Differential accumulation of oocyte nuclear proteins by embryonic nuclei of *Xenopus*

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Summary

Oocyte nuclear proteins of *Xenopus* are distributed into the cytoplasm of the maturing egg after germinal vesicle breakdown. Later they are found in all cell nuclei of the embryo. At early stages of development, different nuclear proteins behave differently. A class of 'early shifting' antigens is accumulated by pronuclei and cleavage nuclei, whereas others appear to be excluded from the nuclei at early stages but are shifted into the nuclei at blastula or during and after gastrulation. Accumulation of 'late-shifting' nuclear antigens is a gradual process and occurs during a period characteristic of each protein. Multiple artificial pronuclei can be formed after injection of sperm nuclei, erythrocyte nuclei or pure λ-DNA into unfertilized eggs. The artificial pronuclei accumulate early- but not late-shifting proteins. Early-migrating proteins rapidly accumulate into the germinal vesicle after *de novo* synthesis in the oocyte, indicating that the efficiency of translocation into nuclei is an intrinsic property of each protein. Artificial extension of the length of the cell cycle before midblastula transition does not lead to accumulation of the late-shifting nuclear antigens investigated.

Key words: *Xenopus*, oocyte, nuclear proteins, protein transport.

Abbreviations: mAB: monoclonal antibody; GV: germinal vesicle; DAPI: 4',6-diamidino-2-phenyl-indol-dihydrochloride; FITC: fluoresceinisothiocyanate; TCA: trichloroacetic acid; MBS-H: modified Barth’s solution.

Introduction

Proteins of the oocyte nucleus of amphibia can be viewed as a maternal store of proteins that will later be found in the somatic nuclei of the embryo. This pool is accumulated during oogenesis, a period lasting several months in *Xenopus*.

We have established a library of monoclonal antibodies (mABs) binding to proteins found in the oocyte nucleus of *X. laevis* and of *X. borealis* and have used these to characterize the corresponding antigens biochemically, and to trace their behaviour in development (Dreyer, Singer & Hausen, 1981; Dreyer, Scholz & Hausen, 1982; Dreyer, Wang, Wedlich & Hausen, 1983; Dreyer, Wang & Hausen, 1985; Dreyer & Hausen, 1983; Wedlich, Dreyer & Hausen, 1985).

In *Xenopus*, egg maturation is accompanied by the breakdown of the oocyte nucleus or germinal vesicle (GV), which leads to a loss of compartment for the GV proteins, except those that are bound to the condensed chromosomes. In the mature egg, GV proteins are distributed in the cytoplasm, mainly in the animal half, where most of the nuclei of the embryo will later form (Dreyer et al. 1982, 1983; Hausen, Wang, Dreyer & Stick, 1985).

During cleavage, the cumulative volume of all nuclei is small as compared to that of the oocyte nucleus. It is not before midblastula that the volume of all nuclei of one embryo comes close to that of one germinal vesicle (Gerhart, 1980). Therefore, it has to be expected that cleavage nuclei cannot contain the total amount of protein derived from the oocyte nucleus. As was previously observed, cleavage nuclei do not contain all of the maternal nuclear proteins in
the same proportion as they are found in the germinal vesicle (Dreyer et al. 1982, 1983). In this paper, I show that at least two classes of protein antigens can be distinguished: a first class that is accumulated by pronuclei and by cleavage nuclei, and a second class that appears to be excluded from the nuclei at early stages, but later will also be accumulated.

For reasons of simplicity, the first class will be called ‘early-’ and the second class ‘late-shifting’ antigens. The second class comprises proteins shifting to the nuclei in late blastula and in late gastrula, and members of this class each shift into the nuclei at a characteristic developmental stage. The shift of any given GV-derived antigen from the egg cytoplasm to the nuclei of the embryo is a gradual process.

Observations made in vivo are complemented by studies on artificial pronuclei formed after injection of nuclei or DNA into unfertilized eggs. The biological meaning and the possible mechanism of selective transport of nuclear proteins are discussed. The parameters for the accumulation of nuclear proteins at early stages of development have also been studied in vitro (Dreyer, Stick & Hausen, 1986; C. Dreyer, unpublished data).

Materials and methods

Immunohistology

Eggs or embryos were fixed for 1 h with either 2 % TCA, or 4 % paraformaldehyde, or according to Romeis (42 % saturated HgCl₂, 1-7 % TCA, 9-3 % formaldehyde). Alternatively, they were fixed by freeze substitution with ethanol as described previously (Dreyer et al. 1982). Usually at least two different fixation procedures were performed with duplicate specimens in each experiment. Detection of nucleoplasmin requires fixation with Romeis (1968) fixative, which is deleterious to most other antigens. The fixed specimens were embedded in low-melting-point wax (Steedman, 1957) and sections, 5 μm thick, were subjected to indirect immunofluorescent staining as described previously (Wedlich et al. 1985).

Injection of nuclei and DNA into unfertilized eggs

Sperm nuclei were isolated essentially as described by Lohka & Masui (1983). Gonadotropin treatment of the male was omitted and cell lysis was at a final concentration of 0-1 % lyssolecithin ml⁻¹. Erythrocyte nuclei were also prepared with lyssolecithin. 20 nl of a suspension containing 2×10⁷ nuclei ml⁻¹ were injected with continuous flow using a micropipette measuring 20 μm at its tip, attached to a motor-driven Hamilton syringe filled with Fluorinert FC70. Eggs were dejellied before injection. Bacteriophage λ-DNA was purchased from Boehringer and 10–20 nl of a solution containing 0-5 mg ml⁻¹ of DNA was injected per egg.

Labelling of oocyte proteins with [³⁵S]methionine

X. laevis oocytes were injected with 30 nl of [³⁵S]methionine in MBS-H (60 mCi ml⁻¹). At 5 and 15 h after injection, oocytes were manually enucleated under the dissection microscope and the nuclei collected in ethanol. Some intact oocytes were homogenized as described previously (Dreyer et al. 1985). Each gel was loaded with 30 nuclei or with protein equivalent to four oocytes after removal of the yolk. After two-dimensional separation of the polypeptides, the gels were analysed by fluorography as described previously (Dreyer & Hausen, 1983). Areas of the gel where prominent nuclear or cytoplasmic proteins were located were excised and the radioactivity was determined by liquid scintillation counting. The ratio of individual radioactively labelled polypeptides in the nucleus as compared to the cytoplasm was calculated.

[³²P]phosphate labelling in oocytes and maturing eggs

Females were injected with 50 units of gonadotropin from pregnant mare serum (Sigma), 3–7 days before being killed. The ovary was dissociated by collagenase treatment (Eppig & Dumont, 1978) and oocytes of stage VI (Dumont, 1972) were induced to mature by addition of progesterone (2 μg ml⁻¹ in MBS-H). 200 oocytes were incubated for 4 h with 1 mCi of carrier-free [³²P]phosphate in a total volume of 1 ml MBS-H. [³²P]phosphate was added 30 min after addition of progesterone. In the experiment shown in Fig. 9, the first polar body appeared between 3-5 and 4-5 h after addition of progesterone. 200 control oocytes without progesterone were incubated under the same conditions. After labelling, the oocytes were washed with MBS-H and homogenized with 0-9 ml 20 mm-sodium phosphate, pH 8-0, 10 mm-dithiothreitol, 0-5 mM-EDTA, 0-15 mg ml⁻¹ phenylmethylsulphonylfluoride. Aliquots of each extract were analysed on two-dimensional gels that were stained with silver according to Morrissey (1981), and subsequently exposed on X-ray films.

Cell cycle arrest

Embryos at the 64-cell stage were incubated with 100 μg ml⁻¹ cycloheximide in 1/10 MBS-H. Alternatively, they were injected each with 40 nl of 100 μg ml⁻¹ aphidicolin in MBS-H. 4 h later, when controls had reached late stage 9 (Nieuwkoop & Faber, 1967), experimental and control embryos were fixed with 2 % TCA, or 4 % paraformaldehyde, or by freeze substitution with ethanol.

Results

(A) Observations in early development

(1) Early- and late-shifting antigens

Fertilized eggs and embryos at different stages of development, from cleavage to neurula, have been analysed for the presence or absence of germinal-vesicle-derived antigens in their nuclei with application of monoclonal antibodies (Dreyer et al. 1985) and indirect immunofluorescent staining as detailed in Materials and methods. Four different fixation procedures have been compared for each antigen...
### Germinal vesicle-derived antigens in early development and in artificially induced pronuclei

<table>
<thead>
<tr>
<th>Polypeptide*</th>
<th>mAB</th>
<th>Developmental stage of translocation into nuclei</th>
<th>Accumulation by pronuclei formed from injected sperm nuclei</th>
<th>erythrocyte nuclei</th>
<th>λ DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleoplasmin</td>
<td>b7-1A9</td>
<td>Cleavage</td>
<td>+++</td>
<td>n.d.</td>
<td>+++</td>
</tr>
<tr>
<td>(30)</td>
<td>b7-1D1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>b2-2B10</td>
<td>Cleavage</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>(3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>b3-5E8</td>
<td>Cleavage</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>(4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>b7-2H4</td>
<td>Cleavage</td>
<td>++</td>
<td>n.d.</td>
<td>+++</td>
</tr>
<tr>
<td>(29)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamin L,m</td>
<td>L6-8A7</td>
<td>Cleavage</td>
<td>+</td>
<td>n.d.</td>
<td>+</td>
</tr>
<tr>
<td>33×10^3 M,</td>
<td>32-4A1</td>
<td>Cleavage</td>
<td>+</td>
<td>n.d.</td>
<td>+</td>
</tr>
<tr>
<td>(7)</td>
<td>b6-6E7</td>
<td>Blastula</td>
<td>n.d.</td>
<td>n.d.</td>
<td>+</td>
</tr>
<tr>
<td>Nucleolar 86</td>
<td>68×10^3 M,</td>
<td>Blastula to gastrula</td>
<td>+</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>(5)</td>
<td>b7-1B4</td>
<td>Gastrula</td>
<td>±</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>(13)</td>
<td>37-1B2</td>
<td>Gastrula to neurula</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(21)</td>
<td>32-5B6</td>
<td>Gastrula to neurula</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses refer to numbering in previous publications (Dreyer et al. 1985).

† Accumulation very strong (+++), strong (++), significant (+), not significant (±), no accumulation (-). n.d.: not done.

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**(2) Reaccumulation of nuclear antigens after mitosis**

After first cleavage, the embryo undergoes a series of eleven more rapid and approximately synchronous divisions. Cell cycles last between 25 and 30 min and seem to consist of S-phase and mitosis only (Graham & Morgan, 1966; Boterenbrood, Narraway & Hara, 1983; Boterenbrood & Narraway, 1986; Newport & Kirschner, 1982). Thereafter, starting at the mid-blastula transition, the cell cycles become longer and more asynchronous (Gerhart, 1980; Newport & Kirschner, 1982).

Nucleoplasmic proteins are dissipated in the cytoplasm during each mitosis and have to be reaccumulated after each division. The same applies to the nuclear lamina which disintegrates and reforms during each mitosis (Gerace & Blobel, 1980).

In Fig. 2, the distribution of nucleoplasmin is shown on sections of cleavage-stage embryos. After the seventh division, a considerable amount of nucleoplasmin is seen in the cytoplasm although its concentration in the interphase nuclei is apparently much higher, as revealed by immunofluorescent staining with mAB b7-1A9 (Fig. 2A,B). As the cells become smaller after the subsequent divisions, nucleoplasmin seems to be quantitatively shifted to the interphase nuclei in each cell cycle.

In Fig. 2C,D, nests of cells with no nucleoplasmin detectable in the cytoplasm can be distinguished from areas with cells containing a significant amount of nucleoplasmin in the cytoplasm. The latter are cells at or shortly after mitosis. Mitotic figures have been identified by counterstaining of the chromosomes with DAPI (not shown). Since we also find cells with nonmitotic nuclei in stained cytoplasm, we conclude...
that the reaccumulation of nucleoplasmin requires a certain amount of time after mitosis. The finding of nests of cells that are either at mitosis, or shortly after, adjacent to cells that are probably shortly before mitosis may be explained by the dynamics of the cleavage waves that have recently been studied in great detail (Boterenbrood et al. 1983; Boterenbrood & Narraway, 1986).

The fact that the cell cycles become more asynchronously after midblastula is also reflected by a more complicated distribution of patches of cells that are early or late in the cell cycle (Fig. 2E,F). The cell-cycle-dependent dissipation and reaccumulation is most prominently seen with nucleoplasmin, which is a very abundant maternal nuclear protein. However, similar patterns can be observed with antigen b6-bE7 at later stages (data not shown).

(B) Observations on artificial pronuclei

Additional pronuclei can be induced by injection of isolated sperm nuclei into unfertilized eggs (Graham, 1966). We have assayed such artificial pronuclei for the uptake of nuclear antigens from the egg cytoplasm. Injected sperm nuclei round up and accumulate nucleoplasmic antigens that are normally found in cleavage nuclei (Figs 3, 4, 5). Concomitantly they assemble a lamina consisting of germinal-vesicle-derived lamin L111. Accumulation of antigen b2-2B10 (N1) can be seen as early as 30 min after injection (Fig. 3C–F). The same is true for lamin L111, and antigens 32-4A1, and b7-1A9 (nucleoplasmin; data not shown). From serial sections of eggs injected with sperm nuclei, we estimate that at least several hundred heavily swollen pronuclei can be formed within a period of 2 h (Fig. 4). Occasionally, some sperm nuclei do not swell in the egg cytoplasm. These do not assemble a lamina nor do they accumulate any of the antigens tested (see Fig. 3E,F for N1). Swollen nuclei contain high amounts of antigens b2-2B10 (N1, Fig. 4A), L6-8A7 (L111, Fig. 4B) 32-4A1 (Fig. 5A) and nucleoplasmin (not shown), antigens 37-1A9 and 35-1A7 in moderate amounts (not shown). Antigens 37-1B2 and 32-5B6 were not significantly accumulated after 2 h (Fig. 5E,F, Table 1). After 8 h, antigen 32-5B6, in contrast to early-migrating antigens, is still not accumulated (not shown). I conclude that additional pronuclei formed after injection of sperm nuclei apparently assemble the same subset of germinal-vesicle-derived proteins that are found in cleavage nuclei (Table 1).

In order to assess whether this selectivity is solely inherent to sperm-derived pronuclei, somatic nuclei have been injected into unfertilized eggs. I have chosen erythrocyte nuclei, since like sperm nuclei they do not contain any of the antigens to be tested. In contrast to sperm nuclei, they contain a lamina of the somatic type (Krohne, Dabauvalle & Franke, 1981). As shown in Fig. 6 and Table 1, erythrocyte nuclei also swell in the egg and accumulate early-shifting, but exclude late-shifting, antigens. Their swelling is accompanied by the assembly of a nuclear lamina that must consist almost exclusively of L111, since the nuclei shown in Fig. 6 have up to ten times the diameter of an original erythrocyte nucleus. Moreover, mAB L6-8A7 stains the oocyte-type lamina more strongly than the original somatic lamina (not shown). The highly variable size of the nuclei is most probably due to the fact that erythrocyte nuclei tend to aggregate upon injection.

Pronucleus-like entities can also be formed after injection of pure DNA into unfertilized eggs (Forbes, Kirschner & Newport, 1983). I have injected λ-DNA, which was subsequently identified on sections from the eggs by staining with DAPI. Staining of the same sections with monoclonal antibodies shows that a lamina is assembled around the DNA and that early-shifting antigens are accumulated (Fig. 7A), whereas late-shifting antigens are excluded (Fig. 7C). Antigens shifting to the nuclei at blastula stages (37-1A9 and b6-6E7) are taken up to a moderate degree (Table 1; Dreyer et al. 1986). The size of the resulting entities is even more variable than after injection of erythrocyte nuclei. This seems to depend on the formation of aggregates of the injected DNA (compare Fig. 7B,D). Nuclei formed from a single molecule of λ-DNA would most probably not be detected.

In conclusion, the unfertilized egg contains all the components required for the formation of multiple pronuclei. Pronuclei are formed not only from sperm nuclei but also from somatic cell nuclei and even bacteriophage DNA. The source of the nuclei and the sequence of the DNA do not seem to influence the selective accumulation of early-migrating nuclear proteins.

(C) Properties of early- and late-shifting nuclear antigens

When proteins isolated from the germinal vesicle are injected into the cytoplasm of the oocyte, they accumulate in the oocyte nucleus (Gurdon, 1970; Bonner, 1975; De Robertis, Longthorne & Gurdon, 1978; Dingwall, Sharnick & Laskey, 1982). Proteins
Fig. 2. Nucleocytoplasmic distribution of nucleoplasmin in embryos of *X. laevis*. Embryos after the 7th (A,B), 11th (C,D), and about the 13th cleavage division (E, F) were fixed according to Romeis and embedded in wax. Sections were stained with mAB b7-1A9 that binds to nucleoplasmin. Details of the sections shown in the left panels (A,C,E) are shown in the right panels (B,D,F). Bar 100 μm.
Fig. 3. Kinetics of pronucleus formation after injection of sperm nuclei into unfertilized eggs of *X. laevis*. Sperm nuclei were isolated and injected into eggs as detailed in Materials and methods. Eggs were fixed with TCA 15 min (A,B), 30 min (C,D), and 60 min after injection, embedded in wax, and sectioned. Sections were stained with mAB b2-2B10, which binds to N1 (A,C,E) and counterstained with DAPI (B,D,F). Nuclei that do not swell do not take up the antigen to a significant degree (E,F). Bar 100 µm.
Fig. 4. Multiple pronuclei formed after injection of sperm nuclei into unfertilized eggs. *X. laevis* eggs were fixed with TCA 2 h after injection of sperm nuclei. Wax sections were stained with mABs b2-2B10 (A) and L6-8A7, which binds to lamin Lm (B). Counterstaining with DAPI showed that all nuclei had taken up both antigens (not shown). Bar 100 μm.
Fig. 5. Selective uptake of early-shifting nuclear antigens into injected sperm nuclei. Eggs were fixed with TCA 2 h after injection of sperm nuclei. Wax sections were stained with mABs 32-4A1 (A), 37-1B2 (C), P3 control antibody IgGl secreted by mouse cell line P3X63 Ag8 (E). Counterstaining of each of these sections with DAPI is shown on the right panels (B,D,F). Bar 50 μm.

N1, N2, N4 and nucleoplasmin have previously been described as GV-proteins that are rapidly reaccumulated by the germinal vesicle in such injection experiments and they are also accumulated early by the nuclei of the embryo. I have labelled oocyte proteins in vivo and have investigated which newly synthesized proteins are accumulated by the oocyte nucleus after a certain period of time. For this purpose, oocytes were manually enucleated either 5 or 15 h after injection of [35S]methionine and the labelled proteins of total oocytes and of their nuclei have been compared by two-dimensional gel analysis (Fig. 8).

After 5 h of labelling, some polypeptides are over-represented in the nuclei as compared to the total complement of labelled polypeptides (Fig. 8A,B). Their concentration in the oocyte nucleus is more obvious after 15 h of labelling (Fig. 8C,D). Among the most-prominent labelled nuclear proteins after a short pulse are N1, N2 and N4, which are also contained in cleavage nuclei and in artificial pronuclei. Nucleoplasmin is not labelled under these conditions. The late-migrating antigens 35-1A7 (5) 37-1A9 (7) and 32-5B6 (21) are labelled, but not accumulated in the nucleus to a significant degree.

The same applies to some prominent cytoplasmic proteins, e.g. tubulin and k.I. Proteins 9, 10 and actin are found in both the nucleus and the cytoplasm. Nucleocytoplasmic concentration ratios have been calculated for a number of prominent polypeptides after excision of the corresponding areas from the two-dimensional gels and determination of radioactivity (Table 2). Early-shifting proteins are characterized by the highest accumulation ratios, as far as they could be assayed by this method. These results correlate to the partition coefficients between nucleus and cytoplasm of germinal vesicle proteins in the steady state, although the latter are much higher than the ratios measured after pulse labelling (Paine, 1982; Table 2). As may be derived from Table 2, early-migrating antigens are among the polypeptides that are most efficiently concentrated in the oocyte nucleus after their synthesis in the cytoplasm.

Egg maturation is accompanied by a tremendous increase in protein phosphorylation (Maller, Wu & Gerhard, 1977). Although nucleoplasmin, and N1 and N2 are among the most prominent substrates of protein kinases in maturing eggs, protein phosphorylation cannot easily explain the difference between early- and late-migrating antigens. The early-shifting protein, N4, is not phosphorylated to a significant degree, whereas the later-shifting proteins 5 and 7 (antigens 35-1A7 and 37-1A9) are phosphorylated during egg maturation (Fig. 9). The phosphorylation of 37-1A9 and of 32-5B6 during egg maturation has been corroborated by immunoprecipitation of the 32P-labelled antigens. Phosphorylation of antigen 32-5B6 is close to the limit of detection (data not shown). In oocytes, no [32P]phosphate label was detectable in antigens 37-1A9 and 32-5B6.
Influence of cell cycle length on the accumulation of nuclear proteins

The data above show that different proteins are accumulated into oocyte nuclei with different efficiencies (Fig. 8; Table 2; Paine, 1982). One hypothesis that follows from this observation is that the short cell cycles of early cleavage stages would allow enough time for efficiently accumulated proteins to translocate into nuclei, but no significant appearance of inefficiently accumulated proteins would occur. This difference could then account for the differences observed between early- and late-shifting antigens.

I have therefore tried to extend the length of the cell cycle artificially by application of inhibitors. Since inhibitors may have cytotoxic side effects that might impair transport of nuclear proteins in general, two inhibitors have been applied that both cause cell cycle arrest, but by different mechanisms. Inhibition of translation by cycloheximide leads to cell-cycle arrest between S-phase and mitosis (Miake-Lye, Newport & Kirschner, 1983). Aphidicolin, an inhibitor of DNA polymerase α, prevents initiation of the next S phase (Pedrali-Noy et al. 1980).

Embryos were transferred to cycloheximide at blastula stage 7. 4h later, when controls had advanced to the late blastula stage 9, embryos were fixed and their nuclei assayed for antigen content on wax sections (Fig. 10). Three classes of antigens have been investigated, antigens normally found in the nuclei at cleavage, antigens gradually shifting to the
Fig. 7. Artificial pronuclei formed after injection of λ-DNA. Unfertilized eggs of *X. borealis* were injected with 5–10 ng of λ-DNA each. 2 h after injection, eggs were fixed with TCA. Wax sections were stained with mABs b2-2B10 (A) and 32-5B6 (C). Counterstaining of each section with DAPI is shown on the right panels (B, D). Bar 50 μm.

Table 2. Nucleocytoplasmic distribution of germinal vesicle proteins

<table>
<thead>
<tr>
<th>Polypeptide*</th>
<th>( \frac{N}{C} ) (5 h pulse)†</th>
<th>( \frac{N}{C} ) (15 h pulse)</th>
<th>( \frac{N}{C} ) concentration ratio at equilibrium‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass ratio</td>
<td>Conc. ratio</td>
<td>Mass ratio</td>
</tr>
<tr>
<td>Early-shifting antigens:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nucleoplasmin</td>
<td>(30)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>( N_1/N_2 )</td>
<td>(3/4)</td>
<td>1.3</td>
<td>9.5</td>
</tr>
<tr>
<td>( N_4 )</td>
<td>(29)</td>
<td>0.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Later-shifting antigens:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-1A7</td>
<td>(5)</td>
<td>0.04</td>
<td>0.3</td>
</tr>
<tr>
<td>32-5B6</td>
<td>(21)</td>
<td>0.07</td>
<td>0.5</td>
</tr>
<tr>
<td>Other nuclear proteins:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-actin</td>
<td>(22)</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>(9)</td>
<td>0.14</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>(10)</td>
<td>0.08</td>
<td>0.6</td>
<td>0.3</td>
</tr>
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<td>Cytoplasmic proteins:</td>
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<tr>
<td>( \beta )-tubulin</td>
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<td>(g)</td>
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<td>(k)</td>
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<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>(l)</td>
<td>0.06</td>
<td>0.04</td>
<td>n.d.</td>
</tr>
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</table>

*Numbers and letters in parentheses refer to labelling by Dreyer & Hausen (1983).
†Mass ratios have been calculated after determination of radioactivity in individual polypeptide spots as shown in Fig. 8. Multiplication of the mass ratios by a factor of 7.3 converts them into concentration ratios, since the volume of the cytoplasm is approximately 7.3 times that of the nucleus. Nucleoplasmin is not labelled with \[^{35}S\]methionine under these conditions.
‡Numbers in parentheses refer to labelling by Paine (1982).
n.d.: not done.
Fig. 8. Nucleocytoplasmic distribution of oocyte proteins synthesized de novo. Oocytes of X. laevis were labelled with [35S]methionine for 5 h (A,B) or 15 h (C,D), manually enucleated and protein preparations of either four total oocytes (A,C) or of 30 nuclei were subjected to two-dimensional gel analysis and fluorography as detailed in Materials and methods. After fluorography, the areas at the position of the prominent nuclear and cytoplasmic proteins indicated in the figure were excised from each of the four gels and the amount of radioactivity in each area determined by liquid scintillation counting. The results are given in Table 2. A, actin; T, β-tubulin; N1, antigen b2-2B10; 5, antigen 35-1A7; 7, antigen 37-1A9; 21, antigen 32-5B6 (Dreyer & Hausen, 1983; Dreyer et al. 1985). Nucleoplasmin is not labelled under these conditions.

nuclei during blastula stages and antigens accumulated during or after gastrulation (Table 1). As shown in Fig. 10C, substantial extension of the interphase length by addition of 100 μg ml⁻¹ of cycloheximide did not result in uptake of late-shifting antigen 32-5B6 into the nuclei, whereas N1 was still detectable in the nuclei by mAB b2-2B10 (Fig. 10D). No significant changes after cell-cycle arrest have been observed for any of the antigens tested. The effect of cycloheximide is easily seen in that the cells and their nuclei are much larger (Fig. 10C,D) than those of the control siblings (Fig. 10A,B). After cell-cycle arrest by injection of aphidicolin, the result was the same, i.e. late-shifting antigens remained excluded from the nuclei (data not shown).

Discussion

The germinal vesicle is an extraordinarily large nucleus in relation to its content of DNA, which amounts to four times the haploid genome. The GV proteins are later found in the nuclei of all somatic cells of the embryo, although certain protein species appear to be excluded at early stages of development.
Fig. 9. Phosphorylation of germinal vesicle proteins in oocytes and maturing eggs of *X. laevis*. Oocytes in the absence (A,B) or presence (C) of progesterone were labelled with $[^{32}\text{P}]$phosphate for 4 h as detailed in Materials and methods. Protein preparations were subjected to two-dimensional gel analysis. The gels were stained with silver and analysed by autoradiography. (A) Oocyte proteins stained with silver. Isoelectric focusing was from right to left, SDS-polyacrylamide gel electrophoresis from top to bottom. (B) Autoradiograph of the gel shown in A. (C) Autoradiograph of gel showing $^{32}\text{P}$-labelled proteins from maturing egg. Protein patterns of oocytes and of maturing eggs were not significantly different after silver staining (not shown). Germinal vesicle proteins are numbered as previously. Nu, nucleoplasmin.
Oocyte nuclear protein accumulation in Xenopus

(Fig. 1; Dreyer et al. 1982, 1983). A similar observation has been made in early development of Drosophila, where certain maternal nuclear antigens are not shifted into the nuclei before the syncytial blastoderm stage (Dequin, Saumweber & Sedat, 1984).

Injections of sperm nuclei, erythrocyte nuclei and of pure bacteriophage DNA into unfertilized Xenopus eggs have each resulted in the formation of artificial pronuclei that accumulate the same complement of nuclear antigens that are normally found in cleavage nuclei (Table 1). Since all injected nuclei swell strongly in the egg cytoplasm, nuclear envelope components of somatic origin, e.g. the somatic lamsins of erythrocyte nuclei, become highly diluted with the result that the composition of the nuclear envelope will ultimately be solely dependent on components provided by the egg cytoplasm. This is probably the reason for the finding that the source of the injected nuclei does not seem to influence their selectivity of protein uptake.

Of the six early-shifting antigens described here, four have been previously described as polypeptides that are most highly concentrated in the germinal vesicle (Paine, 1982). The same polypeptides appear to reaccumulate rapidly in the germinal vesicle after injection into the cytoplasm (Bonner, 1975; De Robertis et al. 1978). Three of these early-shifting polypeptides are labelled with $^{35}$S methionine and are rapidly translocated to the germinal vesicle after their synthesis in the cytoplasm, whereas other nuclear polypeptides appear to be accumulated more slowly.

Thus, the efficiency of accumulation of a protein in the germinal vesicle seems to correlate positively with its ability to translocate to the nuclei at an early stage of development. Although this correlation is limited by the fact that not all of the antigens studied here could be identified on the gels shown by Paine (1982) and on the autoradiographs shown in Fig. 8, it suggests that the efficiency of translocation into nuclei is an inherent property of each protein.

The signal hypothesis has predicted that nuclear proteins contain a targeting sequence that guides them into their compartment (De Robertis, 1978). Such signals are most probably integral and permanent parts of nuclear proteins, which have to be reassembled into their compartment after each mitosis. There is no indication of covalent changes on late-migrating nuclear proteins before and after their shift into embryonic nuclei (Dreyer et al. 1982; Dreyer & Hausen, 1983). Proteins translated in vitro, without post-translational modifications, fulfill the requirements for being accumulated by oocyte nuclei (Dabauvalle & Franke, 1982).

There is, however, an enhanced phosphorylation of nuclear proteins upon germinal vesicle breakdown (Fig. 9; Maller et al. 1977), and reaccumulation of lamins and of nucleoplasmin after mitosis is accompanied by dephosphorylation (Gerace & Blobel, 1980; Newmeyer, Lucoq, Bürglin & De Robertis, 1986). Although several of the early-migrating proteins are very acidic proteins that are intensely phosphorylated during egg maturation, there is no unambiguous relationship between phosphorylation and time of nuclear translocation of a protein (Fig. 9). Phosphorylation of nucleoplasmin is thought to increase its binding capacity for histones (Sealy, Cotten & Chalkley, 1986).

Several signal sequences have recently been defined in nuclear proteins of viruses, yeast and in N$_1$, N$_2$, and nucleoplasmin of X. laevis using spontaneous and artificial mutants and fusion proteins (Hall, Hereford & Herskowitz, 1984; Lanford & Butel, 1984; Kalderon, Richardson, Markham & Smith, 1984; Dingwall et al. 1986; Dingwall & Laskey, 1986; Kleinschmidt, Dingwall, Maier & Franke, 1986; Bürglin et al. 1987; for review see Dingwall & Laskey, 1986). Their heterogeneity could explain why different nuclear proteins are accumulated with different efficiencies. Moreover, nucleoplasmin as an avidly accumulated protein contains several sequence elements resembling the signal sequence identified in viral and in yeast nuclear proteins (Dingwall et al. 1986; Bürglin et al. 1987). They are all contained in a proteolytic fragment of nucleoplasmin that can migrate into nuclei on its own (Dingwall et al. 1982) and that can guide large gold particles through the nuclear pores (Feldherr, Kallenbach & Schultz, 1984). Another signal has been tentatively identified on N$_1$/N$_2$ (Kleinschmidt et al. 1986).

Nuclear targeting sequences with different efficiencies could explain why proteins that translocate rapidly into the oocyte nucleus after their synthesis (Fig. 8) are also quickly accumulated by cleavage nuclei. The hypothesis that different nuclear targeting sequences are present in different classes of nuclear proteins will now be testable, since cDNA clones representing sequences of two late-shifting proteins have been selected from a $\lambda$-gt11 expression gene library (Eastman, Dreyer, Hausen & Etkin, 1986). If different nuclear proteins contain nuclear targeting sequences of different efficiency or multiplicity, the length of the cell cycle may play a crucial role for the complement of nuclear proteins that are

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**Fig. 10.** Cell cycle arrest with cycloheximide. Embryos of X. laevis were transferred to $100\mu$g ml$^{-1}$ of cycloheximide in MBSH at the 64-cell stage and incubated therein for 4 h, when controls had reached stage 9$^*$. Experimental and control embryos were fixed by freeze substitution, and wax sections were stained with mAB 32-5B6 (A,C) and b2-2B10 (B,D). (A,B) Controls; (C,D) embryos treated with cycloheximide. Bar 100$\mu$m.
accumulated in embryonic nuclei. A cell-cycle length of 25 min at cleavage might only allow accumulation of the proteins containing the most potent signals. After the midblastula transition, cell-cycle length gradually increases to 1 h after the 13th and to 2-5 h after the 14th cleavage (Boterenbrood et al. 1983). During this period, antigens 37-1A9 and b6-6E7 are accumulated (Table 1; Dreyer et al. 1982). Between gastrula and neurula, cell cycles take about 3-5 h in the ectoderm. During this period, the latest antigens are accumulated (Table 1). Artificial elongation of the cell cycle using cycloheximide or aphidicolin did not significantly change the complement of nuclear proteins before gastrulation (Fig. 10). The evidence from these experiments might, however, be limited due to possible side effects of the inhibitors on embryos. In fibroblasts derived from X. laevis tadpoles, nuclear lamin antigens are reaccumulated after each mitosis during telophase. No significant difference could be observed in the kinetics of reaccumulation of lamin and and of different nuclear antigens, irrespective of their nucleocytoplasmic distribution in the early embryo (B. Bühler unpublished data).

Therefore, we suppose that the length of the cell cycle in combination with different nuclear targeting sequences is not sufficient to explain the differential behaviour of early- and late-migrating proteins in the embryo. Late-shifting proteins might require a factor for transport (Mattaj & De Robertis, 1985) that is not active in early embryogenesis. This question could be addressed by using an in vitro system (Dreyer et al. 1986; C. Dreyer, unpublished data). Alternatively, the structure of the nuclear envelope may change in development. A cascade of gradual changes has recently been described for the nuclear lamina composition in embryos of X. laevis (Stick & Hausen, 1985). This is probably not the cause for the exclusion of late nuclear antigens at early stages, since the germinal vesicle accumulates all of the antigens studied and contains only lamin L_{II}. Nevertheless, the structural changes of lamina composition might be a paradigm for other changes in development. So far, it is not known whether the nuclear pores change in structure or in number per nucleus with development.

The biological significance of early- and late-shifting proteins most probably pertains to the exceptional physiological state of the early embryonic cells. In X. laevis, the embryo cleaves with an average cell-cycle length of less than 30 min up to the midblastula transition (Newport & Kirschner, 1982; Boterenbrood et al. 1983). During this period rapid DNA replication but no significant transcription is observed (Brown & Littna, 1966; Bachvarova & Davidson, 1966). The cell cycle is probably reduced to a sequence of S-phases and mitoses (Graham & Morgan, 1966). After the midblastula transition, RNA polymerases II and III resume transcription (Gurdon & Woodland, 1969; Newport & Kirschner, 1982). Transcription of ribosomal RNA does not start before gastrulation, when newly formed nucleoli can be cytologically identified (Gurdon & Brown, 1965).

It is therefore obvious to assume that early-migrating nuclear antigens serve a function required from the first cell cycle onward whereas the function of later-migrating nuclear proteins has to be sought in the context of transcription, processing and transport of newly formed RNA, or other activities resumed at later stages. Of the early-migrating antigens described here, N_{1}, N_{2} and nucleoplasmin are thought to complex histones and to aid nucleosome assembly (Laskey, Honda, Mills & Finch, 1978; Kleinschmidt & Franke, 1982; Kleinschmidt et al. 1985; for review see Laskey, 1985). These are obviously functions required at the onset of chromosome replication. The lamin L_{II} is the only nuclear lamin found in cleavage nuclei before stage 8 (Stick & Hausen, 1985). The functions of the other early-migrating antigens 32-4A1 and b7-2H4 (N_{4}) are so far unknown.

The functions of the later-migrating antigens studied here are not known. However, antigen b6-6E7 is a DNA-binding nucleolar protein. Injection of the corresponding antibody into oocyte nuclei inhibits nucleolar RNA synthesis (Retzbach, 1987). Antigens 32-5B6, b7-1B4 and 37-1A9 are later highly enriched in the nuclei of specific cell types (Dreyer et al. 1981, 1983; Wedlich & Dreyer, 1987). Proteins that bind to small nuclear URNA are stored in the cytoplasm early in embryogenesis, but are translocated to the nuclei after the onset of transcription of the corresponding RNA. They are concentrated in the nuclei of gastrula (De Robertis, 1983; Zeller, Nyffenegger & De Robertis, 1983; Fritz, Parisot, Newmeyer & De Robertis, 1984). The proteins shifting to the nuclei in Drosophila at blastoderm have been characterized as associated with hnRNP (Risau, Symmons, Saumweber & Frasch, 1983).

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References


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