. They were then transferred after 1 min to 5 ml methanol at −70 °C, in which they were left for 3 days or longer at −70 °C before being transferred in the same bottle of methanol to −20 °C (3 h), 0° C (3 h), and 18 °C (3 h); they were then changed into xylene before wax embedding. Slides carrying sections of the freeze-substituted material were immersed in a solution containing RNase A at 1 mg/ml, RNase T_1 at 4 μg/ml, in 0·1 M-KH_2PO_4/K_2HPO_4 buffer pH 7·3. Incubation was carried out under a coverslip for 6 h at 37 °C; slides were rinsed in 10 % trichloroacetic acid for 1 h at 4 °C and finally washed for 1 h. They were then treated like other slides for autoradiography by standard procedures, using Ilford (Essex) K2 dipping emulsion.

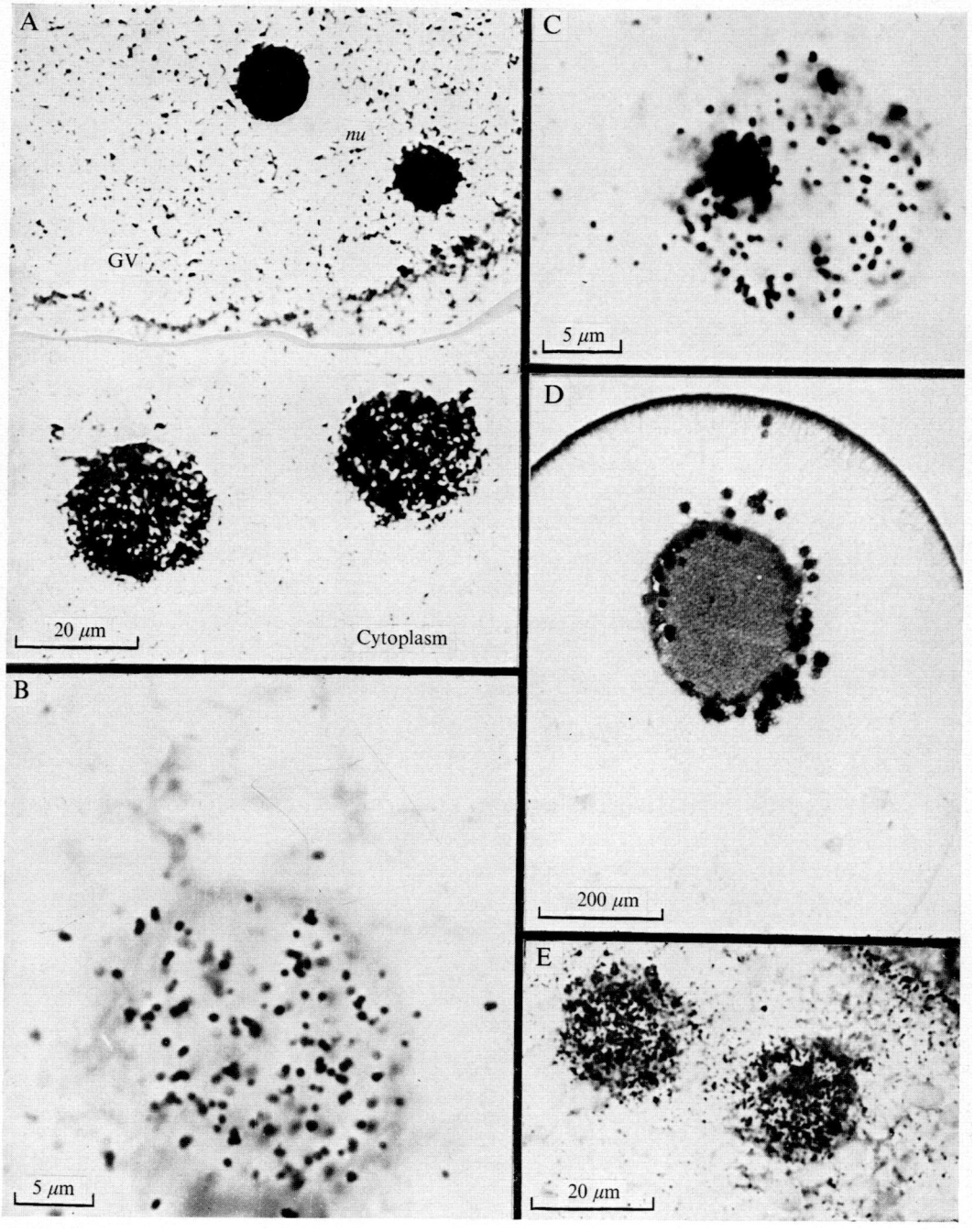
Reference is made in the text and in Fig. 2 to the total number of autoradiographic grains per nucleus. This value is intended to represent the total RNA synthesizing activity per nucleus, and was calculated as follows. The volume of a nucleus (calculated from its maximum diameter, assuming it to be a sphere) was multiplied by the number of grains per μm^2 of its area (taken from Table 1). The grain count per μm^2 is considered to be equal to the same value per μm^3, because the [^{3}H]path length is assumed to be 1 μm.

RESULTS

RNA synthesis

When oocytes containing HeLa nuclei are incubated in [^{3}H]U or [^{3}H]G, or are injected with [^{3}H]GTP, the injected nuclei are always labelled well above the level of the surrounding cytoplasm (Fig. 1A, B, and Table 1). Similar labelling has been seen in the nuclei of human HeLa cells, African green monkey cells, mouse myeloma cells, and even in the nuclei of HeLa cells infected 12 h previously with adenovirus (see Methods); labelling would presumably be observed with all kinds of vertebrate nuclei. The labelling obtained with the isotopes mentioned above is almost entirely eliminated if sections are treated with RNase before autoradiography (Table 1). Labelling of injected nuclei is also largely eliminated if α-amanitin is injected with the nuclei; the dose of α-amanitin used has been previously shown to inhibit non-ribosomal RNA synthesis in *Xenopus* oocytes (see Methods). We conclude that injected nuclei actively synthesize RNA in oocytes.

Injected nuclei which look morphologically healthy are always labelled with RNA precursors (Table 1). Even nuclei which had been in oocytes for 28 days after injection were labelled when subsequently incubated in [^{3}H]U + [^{3}H]G. The level of labelling of injected nuclei is always about the same as, or a little lower than, that of the GV nucleoli, but is very much higher than that of the



GV plasm or cytoplasm (Table 1). Nuclei which have become pycnotic or which appear as condensed chromatin are not labelled above the background level (Table 1).

The rate of RNA synthesis by injected nuclei appears to increase enormously with volume. The number of grains in a given area over sectioned nuclei decreases slightly with increasing nuclear diameter. But when the *total number* of autoradiographic grains per nucleus is related to nuclear volume (see Methods), it is clear that larger nuclei are enormously more heavily labelled than small nuclei in the same oocyte (Fig. 2). Within the limited accuracy of such measurements, the increase in nuclear labelling is nearly proportional to increasing nuclear volume. For a tenfold increase in volume, there is a $7\frac{1}{2}$ times increase in labelling (Fig. 2). The number of grains seen over a nucleus represents the labelled RNA present *in* the nucleus at the time of fixation and will depend on the rate of RNA synthesis, the rate of RNA breakdown or export from the nucleus, and on other factors. But if the amount of autoradiographic labelling is a true reflexion of the rate of RNA synthesis, we may conclude that the enlargement of injected nuclei is accompanied by a very great increase in their rate of gene transcription.

We have assumed so far that the labelled RNA seen in injected nuclei has been synthesized in them and not accumulated by them after synthesis in the oocyte's GV or follicle cells. This assumption is justified by two kinds of experiments. If

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FIGURE 1

(A) An autoradiograph of a [^{3}H]uridine-labelled oocyte with injected HeLa nuclei. The oocyte was injected with HeLa nuclei, incubated in unlabelled medium for 4 days at 25 °C, then incubated in [^{3}H]uridine-containing medium for 24 h before fixation. The autoradiograph was exposed for 3 weeks, and shows that the injected nuclei are much more strongly labelled than the cytoplasm and the germinal vesicle plasm (GV). The germinal vesicle nucleoli (*nu*) are very strongly labelled. The upper and lower halves of the figure (corresponding to GV and cytoplasm) were photographed independently in the same oocyte, because grains were not in the same focal plane in both oocyte sections.

(B) An autoradiograph of an injected HeLa nucleus, after labelling with [^{3}H]uridine as described under Fig. 1 A above. The autoradiograph was exposed for only 4 days. It shows even labelling over the nucleus, without any concentration of grains over the nucleolus which is out of focus in the middle of the nucleus.

(C) An autoradiograph of a HeLa nucleus prelabelled with [^{3}H]uridine. HeLa cells were incubated in medium containing [^{3}H]uridine for 6 h before nuclei were prepared and injected into an oocyte. The injected oocytes were fixed 3 days later. The nucleolus of the injected nucleus (left-hand side of nucleus in the figure) is intensely labelled, compared to the rest of the nucleoplasm.

(D) An autoradiograph of an oocyte with injected HeLa nuclei. After unlabelled nuclei were injected, the oocyte was incubated in unlabelled medium for 5 days, and then injected with [^{125}I]histones (for preparation, see Methods). 16 h later the oocyte was fixed, sectioned, and autoradiographed. The labelled histones have become highly concentrated in the GV and even more so in the injected nuclei.

(E) An enlarged view of an autoradiograph prepared as in D above.

Table 1. *RNA synthesis by nuclei injected into* Xenopus *oocytes*

Type of nuclei*	Special conditions†	Labelling (days after injection‡)	Diameter of nucleus (μm)	Grain counts per 100 μm²§ Injected nuclei	GV nucleoli	GV plasm	Cytoplasm
HeLa	—	3	25	220	190	68	33
	—	5	18	170	220	77	57
Monkey	—	9	20	130	190	21	4
HeLa	—	14	30	153	121	28	11
HeLa	—	22	29	102	145	42	9
HeLa	—	22	22	113	122	27	21
HeLa	—	28	38	135	200	42	32
		Average (%)		146 (86%)	170 (100%)	44 (26%)	24 (14%)
HeLa	GV dispersed	4	43	119	—	—	27
HeLa	12 h after Adeno-5 infection	4	20	220	160	38	13
HeLa	Damaged nuclei as condensed chromatin	7–28	10–15	17	140	32	17
Monkey and HeLa	RNase before autoradiography	9	22	2·0	1·4	1·6	0·4
HeLa	GV enucleated	4	17	48	—	—	9·1

* No difference was observed in the results obtained with the nuclei of human HeLa, monkey MA-134 or CV-1, and mouse myeloma cells.

† See Methods for details of treatment.

‡ Nuclei were injected into oocytes, which were cultured for the number of days stated in unlabelled medium; they were then transferred to medium containing [³H]G and/or [³H]U at 1 mCi/ml for 16–24 h at 19° C (except for 28 day labelling which was for 52 h) and immediately fixed.

§ Grains were counted over 25 μm² areas for nuclei, and over 100 μm² areas for GV plasm and cytoplasm. Each value is the average of at least 10 grain counts. Slide background counts of 1–2 grains per area have been subtracted.

injected nuclei acquire labelled RNA by transfer from elsewhere in the cell, they would be weakly labelled relative to the source of the RNA (GV or follicle cells) when fixed at short times after the start of labelling. Oocytes were injected with nuclei, incubated for 4 days, and fixed 1, 2, 4, 8 and 16 h after a further injection of [³H]GTP. Autoradiographs showed that even after a 1 h labelling period, the injected nuclei were as strongly labelled relative to the GV nucleoli, GV plasm, and follicle cells as at all later times. In the second experiment, oocytes were enucleated, injected with HeLa nuclei, incubated in unlabelled medium for 3 days, and then injected with [³H]GTP or incubated in [³H]U and [³H]G for 24 h. As seen in Table 1, the injected nuclei were labelled well above (five times) their cytoplasmic background level. To be successfully enucleated and subsequently healed, oocytes must first be defolliculated, and this reduces

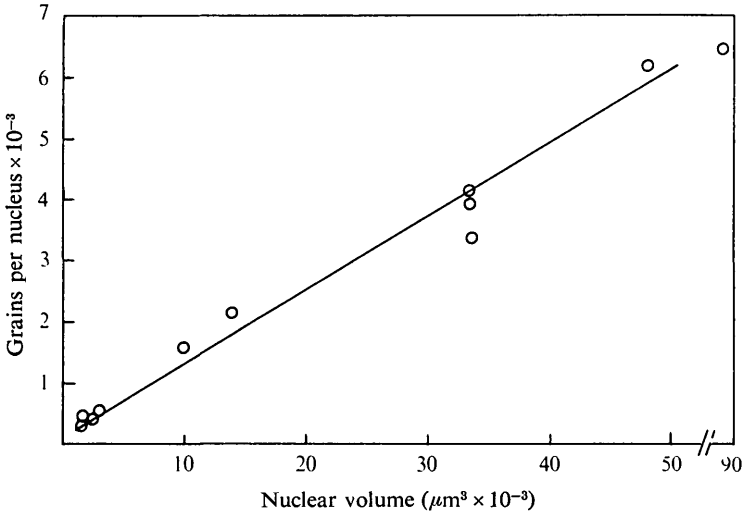

Fig. 2. The relationship of nuclear volume to rate of RNA synthesis as judged autoradiographically by [^{3}H]uridine incorporation into RNA. Nuclear volume was calculated from the diameter of large nuclei measured in sections. The grain number per nucleus was calculated as described in the Methods.

the rate of [^{3}H]U incorporation into RNA (but not the amount of [^{3}H]U phosphorylation). This effect probably accounts for the lower level of nuclear labelling in enucleated, compared to normal, oocytes. We conclude from these tests that the labelled RNA seen in injected nuclei is synthesized by them and not transferred from elsewhere in the oocyte.

Release of protein and RNA by injected nuclei

We have asked whether the increasing rate of RNA synthesis by enlarging nuclei is accompanied by the loss of nuclear proteins. We have therefore labelled the proteins of HeLa nuclei before injection (by incubating HeLa cells in [^{3}H]leucine – see Methods) and determined by autoradiography the amount of label remaining in enlarged nuclei a few days after injection. There is a substantial dilution of label due solely to nuclear volume increase and a considerable variation in the extent to which nuclei of similar size are labelled. For these reasons we have found that the most reliable way of recognizing the loss of labelled proteins in enlarged nuclei is to compare this to the loss of labelled DNA and RNA in other enlarged nuclei. Labelled DNA should not be lost from intact nuclei, and such tests as have been done (Gurdon, 1976) indeed indicate that it is not. On the other hand, RNA would be expected to be lost from nuclei.

The results of grain counting over sectioned nuclei are given in Table 2. The grain counts over donor nuclei are not directly comparable to grain counts over nuclei injected into oocytes. This is because donor nuclei were dried on to slides where they became flattened, so that most of their radioactivity reached

Table 2. *Retention or loss of labelled nucleic acids and proteins by HeLa nuclei in* Xenopus *oocytes*

Labelled component of nucleus	Labelling conditions*	Grain counts per 100 μm^2†					No. of oocytes scored	Retention of label‡
		Donor nuclei	Injected nuclei	GV nucleoli	GV plasm	Cyto-plasm		
DNA	[^{3}H]thymidine (44 h)	95	96	4	3·5	3·0	5	100 %
RNA	[^{3}H]uridine (6 h)	432	109	30	6·0	5·5	8	25 %
Protein	[^{3}H]leucine (45 h)	360	54	1·6	1·7	0·2	5	15 %
Protein	[^{3}H]leucine (6 h)	101	18	9	11	10	6	18 %

* HeLa cells were labelled with the isotope specified, for the number of hours stated, immediately before preparation of nuclei for injection. For labelling conditions, see Methods.

† Donor nuclei were dried onto a slide; all other grain counts were carried out on sections of oocytes fixed 3 days at 19 °C after the injection of nuclei. Grains over nuclei were usually counted in 25 μm^2 areas. Each value in the table represents the average of counts over at least 20 areas for nuclei in several different oocytes. At least 100 grains were counted for GV nucleoli, GV plasm, and cytoplasm. Injected nuclei of diameter 17–22 μm were scored. For reasons explained in the text (p. 548), autoradiographs of donor nuclei were exposed for 1/10 of the time given to oocyte sections. The grain count values of 432 and 360 were calculated from actual counts made on autoradiographs exposed for four times less than other preparations of donor nuclei.

‡ Since the grain counts for donor nuclei and injected nuclei are not exactly comparable (see page 547), the percentage retention of label wasestimatedby assuming that the value for DNA (96:95) represents 100 %. The values for retention of RNA and protein are calculated in the same way as for DNA.

the overlying autoradiographic film. Nuclei injected into oocytes are fixed and sectioned at 10 μm; only [^{3}H]radioactivity in the top 1 μm of each section reaches the autoradiographic film. For this reason, dried donor nucleus preparations were given shorter autoradiographic exposures than sectioned nuclei (details in the legend to Table 2). As a result of this adjustment, the grain counts over [^{3}H]thymidine-labelled donor nuclei are comparable to the grain counts over [^{3}H]thymidine-labelled nuclei in sectioned oocytes, a result expected if there is no loss of DNA. In contrast, the equivalent comparison shows a substantial reduction in grain counts over sectioned nuclei compared to donor nuclei, when the label is in RNA or protein (Table 2).

Compared to DNA, about three-quarters of the labelled RNA and rather more of the labelled protein is lost from nuclei which have enlarged in oocytes. The results with RNA are a little surprising, since nuclei which are actively synthesizing RNA might be expected to have lost all of their RNA labelled 3 days ago. The possibility that the residual label represents the synthesis of new

RNA from degradation products of the labelled RNA carried in with the injected nuclei can be largely eliminated by the distribution of label over nuclei. As shown in Fig. 1C, prelabelled nuclei show a very high concentration of grains over the nucleolus, as do the [^{3}H]U prelabelled donor nuclei. This is in marked contrast to nuclei labelled after injection into oocytes which show no concentration of label over the nucleoli (Fig. 1B). Clearly there is little, if any, export or breakdown of nucleolar RNA in injected nuclei. This may be connected with the reduced size and apparent inactivity of nucleoli in enlarged HeLa nuclei; indeed nucleoli eventually disappear (Gurdon, 1976). The residual label over the nucleoplasm of injected nuclei cannot be wholly due to re-synthesis of RNA from breakdown products, because if this had been so, the GV nucleoli would have been at least as heavily labelled as the injected nuclei; this is the case in oocytes with nuclei labelled *after* injection, as was described above. In fact GV nucleoli are labelled three to four times less than the nucleoplasm of prelabelled injected nuclei (Table 2).

As far as nuclear proteins are concerned, a clear result is that the majority of these are broken down in, or exported from, injected nuclei. This could be connected with the increased dispersion of their chromatin. There is no reason to believe that the residual protein label results from re-synthesis. At least part of it may reasonably be attributed to histones, which, as shown below, are strongly concentrated in injected nuclei.

The main conclusion from these studies is that most, but not all, of the non-nucleolar RNA and nuclear protein is lost from HeLa nuclei which enlarge after injection into oocytes.

The uptake of proteins by injected nuclei

This has been followed autoradiographically in two ways: by observing the uptake of cytoplasmically synthesized (non-histone) proteins and the uptake of [^{125}I]labelled histones injected into oocytes.

Cytoplasmic proteins were labelled by incubating oocytes, injected 5 days previously with HeLa nuclei, in [^{3}H]leucine for 2 h, and then fixing them after a further incubation of 0·5 or 16 h in unlabelled medium. Subsequent autoradiography showed that the injected nuclei were labelled well above the cytoplasmic background (Table 3). We assume that this represents the uptake of cytoplasmically synthesized proteins, since there is no reason to believe that proteins are ever synthesized in HeLa or oocyte nuclei, and the migration of cytoplasmic proteins into nuclei in *Xenopus* eggs is not inhibited by puromycin (Arms, 1968; Merriam, 1969). The results were the same, whether or not oocytes were incubated in unlabelled medium after labelling and before fixation. Since histones represent only about 0·4 % of all proteins synthesized by oocytes (Adamson & Woodland, 1974), it is very likely that the proteins taken up by injected nuclei were not only histones. This conclusion is strongly supported by the fact that injected nuclei were also labelled well above their cytoplasmic

Table 3. *Uptake of proteins by HeLa nuclei in* Xenopus *oocytes*

Labelled proteins	Diameter of injected nuclei (μm)	Nuclear Volume (μm^3)	Grain counts per 100 μm^2‡ Injected nuclei	GV nucleoli	GV plasm	Cyto-plasm	Total grains per nucleus§
Oocyte cytoplasmic;	12–16	1437	93	—	—	—	1336
[^{3}H]leu labelled*	18–19	3316	116	42	34	14	3846
	20–25	5966	103	—	—	—	6145
Injected							
calf thymus histones;	11–12	695	145	—	—	—	1008
[^{125}I]labelled†	13–14	1147	161	300	240	6	1847
	16–17	2564	182	—	—	—	4665

* Proteins were labelled by incubating oocytes in [^{3}H]leucine for 2 h, then in unlabelled medium (p. 549). The oocytes had been injected with HeLa nuclei and incubated in unlabelled medium for 5 days, before transfer to [^{3}H]leucine-containing medium.

† Calf thymus histones, labelled *in vitro* (see Methods), were injected into oocytes, which had received HeLa nuclei 5 days before. Oocytes were fixed 16 h after the injection of labelled histones.

‡ The grain counts shown for injected nuclei are the average of counts over 7–10 nuclei. The values for the GV and cytoplasm are the average of counts over at least 10 different areas.

§ Calculated as nuclear volume (μm^3) × grain counts per μm^2 of injected nuclei. The reasons for making the calculation in this way are described in the Methods section.

background when [^{3}H]tryptophan (which is not incorporated into histones) was used for an experiment of the same design as that just described.

In the second experiment, histones were purified from calf thymus (see Methods) in such a way as to lack F_1. The histones were iodinated (see Methods), and SDS gel electrophoresis showed that radioactivity was present in histones F_3:F_{2a2}:F_{2b}:and F_{2a1} in the ratio of about 1·0 : 1·5 : 1·0 : 1·2, as expected if these four kinds of histones were present in about equal abundance. Oocytes were injected with HeLa nuclei, incubated for 5 days, injected again with [^{125}I]histones, and then fixed after 16 h of further incubation. Autoradiography showed that the injected nuclei were intensely labelled (Fig. 1 D, E). as was the oocyte's GV (Table 3). Oocytes contain their own histones which they synthesize at a low rate (Adamson & Woodland, 1974); presumably these are also taken up by injected nuclei.

These experiments indicate that HeLa nuclei injected into oocytes take up histone and non-histone proteins from the surrounding oocyte cytoplasm. Of special interest is the observation that the amount of histone and non-histone protein taken up increases substantially with nuclear volume (Table 3). This suggests that protein-binding sites, some of which might be chromosomal, are made available progressively as nuclei enlarge and as their chromatin undergoes dispersion.

DISCUSSION

It appears that all morphologically healthy nuclei in oocytes actively synthesize RNA. All kinds of nuclei so far tested (HeLa, monkey, and mouse cells), and HeLa nuclei when tested at any time from 1 to 28 days after injection, are rapidly labelled by RNA precursors.

The labelling of injected nuclei by RNA precursors increases nearly in proportion to nuclear volume. Since HeLa nuclei in oocytes commonly undergo a 30-fold volume increase, and sometimes enlarge several hundred times, injected nuclei which enlarge evidently undergo a very substantial activation of RNA synthesis. This apparent activation of RNA synthesis seems to be correlated not only with an increase in nuclear volume, but also with an increase in chromatin dispersion, and perhaps more significantly, with an increasing exchange of proteins between nucleus and cytoplasm. It seems possible that the conditions or factors responsible for these cytoplasmically induced changes in injected mammalian nuclei may also be responsible for the highly dispersed, and transcriptionally very active chromatin of oocyte nuclei (GVs).

The apparent increase in rate of RNA synthesis seen in HeLa nuclei which enlarge in oocytes could be regarded in one of two ways. It could represent a real increase in RNA synthesis, so that enlarged HeLa nuclei are more transcriptionally active in oocytes than they are in growing HeLa cells. Alternatively, HeLa nuclei might undergo a large reduction in rate of RNA synthesis as a result of isolation, injection into oocytes, and incubation at 19–25 °C; enlargement would then amount to a recovery of their original rate of transcription. The absolute rate of RNA synthesis by injected HeLa nuclei cannot be estimated at all accurately because we know too little about RNA turnover and export in injected nuclei. If, however, the amount of nuclear labelling, seen autoradiographically, reflects the rate of RNA synthesis, then we can conclude that injected HeLa nuclei which have *not* yet enlarged synthesize RNA at nearly the same rate as oocyte nucleoli, the two being the same size and similarly labelled. The rate of stable RNA synthesis by oocyte nuclei has been estimated at 1–4 ng/h (Scheer, 1973; La Marca, Smith & Strobel, 1973). Since most of the nuclear RNA in one oocyte is synthesized by about 1000 nucleoli, each nucleolus must synthesize about 2 pg/h, and each injected nucleus nearly as much. Since a whole HeLa cell in culture doubles its content of RNA ($\sim$ 50 pg) in about 24 h, it must synthesize about 2 pg stable RNA/h. There are many respects in which this calculation may prove wrong. Nevertheless it appears that *unenlarged* HeLa nuclei in oocytes are about as active in transcription as are the same nuclei in growing HeLa cells, and therefore that a real increase in RNA synthesis takes place as a result of enlargement. If, as is suggested by the disappearance of the nucleolus (Gurdon, 1976) and the known effect of incubating HeLa cells at 20 °C (Stevens & Amos, 1971), HeLa nuclei do not synthesize ribosomal RNA in oocytes, the activation of RNA synthesis in the rest of the HeLa genome is likely to be very substantial.

Conclusions similar to those reported here have been reached from previous experiments in which nuclei have been introduced experimentally into cells of different types or metabolic conditions. For example, hen erythrocyte nuclei fused into cultured mammalian cells show a progressive enlargement, chromatin dispersal, and reactivation of RNA synthesis in the course of a few days (Harris, 1967; Bolund, Ringertz & Harris, 1969). During this process, proteins are taken up, partly from the host nucleus (Ringertz, Carlsson, Ege & Bolund, 1971). In Amoeba, Legname & Goldstein (1972) and Goldstein (1974) have documented the exchange of proteins and RNA between transplanted nuclei and components of their host cell. Transplanted nuclei to amphibian *eggs* undergo a rapid enlargement and dispersion of chromatin (Graham, Arms & Gurdon, 1966), and during this process proteins are taken up by nuclei from, and released by nuclei into, their surrounding cytoplasm (Arms, 1968; Merriam, 1969; Gurdon, 1970; Di Berardino & Hoffner, 1975). In this case, however, nuclei are induced to synthesize DNA but not RNA. The generalization suggested by these results is that nuclei come to resemble both morphologically and metabolically the cells into whose cytoplasm they are introduced. There is special interest in the transfer of nuclei to oocytes because, unlike the other systems referred to above, the introduced nuclei remain in a transcriptionally active state for several weeks while undergoing no division whatsoever, We believe that this situation, together with the fact that the injection procedure used permits purified macromolecules as well as nuclei to be introduced into oocytes, may make the experimental system described here particularly favourable for further analysis.

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REFERENCES

ADAMSON, E. D. & WOODLAND, H. R. (1974). Histone synthesis in early amphibian development: histone and DNA synthesis are not coordinated. *J. molec. Biol.* **88**, 263–285.

ARMS, K. (1968). Cytonucleoproteins in cleaving eggs of *Xenopus laevis*. *J. Embryol. exp. Morph.* **20**, 367–374.

BOLUND, L., RINGERTZ, N. R. & HARRIS, H. (1969). Changes in the cytochemical properties of erythrocyte nuclei reactivated by cell fusion. *J. Cell. Sci.* **4**, 71–87.

COLMAN, A. (1974). Synthesis of RNA in oocytes of *Xenopus laevis* during culture *in vitro*. *J. Embryol. exp. Morph.* **32**, 515–532.

COLMAN, A. (1975). Transcriptions of DNAs of known sequence after injection into the eggs and oocytes of *Xenopus laevis*. *Europ. J. Biochem.* **57**, 85–96.

DI BERARDINO, M. A. & HOFFNER, N. J. (1975). Nucleo-cytoplasmic exchange of non-histone proteins in amphibian embryos. *Expl Cell Res.* **94**, 235–252

FORD, C. C. & GURDON, J. B. (1977). A method for enucleating oocytes of *Xenopus laevis*. *J. Embryol. exp. Morph.* (in press).

GOLDSTEIN, L. (1974). The shuttling activity of nuclear RNAs is not an artefact of experimental manipulation. *Expl Cell Res.* **85**, 159–166.

GRAHAM, C. F., ARMS, K. & GURDON, J. B. (1966). The induction of DNA synthesis by frog egg cytoplasm. *Devl Biol.* **14**, 349–381.

GREENWOOD, F. C., HUNTER, W. M. & GLOVER, J. S. (1963). The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* **89**, 114–123.

GURDON, J. B. (1970). Nuclear transplantation and the control of gene activity in development. *Proc. R. Soc.* B, **176**, 303–314.

GURDON, J. B. (1976). Injected nuclei in frog oocytes: fate, enlargement and chomatin dispersal. *J. Embryol. exp. Morph.* **36**, 523–540.

GURDON, J. B., DE ROBERTIS, E. M. & PARTINGTON, G. (1976). Injected nuclei in frog oocytes provide a living cell system for the study of transcriptional control. *Nature, Lond.* **260**, 116–120.

HARRIS, H. (1967). The reactivation of the red cell nucleus. *J. Cell Sci.* **2**, 23–32.

LA MARCA, M. J., SMITH, L. D. & STROBEL, M. C. (1973). Quantitative and qualitative analysis of RNA synthesis in stage 6 and stage 4 oocytes of *Xenopus laevis*. *Devl Biol.* **34**, 106–118.

LEGNAME, C. & GOLDSTEIN, L. (1972). Proteins in nucleocytoplasmic interactions. VI. Is there an artefact responsible for the observed shuttling of proteins between cytoplasm and nucleus in *Amoeba proteus*? *Expl Cell Res.* **75**, 111–121.

MERRIAM, R. W. (1969). Movement of cytoplasmic proteins into nuclei induced to enlarge and initiate DNA or RNA synthesis. *J. Cell Sci.* **5**, 333–349.

RINGERTZ, N. R., CARLSSON, S.-A., EGE, T. & BOLUND, L. (1971). Detection of human and chick nuclear antigens in nuclei of chick erythrocytes during reactivation in heterokaryons with HeLa cells. *Proc. natn. Acad. Sci., U.S.A.* **68**, 3228–3232.

ROEDER, R. G. (1974). Multiple forms of deoxyribonucleic acid dependent ribonucleic acid polymerase in *Xenopus laevis*. Isolation and partial characterization. *J. biol. Chem.* **249**, 241–248.

SCHEER, U. (1973). Nuclear pore flow rate of ribosomal RNA and chain growth rate of its precursor during oogenesis of *Xenopus laevis*. *Devl Biol.* **30**, 13–28.

STEVENS, R. H. & AMOS, H. (1971). RNA metabolism in HeLa cells at reduced temperature. I. Modified processing of 45S RNA. *J. Cell Biol.* **50**, 818–829.

TOCHINI-VALENTINI, G. P. & CRIPPA, M. (1970). RNA polymerases from *Xenopus laevis*. *Cold Spring Harb. Symp. quant. Biol.* **35**, 737–742.

VAN DER WESTHUYZEN, D. R. & VON HOLT, C. (1971). A new procedure for the isolation and fractionation of histones. *FEBS Letters* **14**, 333–337.

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