

PROTEIN MIGRATION INTO NUCLEI

II. Frog Oocyte Nuclei Accumulate a Class of Microinjected Oocyte Nuclear Proteins and Exclude a Class of Microinjected Oocyte Cytoplasmic Proteins

WILLIAM M. BONNER

From the Medical Research Council, Laboratory of Molecular Biology, Cambridge, England. Dr. Bonner's present address is National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014.

ABSTRACT

Nuclear contents or cytoplasm from *Xenopus* oocytes labeled with [³⁵S]methionine or [³H]proline (donor oocytes) were reinjected into unlabeled oocytes (recipient oocytes). The radioactivity injected as nuclear contents was found to enter and accumulate in the recipient oocyte nucleus. In contrast, the radioactivity injected as cytoplasm was found to enter but not to accumulate in the recipient oocyte nucleus.

Sodium dodecyl sulfate (SDS) gel electrophoresis of the nucleus and cytoplasm of donor oocytes revealed the existence of three classes of labeled proteins in these oocytes: those proteins found predominantly in the nucleus (N proteins), those found predominantly in the cytoplasm (C proteins), and those found in both the nucleus and cytoplasm at similar concentrations (B proteins).

SDS gel electrophoresis of the nucleus and cytoplasm of recipient oocytes showed that N proteins entered and accumulated in the nucleus but that B proteins partitioned about equally between the nucleus and cytoplasm. A similar analysis of oocytes injected with labeled cytoplasm showed that C proteins did not enter the nucleus but again B proteins partitioned about equally between the nucleus and cytoplasm.

Proteins that migrate between the cytoplasm and nucleus of a cell are interesting because they may be involved in control processes important for cellular maintenance, growth, and differentiation. Examples of this class of proteins have been reported in *Amoeba* (1, 2), *Chironomus* (3), and *Xenopus* (4-7).

In *Xenopus laevis*, Gurdon has shown that when [¹²⁵I]histone is microinjected into oocytes it concentrates in the nucleus (6). In the accompanying paper (7), it is shown that injected non-nuclear

[¹²⁵I]proteins may enter the nucleus but do not concentrate there, but that histones, including purified fractions, concentrate in the nucleus. Using techniques developed in that paper, this study shows that not only histones but a large class of labeled oocyte nuclear proteins are able to reenter and concentrate in the oocyte nucleus.

MATERIALS AND METHODS

The protocol of the experiments presented in this paper is shown in Fig. 1. To obtain radioactive nuclear and

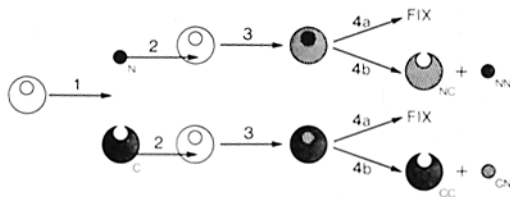


FIGURE 1 Protocol of the experiments in this paper. The steps are described in the text. The stippling indicates the presence of radioactive proteins.

cytoplasmic proteins, large oocytes (stages 5 and 6 of Dumont [8]) of *X. laevis* were incubated for 24 h at 19°C in modified Barth saline (9) (2 μ l/oocyte) containing 1 mCi/ml of [³H]proline (Amersham/Searle Corp., Arlington Heights, Ill., 10 Ci/mmol) or 1 mCi/ml of [³⁵S]methionine (Amersham/Searle Corp. 156 Ci/mmol), and then for 4 h in modified Barth saline without label. These donor oocytes were then manually enucleated in 0.05 M NaCl (Fig. 1, step 1) (7). The isolated nuclei contained 9–11% of the total oocyte radioactivity. 98% of the nuclear radioactivity and 90% of the cytoplasmic radioactivity were cold TCA precipitable.

In order to minimize the leakage of radioactive material from isolated organelles, the nuclear contents or the cytoplasm of donor oocytes were sucked into microinjection pipettes within 2 min of enucleation. These materials were then injected into the cytoplasm of other unlabeled oocytes (Fig. 1, step 2). These oocytes, hereafter called recipient oocytes, were then incubated in modified Barth saline for 20 h unless indicated otherwise (Fig. 1, step 3).

The recipient oocytes which had been injected with [³H]proline-labeled material were fixed overnight in Perenyi's solution (10), embedded in paraffin wax, sectioned at 7 μ m, and autoradiographed with Ilford K2 emulsion for 3 days (Fig. 1, step 4a).

The recipient oocytes which had been injected with [³⁵S]methionine-labeled material were manually enucleated (Fig. 1, step 4b). The nuclear and cytoplasmic fractions were homogenized in 0.05 M Tris-HCl pH 6.8 and the amount of labeled protein in each fraction was determined by liquid scintillation counting of aliquots precipitated in 10% TCA-10 mM L-methionine and collected on Whatman GF/C glass fiber filters.

Aliquots of the six fractions noted in Fig. 1 were analyzed by SDS acrylamide gel electrophoresis using 10% acrylamide and 0.13% bisacrylamide in the resolving gel, 3.3% acrylamide and 0.16% bisacrylamide in the stacking gel, and the discontinuous buffer system of Laemmli (11).

Samples of nuclear and cytoplasmic fractions were loaded onto gel slots so that a protein of equal radioactive concentration in the nucleus and cytoplasm would yield similarly dense autoradiographic bands in the two slots. This loading is 0.12 of an oocyte cytoplasm for every oocyte nucleus (7).

It was not found necessary to remove nuclear mem-

branes from the isolated nuclei, because isolated nuclear membranes were found to contain insignificant amounts of labeled proteins. No differences were seen in the autoradiographic gel patterns of whole nuclei and nucleoplasm.

It was found necessary to centrifuge the cytoplasmic homogenate at 3,000 g for 5 min to remove yolk platelets since these would overload the gel. If the homogenate contained at least 20 μ l of buffer per oocyte and was centrifuged before freezing, 85% of the radioactive proteins remained in the supernate. Removing the yolk from the cytoplasmic fractions did not alter the autoradiographic band pattern after SDS gel electrophoresis. Nuclear and centrifuged cytoplasmic fractions were stored at -20°C.

After electrophoresis, the gel was attached to paper, dried under vacuum at 100°C, and autoradiographed with Kodirex AP54 film. In order to quantitate the partitioning of various proteins between the nucleus and cytoplasm, certain of the radioactive bands were located on the dried gel by superimposing the autoradiographs over it. Slices (1 \times 10 mm) were taken from these areas, digested in alkaline H₂O₂, and their radioactivity was determined.

RESULTS

When the nuclear contents of donor oocytes were microinjected into the cytoplasm of recipient oocytes, the radioactivity migrated into the recipient oocyte nucleus and concentrated there. In contrast, when the cytoplasm of labeled donor oocytes was microinjected into recipient oocytes, the radioactivity entered the nucleus but did not concentrate there. These results were obtained both by autoradiography of oocyte sections and by manual enucleation of oocytes.

Autoradiography

Autoradiographs (Fig. 2) of recipient oocytes injected with nuclear contents or cytoplasm from labeled donor oocytes clearly show how differently these two fractions partition between the nucleus and cytoplasm. That the process being observed is a partitioning between the nucleus and cytoplasm is shown by the presence of grains *inside* the nucleus and in the cytoplasm with no local accumulation on the nuclear membrane. Grains in the nucleus seem to be randomly distributed.

Table I presents quantitative results from two typical microinjection experiments. The results, after correction for the excluded cytoplasmic volume due to yolk platelets (7), show that radioactivity injected as nuclear contents has entered and concentrated four- to ninefold in the recipient oocyte nucleus while radioactivity injected as cyto-

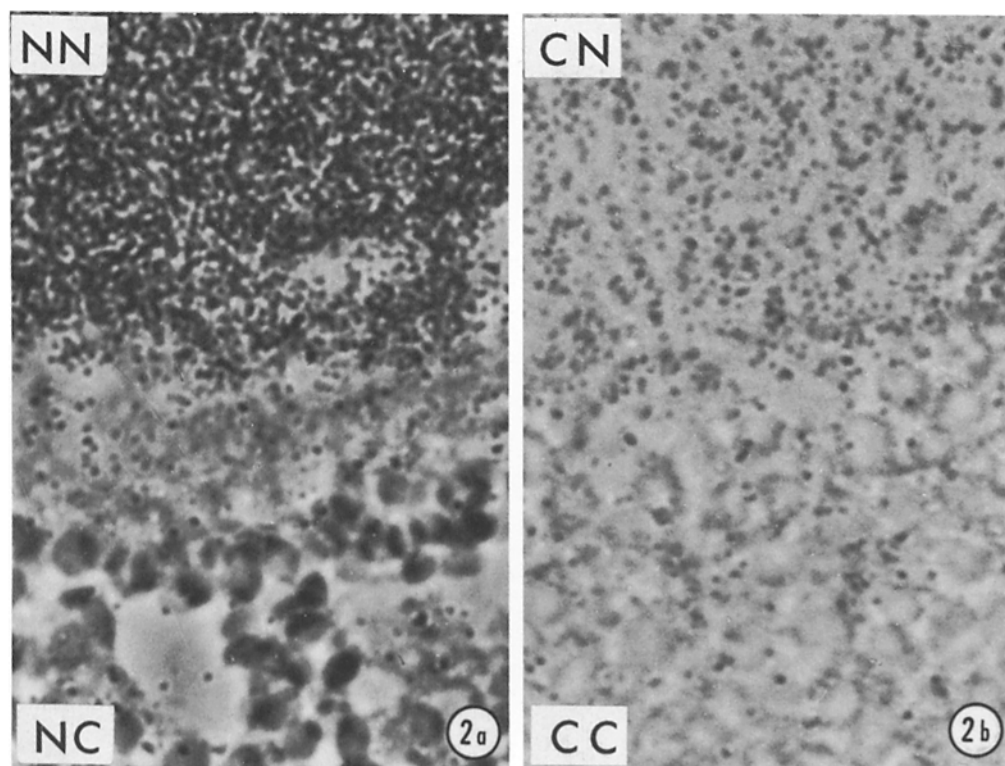


FIGURE 2 Autoradiographs of sections through nucleus (top) and adjacent cytoplasm (bottom) of recipient oocytes injected with nuclear contents (a) or cytoplasm (b) from [^3H]proline-labeled donor oocytes. See Materials and Methods for description of manipulations. Some silver grains over the cytoplasm are out of the plane of focus and are not visible in these photographs.

plasm has entered the nucleus but has not noticeably accumulated there. In these two experiments the nuclear-cytoplasmic concentration ratios for injected nuclear contents should not necessarily agree since these ratios, like those for histone accumulation (7), depend on the total amount of material injected.

Manual Enucleation

Autoradiography is useful for determining the localization of a material but not its identity. By using the techniques developed and tested previously (7), it is possible to determine the number and kind of newly synthesized proteins that make up the nuclear and cytoplasmic fractions of oocytes.

The results presented in Table II show that the phenomenon observed with [^3H]proline labeling and autoradiography of oocytes is also observed with [^{35}S]methionine labeling and manual enucleation. Radioactivity injected as nuclear contents has entered and concentrated 4.8- to 15-fold in the

recipient oocyte nucleus while radioactivity injected as cytoplasm has entered but has not concentrated in the nucleus. These experiments of injected nuclear material are not duplicates and the values should not necessarily be the same. Since one is presumably studying a "saturable" system, similar to that with the histones (7), the nuclear-cytoplasmic ratio should depend on the total amount injected. If it is assumed that the specific activities of the injected proteins in the different experiments are similar, these values are consistent with a saturation phenomenon in that the nuclear-cytoplasmic ratio increases as less radioactivity is injected.

Gel Analysis of Labeled Nuclear and Cytoplasmic Proteins

To further analyze this phenomenon the number and size of the newly synthesized protein chains comprising the nuclear and cytoplasmic fractions (*N* and *C* of Fig. 1) were compared by SDS gel electrophoresis (Fig. 3). The newly synthesized

TABLE I
Partitioning of [³H]Proline-Labeled Nuclear and Cytoplasmic Material in Oocytes

Material injected	Number of oocytes	Grains/area		Nucleus*		Nucleus§
		Nucleus	Cytoplasm	cytoplasm (+yolk)	% nuclear†	
Cytoplasm	1	17.0	4.8	3.3	12.9	1.2
	1	33.5	12.5	2.6	10.1	0.9
Nucleus	1	87.4	3.5	25.0	51.3	8.9
	1	80.0	6.4	12.5	34.0	4.5

Oocytes were microinjected with [³H]proline-labeled materials, incubated, and processed for autoradiography as described in Materials and Methods. Grain counts of nuclear and cytoplasmic regions were corrected for a background of 0.5 grains/area for the labeled cytoplasm and 0.2 grains/area for the labeled nuclear material.

* This ratio is the nuclear-cytoplasmic concentration ratio uncorrected for yolk platelet volume, assuming that this ratio is the same as the ratio of the grain density in the two regions.

† The percent nuclear radioactivity (%N) is calculated from the formula

$$R = \frac{\%N}{4\%} \div \frac{100 - \%N}{96\%}$$

where *R* is the uncorrected nuclear-cytoplasmic concentration ratio. 4% and 96% represent the percentage of the oocyte volume in the nucleus and cytoplasm, respectively. These values were obtained from measurements of the nucleus and cytoplasm dimensions in sections of fixed oocytes.

§ This ratio is the nuclear-cytoplasmic concentration ratio corrected for the yolk platelet volume which is inaccessible to injected proteins. It equals the uncorrected ratio divided by 2.8. The factor 2.8 is obtained from experiments of the partitioning of [¹²⁵I]myoglobin between the nucleus and cytoplasm (7).

TABLE II
Partitioning of [³⁵S]Methionine-Labeled Nuclear and Cytoplasmic Material in Oocytes

Material injected	Experiment number	Number of oocytes				% nuclear radioactivity	Nucleus*	Nucleus‡
			Nucleus	Cytoplasm	Total		cytoplasm (+yolk)	cytoplasm -yolk
Cytoplasm	1	10	5,900	51,000	56,900	10.4	2.8	1.0
	2	3	9,380	117,700	127,000	7.3	1.9	0.7
	3§	9	3,280	35,000	38,300	8.5	2.2	0.8
Nucleus	1	6	130,000	233,000	364,000	36	13.5	4.8
	2	3	96,000	129,700	226,600	42.5	17.9	6.4
	3§	6	19,500	11,000	30,500	64	42.6	15

* Nuclear-cytoplasmic concentration ratio uncorrected for yolk platelet volume. See footnotes * and ‡ of Table I. *R* in this case is calculated from the formula with %N known.

‡ Nuclear-cytoplasmic concentration ratio corrected for the yolk platelet volume. See footnote § of Table I.

§ In this experiment, the oocytes were labeled with [³⁵S]methionine for 7 h and chased with cold L-methionine (10 μM) for 15 h. After injection, the recipient oocytes were incubated for 30 h.

proteins fall into three classes: (a) Those characteristic of the nucleus (N proteins); (b) those characteristic of the cytoplasm (C proteins); (c) those found in both compartments (B proteins).

The difference between the newly synthesized proteins of the oocyte nucleus and cytoplasm are most obvious among proteins heavier than 25,000 daltons. Radioactive bands due to lighter protein chains are found on higher percentage gels, but

these are in general less well resolved and not labeled as heavily as some of the protein bands of higher molecular weight.

That these radioactive bands are, in fact, protein is shown by the findings that the same autoradiographic pattern is obtained when [¹⁴C]amino acid hydrolyzate is substituted for [³⁵S]methionine and that no radioactive bands are found on gels in which the samples were pretreated with Pronase.

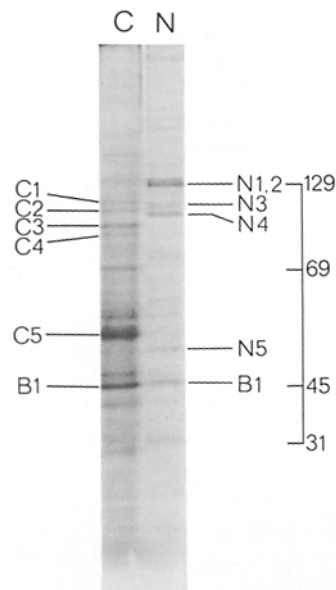


FIGURE 3 Autoradiograph of 10% SDS gel of the nucleus (*N*) and cytoplasm (*C*) of donor oocytes labeled with [³⁵S]methionine. Radioactive protein bands of interest are indicated. The scale at right indicates the approximate molecular weight ($\times 10^{-3}$).

On the other hand, RNase and DNase treatment did not alter the band pattern.

Gel Analysis of Reinjecting Nuclear Contents

How the various radioactive protein chains in the nucleus and cytoplasm are distributed when they are injected into recipient oocytes is shown in Fig. 4.

As shown before (Tables I and II), when labeled nuclear contents are injected into recipient oocytes, the radioactivity concentrates in the nucleus. Fig. 4 *a* slots *NN* and *NC* show that all the *N* protein species concentrate in the nucleus, while *B1* enters but concentrates to only a slight extent. Concentration ratios can be roughly quantitated by slicing the relevant sections of slots like *NN* and *NC* in Fig. 4 *a*, and determining the radioactivity in certain bands. After correcting for the cytoplasmic volume of yolk platelets (Table I, footnote §), the nuclear-cytoplasmic concentration ratio is typically about 50 for band *N1, 2* and between 10 and 50 for bands *N3*, *N4*, and *N5*. How these concentration ratios for *N* proteins vary with incubation time was not studied, so it is not known whether they are equilibrium ratios. It is possible that these ratios would increase still further.

In contrast to the *N* proteins, proteins *B1* and *B2* injected in the nuclear contents have concentration ratios corrected for yolk platelet volume between 1.5 and 2.5.

The labeled proteins injected as nuclear contents into oocytes are not degraded and the liberated labeled [³⁵S]methionine incorporated into oocyte proteins. Since 90% of the labeled protein in an oocyte is cytoplasmic, the liberated or free [³⁵S]methionine should preferentially label cytoplasmic proteins. The protein bands that can be seen in slot *NC* of Fig. 4 *a* are *N* proteins or *B* proteins, *B1* being the strongest, but no *C* proteins are seen in significant amounts. Therefore, the injected nuclear proteins are stable in oocytes.

Gel Analysis of Reinjecting Cytoplasm

In contrast to the nuclear concentration of injected nuclear proteins, some labeled proteins from injected cytoplasm enter the recipient oocyte nucleus but there is no accumulation of the cytoplasmic radioactivity as a whole in the nucleus. Slots *CN* and *CC* (Fig. 4 *b*) show that the proteins present in the nucleus and cytoplasm of a recipient oocyte injected with labeled cytoplasm differ from each other. The proteins found in the nucleus are *N* and *B* proteins, while the proteins found in the cytoplasm are *C* and *B* proteins. Therefore the 8–10% of the label from injected

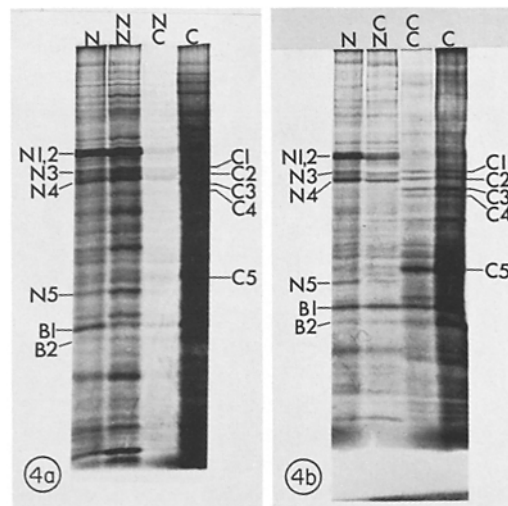


FIGURE 4 Autoradiograph of 10% SDS gel of the nucleus and cytoplasm of recipient oocytes which have been injected with [³⁵S]methionine-labeled nuclear contents (*a*) or cytoplasm (*b*). For identification of the relationship between slots see Fig. 1. The slots *N* and *C* are included as markers.

cytoplasm recorded as being in the nucleus in Tables I and II is due to nuclear (N and B proteins) rather than cytoplasmic proteins.

Although the labeled cytoplasm contains 10% of the label as TCA-soluble material, the labeled nuclear proteins probably do not arise by synthesis from injected free [³⁵S]methionine because a long chase with cold methionine (Table II, exp. 3) did not cause a decrease in the percentage of labeled material entering the nucleus. It is more likely that the cytoplasm contained a small fraction of the labeled N proteins either as a normal component or as nuclear contaminants transferred during manual enucleation.

Because no C proteins are found in the recipient oocyte nucleus, it is not possible to compute concentration ratios for them. However, the B proteins in the injected cytoplasm enter the nucleus and have concentration ratios between 1.0 and 1.5, as compared to 1.5–2.5 for the same proteins injected as nuclear contents.

DISCUSSION

When labeled cytoplasm or nuclear contents are reinjected into a recipient oocyte, the distribution of each injected protein between the nucleus and cytoplasm is the same as in the labeled donor oocyte. Labeled cytoplasmic proteins remain in the cytoplasm possibly because they are part of structures too large to enter nuclei or because they are actively excluded. Their behavior, which is similar to that of injected bovine serum albumin (BSA) or γ -globulin, could be explained on the basis of passive diffusion throughout the cytoplasm. Whether the cytoplasmic proteins would migrate to and accumulate in the cytoplasm if they were injected into the nucleus is not known and cannot easily be tested in this system. On the other hand, labeled nuclear proteins reenter and concentrate in the recipient oocyte nucleus.

In addition to the cytoplasmic and nuclear proteins there is a group of newly synthesized proteins in the oocyte, the B proteins, which are equally concentrated in the nucleus and cytoplasm. B proteins found in both the nucleus and cytoplasm of the oocyte are redistributed to both the nucleus and cytoplasm of the recipient oocyte whether they are injected as part of the nuclear contents or cytoplasm.

Some of the nuclear proteins that enter and accumulate in the recipient oocyte nucleus have SDS molecular weights of at least 130,000 daltons.

If entry is through the pores in the nuclear membrane, then proteins at least as large as 130,000 daltons can enter. However, it is not known whether these proteins are entering the nucleus as free protomers or as part of a larger structure. Many of these proteins have isoelectric points between 4.5 and 6.0 (unpublished observations), so they are not basic like histones, even though both classes of proteins accumulate in the oocyte nucleus.

It is striking that none of the injected N protein *species* remain more concentrated in the cytoplasm than in the nucleus. Possibly, this behavior is typical of all nuclear proteins, particularly in light of evidence that histones (12–14) and therefore possibly all nuclear proteins are synthesized in the cytoplasm. However, since the newly synthesized N proteins studied here may be only a tiny fraction of the kinds of proteins *present* in the nucleus, it is possible that they are not typical of total nuclear proteins, but are a special class with the ability to migrate into nuclei and concentrate there. Therefore the results obtained with this class of nuclear proteins should not be generalized to all nuclear proteins without further study. In fact, evidence that some microinjected nuclear proteins do not migrate from cytoplasm to nucleus comes from experiments in which the oocyte nuclear contents were labeled *in vitro* with ¹²⁵I, a procedure which labels proteins present in the nucleus and not only newly synthesized proteins. When these nuclear contents were injected into recipient oocytes, some of the ¹²⁵I-labeled nuclear protein species remained in the cytoplasm (unpublished observations).

In these studies, the nuclei and cytoplasm are in contact with the surrounding medium for 1–2 min, during which time components may have been lost from them. However, results from gamma counting of single oocytes injected with [¹²⁵I]myoglobin or [¹²⁵I]BSA followed by gamma counting of the isolated nucleus and cytoplasm from those oocytes show that recoveries of these two proteins were over 90%. Since the [³⁵S]methionine-labeled proteins were isolated in the same manner as the ¹²⁵I-labeled proteins, losses of the former during manual enucleation of live oocytes should also be less than 10%.

In *Amoeba* there exists a class of proteins that migrate into and concentrate in the nuclei. Legname and Goldstein (2) have shown that this class of proteins can accumulate in the nucleus to a concentration five to ten times that in the cyto-

plasm. These nuclear-cytoplasmic ratios are similar to those obtained in this work for the accumulation of N proteins in the nuclei for *Xenopus* oocytes.

Jelinek and Goldstein (1) have recently characterized some of those *Amoeba* proteins. One of these, an acidic protein of 2,300 daltons, was purified to homogeneity. When this protein was microinjected into the cytoplasm of *Amoeba*, it migrated into and accumulated in the nucleus. The proven existence of these migrating proteins in two organisms, *Amoeba* and *Xenopus*, indicates that this type of protein probably exists in all eucaryotic cells.

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