Nuclear-envelope vesicles as a model system to study nucleocytoplasmic transport

Specific uptake of nuclear proteins

Norbert RIEDEL* and Hugo FASOLD

Institut für Biochemie der Johann-Wolfgang-Goethe-Universität, Haus 75A des Klinikums, Theodor-Stern-Kai 7, D-6000 Frankfurt am Main 70, Federal Republic of Germany

In the preceding paper [Riedel & Fasold (1987) *Biochem. J.* **241**, 203–212] we have described a procedure for the preparation of nuclear-envelope vesicles (NE vesicles) from rat liver nuclei. These vesicles, which are largely free of components of the nuclear interior, were employed in an assay system *in vitro* to study protein translocation across the NE. We found that nuclear proteins such as histones, high-mobility-group proteins and acidic chromosomal proteins are specifically taken up and accumulated in the NE vesicles, whereas there is little or no affinity for non-nuclear proteins like immunoglobulin, myoglobin and cytochrome *c*. The kinetics of histone uptake into the NE vesicles are similar to those obtained for whole rat liver nuclei, and comparative studies with non-vesicular NEs prepared by deoxyribonuclease I-treatment (DNAase-NEs) indicate that the NE of the vesicles affects the uptake kinetics and increases the capacity for nuclear proteins. The uptake of histones into NE vesicles, but not the binding to DNAase-NEs, can be stimulated by GTP and GDP. Furthermore, we found that even very large molecules can be entrapped in the vesicles during their preparation. These results indicate that the NE vesicles might provide a useful system *in vitro* with which to investigate the structures and mechanisms involved in protein translocation across the NE.

INTRODUCTION

The nuclear envelope (NE) is a characteristic structure of the eukaryotic cell. It consists of an inner and an outer membrane which are separated by the nuclear space [1]. The outer membrane is connected to the endoplasmic reticulum, whereas the inner membrane faces the nucleoplasm and is tightly associated with the fibrous lamina [2]. The lamina is spaced between the inner nuclear membrane and the peripheral chromatin and is found in most eukaryotes [3].

Other characteristic features of the NE are the pore complexes. The structure and composition of the pore complexes have not been completely analysed. Under the electron microscope the pores appear as cylinders that span the NE. They show an octagonal symmetry around a central axis perpendicular to the membrane. Eight globular subunits with diameters of approx. 22 nm form an outer and an inner ring around the pore. A central 'plug' is located in the centre of the pore and is connected with the periphery via 'spokes'. The whole pore complex, which has an outer diameter of 120 nm [4,5] can be described as a 'triple-layered sandwich' consisting of the spokes and the central plug between the outer and inner ring.

The NE is of particular interest because of its likely role in nucleocytoplasmic transport processes. The pore complexes are the most prominent candidates as pathways mediating the exchange of molecules between the nucleus and the cytoplasm. Early microinjection

experiments with amoebae using gold particles showed that these were taken up into the nucleus through the pore complexes [6]. Measurements with oocytes using labelled proteins [7,8,9] or dextrans of different size [10] showed that the diameter of the pore is of the order of 9 nm [10].

Small molecules and ions can move across the NE via diffusion [11,12]. Measurements with larger molecules, like myoglobin, γ -globulin, ovalbumin and bovine serum albumin showed that the size of the protein seems to be a decisive parameter for passage through the nuclear envelope [8]. Proteins with a size in the range of the pore diameter need hours to enter the nucleus [7]. However, the uptake of at least some large nuclear proteins seems to be mediated and regulated by the pore complexes, which is shown by the fact that their uptake into the nucleus is about 20 times faster than could be explained by a diffusional process [13]. For nucleoplasmin, a pentamer of M_r 165000, it could be demonstrated that a region of the monomer corresponding to M_r 12000 is necessary for selective transport into the nucleus [14]. For the nuclear proteins N1–N4 it was shown that the cytoplasmic precursors and the accumulated proteins in the nucleus are identical, indicating that the signal sequence that directs these proteins to the nucleus is an internal part of the translational product [15]. This is also supported by experiments using simian-virus-40 large T antigen, which show that a small region of the protein serves as a signal sequence for directed transport into the nucleus. Fusion proteins containing the signal

Abbreviations used: NE, nuclear envelope; DNAase-NEs, deoxyribonuclease I-prepared nuclear envelopes; HMG proteins, high-mobility-group proteins; NTPase, nucleoside triphosphatase (EC 3.6.1.15); mRNP, messenger ribonucleoprotein.

* Present address and address for correspondence and reprint requests: Department of Cancer Biology, Harvard University School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA.

sequence and typical cytoplasmic or bacterial proteins were accumulated in the nucleus [16,17].

In the preceding paper we described the preparation and characterization of NE vesicles. These vesicles contain the components of the NE, i.e. the lamina and the pore complexes, in the same orientation as in whole nuclei. In contrast, they contain only 1.5% of the DNA of the nucleus and are mostly free of the components of the nuclear interior. By means of microscopic and biochemical procedures we showed that the preparations contain about 80% sealed vesicles and are enriched in the Mg^{2+} -dependent NTPase [18] that mediates nucleocytoplasmic transport processes. Here we describe protein-uptake measurements performed with these vesicles using radiolabelled nuclear and non-nuclear proteins and show that the characteristics for their uptake are very similar to those of whole nuclei. Our results indicate that the NE itself affects the uptake kinetics and capacity for nuclear proteins. We believe that these vesicles provide a valuable model system for further elucidating the mechanism and regulation of transport processes across the NE.

MATERIALS AND METHODS

Nucleoside phosphates, polylysine (M_r 15000–25000), sperm-whale myoglobin, γ -globulin and cytochrome *c* were purchased from Sigma Chemical Co. Proteins were labelled in 50 mM- $NaHCO_3$ solution by addition of ^{14}C -labelled acetic anhydride (Amersham Buchler Co.) with a specific radioactivity of 120 mCi/mmol. The proteins were then dialysed against the buffer for transport measurements. The final specific radioactivity amounted to 30000–50000 c.p.m./mg. Thus between one and two side chains per M_r -15000 protein were modified.

Rat liver nuclei, NE vesicles, and DNAase-NEs were prepared by using the protocols described in the preceding paper [18a].

Polyacrylamide-gel electrophoresis was performed as described by Laemmli [19].

Influx and efflux measurements

These measurements were performed by using a modification [18] of the procedure of Klingenberg & Pfaff [20] to measure the uptake or efflux even after very short incubation times, i.e. of the order of 5 s. NE vesicles were suspended at a protein concentration of 2 mg/ml in an incubation buffer containing 50 mM-Tris-HCl, pH 7.4, 25 mM-KCl, 2.5 mM- $MgCl_2$, 0.5 mM- $CaCl_2$, 5 mM-NaCl, 2.5 mM- Na_2HPO_4 and 5 mM-spermidine. Portions (0.15 ml each) were layered on 0.04 ml of silicone oil of relative density (*d*) 1.03 in 0.4 ml polypropylene Microfuge tubes, containing 10 μ l of $HClO_4$ (*d* 1.06) at the bottom. After addition of 10 μ l of the labelled protein solutions and incubation for the appropriate time the uptake was stopped by starting the Microfuge and separating the vesicles from the incubation medium. After aspiration of the top layer and the silicone oil the pellets were transferred to scintillation vials and counted for radioactivity in a liquid-scintillation counter (Beckman LS 3155 T).

For efflux measurements, 0.15 ml of the vesicle suspension was layered on top of the silicone oil and the efflux stopped by starting the centrifuge. In these cases the radioactivity in 0.13 ml of the incubation medium and in the pellets was measured.

Preparation of histones

The procedure of Johns [21] was used for the preparation of histones. In brief, calf thymus was minced and homogenized several times in 0.9% NaCl and then in ethanol. Histones H2A, H3 and H4 were then extracted by using a mixture of ethanol and HCl, followed by the extraction of histones H1 and H2B with HCl alone. The different histones were then separated by selective precipitation. For the uptake measurements a mixture of the five different histones was used.

Preparation of HMG proteins and acidic chromosomal proteins.

HMG proteins were prepared by using the procedure of Sanders [22]. The HMG proteins, which have M_r values below 30000 and a high content of acidic and basic amino acids, can be extracted from chromatin with $HClO_4$. After precipitating out histone H1, the HMG proteins can be precipitated with acetone. Further purification can then be achieved by using CM-Sephadex C-25 chromatography, yielding a fraction that is highly enriched in the HMG proteins 1, 2, 14 and 17.

The preparation of acidic chromosomal proteins was preceded by the preparation of rat liver nuclei as described above. The chromatin fraction was then isolated as described by Spelsberg & Hnilica [23], followed by the extraction of the acidic chromosomal proteins as described by Wilson & Spelsberg [24].

RESULTS

Flux measurements across the NE of the vesicles

Whole nuclei contain chromatin and the nuclear matrix. Microinjection experiments and experiments with isolated whole nuclei therefore make it difficult to determine whether a nuclear accumulation of proteins is caused by specific binding to components of the nuclear interior or by transport processes regulated by components of the NE itself.

NE vesicles have the important advantage that they are free of most of the nuclear contents, but retain the components of the NE. Therefore we wanted to investigate the uptake kinetics of some nuclear and non-nuclear proteins.

Measurements with ^{14}C -labelled histones

Fig. 1 shows a polyacrylamide gel of a histone mixture prepared as described by Johns [21]. The mixture consists of the histones H1, H2A, H2B, H3 (in low concentration) and H4, which were labelled with ^{14}C acetic anhydride. The specific radioactivity of the histones, and also of the proteins described below, was in the range of 30000–50000 c.p.m./mg. Thus between one and two side chains per 15000 protein M_r were modified. The low specific radioactivity was chosen to eliminate the possibility that the modification could affect the binding characteristics of the proteins.

To prove the usefulness of the histone mixture for transport measurements, we first performed uptake measurements with whole rat liver nuclei. Earlier experiments showed that histones can be accumulated in the nucleus [25] and that their uptake under '*in vivo*' conditions using erythroblasts [26] or isolated nuclei from HeLa cells [27] is a very fast process. Our measurements using isolated rat liver nuclei showed a

Fig. 1. Polyacrylamide (18%) gel of the histone mixture used in the described uptake measurements

Portions (10, 20 and 30 μg , left to right) were loaded and run together with the M_r markers ovalbumin (44 600) and trypsin inhibitor (20 100) shown at the very left and the very right respectively.

very rapid uptake of the histones, reaching a maximum at approx. 90 s (results not shown).

Kinetics of histone uptake into NE vesicles

The kinetics of histone uptake into NE vesicles is shown in Fig. 2 in comparison with NEs prepared by using DNAase I. The uptake of histones is seen to be a very fast process, having its maximum at 2 min, after which a plateau is reached. On the basis of the DNA content of the vesicles we can estimate that the amount of histones taken up is 2.5 $\mu\text{g}/\mu\text{g}$ of DNA under the conditions used. NEs prepared with DNAase I [28] do not show similar kinetics. Whereas the uptake of histones into the vesicles increased by 140% in the first 300 s, the equivalent value in the case of the DNAase-NEs was only 48%. This probably reflects an absorption to components of the NE, and it reached a plateau after 50 s. On the basis of the DNA content, the capacity for histones was 45% higher in the case of the NE vesicles. Accumulation of histones by binding to the vesicular interior alone (and here most likely to residual DNA) cannot account for the observed uptake kinetics, because the non-vesicular NEs prepared by DNAase I did not show very similar kinetics. Thus we believe that the NE itself contributes to the difference in uptake kinetics and the much higher capacity of the vesicles for histones (see below).

We tested a variety of nucleotides, e.g. GTP, GDP, GMP, cGMP, ATP, cAMP and CTP, as well as guanosine

Fig. 2. Kinetics of histone uptake into NE vesicles in the presence (○) or absence (●) of 10 mM-GTP

In contrast with the vesicles, the kinetics for the DNAase I-treated NEs is very different (+), shows no GTP effect (×) and seems to reflect a simple adsorption process.

for their effect on histone uptake. As Fig. 2 shows, GTP at a concentration of 10 mM stimulated the uptake of histones into the vesicles, whereas no effect on the DNAase-NEs was measurable. None of the other nucleotides tested had an effect.

NE vesicles were incubated with radiolabelled histones for 20 min in the presence or absence of GTP to obtain maximum uptake (set as 100%). The exchange rate was then measured by adding a 10-fold excess of non-radioactive histones followed by separation of the vesicles by centrifugation at different time points between 5 and 300 s and measurement of the radioactivity in the supernatants and in the vesicle pellets. In the absence of GTP the exchange-efflux of radioactivity from the vesicles reached a plateau after approx. 120 s, at which time only 65% of the maximum radioactivity remained associated with the vesicular pellet (results not shown). This indicates that approx. 35% of the radioactivity taken up by the vesicles was not stably bound to components of the vesicular interior. Histones were recovered from these supernatants and analysed by polyacrylamide-gel electrophoresis. The composition was found to be indistinguishable from the starting material used to 'load' the vesicles. The remaining radioactivity is in the range of the values observed for the DNAase-NEs, indicating similar binding capacities for the two preparations. We estimate that the internal volume of the vesicles in the incubation medium is approx. 1.7% (see the preceding paper [18a]). Thus these results suggest that the NE of the vesicles is involved in

the accumulation of histones. The exchange-efflux in the presence of GTP was only 5% of the maximum radioactivity over the time course of 300 s, showing that GTP causes a retention of the histones in the vesicular interior (results not shown).

We do not know how GTP stimulates histone uptake and inhibits histone efflux. We have presently been using radiolabelled GTP and GTP analogues followed by a subfractionation of the NE vesicles in order to find components of the NE that have affinity for GTP and might be responsible for the increased uptake of histones.

Fig. 3 shows that the observed uptake of histones is not due to an adsorption to the outside of the vesicles. NE vesicles were first incubated with ^{14}C -labelled histones for 20 min to complete the uptake. All excess radioactivity was then removed by centrifugation through 30%-(w/v)-sucrose cushions. After resuspension of the vesicles, the radioactivity was measured and set as 100%. The vesicles were subsequently incubated with 1% Triton X-100 at 4°C for 10 min to remove the nuclear membranes, followed by a second centrifugation. Only 6.7% of the radioactivity was associated with the membrane-containing supernatant, while the remainder was still associated with the vesicular suspension.

Uptake measurements with histones using vesicles that were first treated with Triton X-100 to remove the membranes were also performed. The uptake kinetics obtained were very similar to those shown in Fig. 2, indicating a minor role for the nuclear membranes (results not shown).

Fig. 3. To demonstrate that the observed histone binding is not due to a simple adsorption to the outside of the vesicles, these were pre-loaded with labelled histones followed by measuring the distribution of the radioactivity after removal of the nuclear membranes using 1% Triton X-100

To exclude the possibility that the measured uptake and binding of histones is due to a non-specific binding to heparin which could remain associated with the NE vesicles, we prepared these vesicles using ^{14}C -labelled heparin. The labelling was performed by a procedure analogous to that used by Bendtzen & Rocklin [29]. A nuclear suspension in lysis buffer was then incubated with the labelled heparin to prepare vesicles and the radioactivity was measured. After the resulting NE vesicles were pelleted, they were washed and resuspended as usual. The vesicle suspension contained 0.056% of the originally added ^{14}C -heparin, which corresponds to 0.553 $\mu\text{g}/\text{mg}$ of vesicle protein. Since all the uptake measurements were performed at histone concentrations of 100 $\mu\text{g}/\text{mg}$ of vesicle protein, the histones were in a 180-fold excess compared with heparin. Thus the effect of heparin itself is probably negligible.

Uptake of HMG-proteins and acidic chromosomal proteins

In mammalian cells, HMG proteins form a group of four different proteins: HMG 1, 2, 14 and 17. Their concentration in the nucleus is within the range 10^5 – 10^6 molecules and they are thought to have gene-regulatory functions [30,31]. They are associated with approx. 10–20% of the nucleosomes, this association being predominantly [32], but not exclusively [30,33,34], with actively transcribed chromatin. HMG 14 and HMG 17 were found to have specific binding sites for nucleosomes [34,35], and HMG 1 and HMG 2 seem to play a direct role in destabilizing double-stranded DNA. In addition, it was shown that HMG 14 and HMG 17 are associated with the nuclear matrix and the NE [31]. Fig. 4 shows

Fig. 4. Polyacrylamide (18%) gel of HMG proteins before and after purification using CM-Sephadex C-25 chromatography

Lanes a and b represent 50 and 100 μg of HMG proteins before, and lanes c, d and e, 12, 24 and 36 μg of HMG proteins after, column chromatography. Lanes f and g show 5 and 10 μg of histone H1 co-purified by the method of Sanders [22].

a polyacrylamide gel of the HMG-protein preparation that was used in the uptake measurements.

In contrast with the well-characterized HMG proteins, the acidic chromosomal proteins comprise a broad spectrum of polypeptides in the M_r range 20 000–200 000. They bind specifically to DNA, but their function is poorly understood.

The kinetics of the uptake of these proteins into vesicles are shown in Fig. 5. In contrast with the histones, the uptake continued for approx. 4 h before a plateau was reached. Also, the binding capacity of the vesicles for these proteins was lower. We also performed uptake measurements in the presence of 10 mM-GTP and 10 mM-GDP respectively, but with no effect. A comparison of the 'uptake' of the acidic chromosomal proteins using DNAase-NEs showed that the process was much faster in reaching a plateau and that approximately the same amount of protein was bound (results not shown). This further indicates that the NE has an effect on uptake or binding kinetics, in this case by delaying the uptake process into the vesicles.

Uptake measurements of non-nuclear proteins

To judge whether the described kinetics indicate a specific process, it is necessary to determine whether the affinity of the vesicles is specific for nuclear proteins and if proteins which have no known function in the nucleus are not at all, or at least to a much lower extent, accumulated.

In the following experiments we used different non-nuclear proteins to measure their affinity for the NE vesicles. The results are shown in Fig. 6.

γ -Globulin has an M_r of 150 000 and is too large to traverse the NE of oocyte nuclei [8]. After microinjection,

Fig. 5. Kinetics of the uptake of HMG proteins (●) and acidic chromosomal proteins (○) into NE vesicles

Further details are given in the text.

Fig. 6. Uptake or binding kinetics of non-nuclear proteins in NE vesicles

Histones (▽), polylysine (△), histone H1 (×), myoglobin (○), γ -globulin (▲) and cytochrome *c* (●) were used at concentrations of 100 μ g per mg of vesicle protein and each value represents the mean of two separate measurements. For further details, see the text.

the nucleus/cytoplasm ratio has a value of 0.08 [8]. As Fig. 6 shows, we were unable to measure an uptake of γ -globulin during a time period of 3 h. The amount of radioactivity bound was only 6% and probably reflects an adsorption to the outside of the nuclear membrane. A value of approx. 6% was also found for the adsorption of histones (Fig. 3) and the ATP analogue *S*-dinitrophenyl-6-mercaptapurine riboside triphosphate. Myoglobin (M_r 17 500) was shown to be accumulated in the nucleus after microinjection into oocytes [7,8]. In the course of 2 h a nucleus/cytoplasm ratio of 2 can be measured and the uptake is due to a binding to intranuclear components [7]. Similar results were obtained by using NE vesicles; however, the myoglobin uptake was much slower compared with the very fast histone uptake and accumulation [Fig. 6], and the amount of myoglobin taken up reached only approx. 40% of the value for histones. We assume that these differences are due to different binding affinities and binding capacities for histones and myoglobin to residual components in the vesicular interior. However, it is noteworthy that histone H1 alone, which has the highest lysine content of all histones, as well as polylysine, show similar uptake kinetics compared with myoglobin, suggesting that, in the mixture of histones H1, H2A, H2B, H3 and H4, intravesicular aggregation of histones might also play a role in the kinetics observed (Fig. 6).

Cytochrome *c*, which has an M_r of 12 500 and an isoelectric point of 10, is a small and very basic protein which seems to be especially suitable for a comparison with histones. Early experiments using oocytes showed that this protein has no affinity for nuclei [7]. After microinjection, a nucleus/cytoplasm ratio of 1 is reached very rapidly and is then stable for several hours, reflecting a diffusion into the nucleus to equilibrium. The measurements using NE vesicles are consistent with this result. The increase of radioactivity from 2.7 to 4.7% probably represents a diffusion into the vesicles, which ends after 5 min.

The uptake measurements of all of the above-

mentioned proteins were also performed in the presence of 10 mM-GTP; however, no stimulating effect for any of the proteins was found.

In conclusion, these results show that the vesicles have a high affinity for nuclear proteins. The measurements using this 'in vitro' system yield results that are similar to those described in 'in vivo' experiments using oocytes [7,8] and in experiments using HeLa-cell nuclei [27].

DISCUSSION

The NE separates the eukaryotic cell into the nuclear and the cytoplasmic compartments. Thus the exchange of molecules occurs across the NE, presumably through the pore complexes, which are involved in regulation of these processes. Although the morphology of the pore complexes has been investigated, very little is known about those components of the NE that participate in the control of uptake and export processes. The uptake of proteins into nuclei by means of microinjection experiments into oocytes is well documented [7-9,13,15,25,36,37]; however, uptake measurements using isolated nuclei of mammalian cells have so far only been described by Cox [27].

We have performed protein-uptake measurements *in vitro* using NE vesicles. Nuclear and non-nuclear proteins showed uptake kinetics similar in many respects to those observed for intact nuclei. Histones were taken up and accumulated very rapidly, consistent with earlier observations using oocytes [8,25] and isolated HeLa-cell nuclei [27]. Triton X-100 removal of the nuclear membranes from vesicles preloaded with histones clearly demonstrated that adsorption to the outside of the vesicles is negligible and that the observed kinetics reflect a true uptake into the vesicular interior with subsequent binding of a portion of the histones to intravesicular components. We have currently been analysing the distribution of histones in the interior of the vesicles by using fluorescent proteins. In addition, our data suggest that the histone uptake into vesicles is only partly due to specific binding to residual intravesicular or NE components. The fact that NEs prepared by DNAase I treatment [28] have very different binding kinetics and a much lower capacity for histones (Fig. 2), in addition to the fact that only the uptake into the vesicles but not the binding to DNAase-NEs is stimulated in the presence of GTP and GDP, indicates that the NE itself has an effect on this process. This is further supported by the results obtained from exchange-efflux measurements, where only 65% of histones were found to be stably bound to intravesicular components and the amount of histones remaining bound corresponds to the binding capacity measured for DNAase-NEs, as shown in Fig. 2. How GTP and GDP stimulate the uptake is not known; the stimulating effect is retained if guanosine 5'-O-[3-methylene]triphosphate is used instead of GTP. Results by Cox [27] also argue against an energy-dependency of the protein uptake. Measurements using non-nuclear proteins showed that the vesicles have a high affinity only for proteins that are specific for the nuclear compartment, with the exception of myoglobin (Fig. 6). These results are also similar to those obtained using oocytes [7-9,36,38] and isolated HeLa-cell nuclei [27].

We believe that the NE-vesicles described here can be useful for further investigating the role of the NE in regulating nucleocytoplasmic transport processes. Pro-

teins below a certain M_r are not detectably retarded and seem to permeate the NE by simple diffusion. However, for larger proteins it was shown that they are taken up into the nucleus much faster than can be explained by simple diffusion. Those proteins contain internal signal sequences which direct them into the nucleus [14-17,39,40]. The nuclear accumulation of small nuclear RNAs requires the binding of specific proteins, which then seems to unmask a karyophilic signal for nuclear migration and accumulation [41]. Since the diameter of an mRNP particle is of the order of 20 nm [42] compared with a pore diameter of 9 nm [10], a model for the transport of these particles has been proposed in which the RNA unfolds in small local regions to interact with a transport mechanism in the NE. The Mg^{2+} -dependent NTPase is thought to be a component of this translocation mechanism, catalysing the active efflux of the mRNP particle with simultaneous hydrolysis of ATP [42]. Although an active transport mechanism has been shown for the efflux of mRNP particles [43-48], it is not yet known how the intrinsic signal sequences of nuclear proteins specify the uptake of these proteins into the nuclear compartment. In addition to the possibility that some such proteins might invade the nucleus during mitosis, at which time the NE breaks down transiently in most eukaryotes, it seems likely that these proteins interact with specific components that are involved in the transport across the NE. Once transported across the NE, these proteins could then be retained and accumulated by specific binding to intranuclear components.

We could also show that the described NE vesicles contain the translocation mechanism for mRNA and that they can catalyse a unidirectional transport of mRNA and poly(A) from the vesicular interior into the medium in the presence of ATP [49]. Thus these vesicles could prove most useful in elucidating the mechanism of nuclear transport and accumulation.

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REFERENCES

1. Afzelius, B. A. (1955) *Exp. Cell Res.* **8**, 147-158
2. Fawcett, D. W. (1966) *Am. J. Anat.* **119**, 129-146
3. Shelton, K. R., Egle, P. M. & Cochran, D. L. (1981) *Biochem. Biophys. Res. Commun.* **103**, 975-981
4. Green, N. M. (1982) *Nature (London)* **297**, 287-288
5. Unwin, P. N. T. & Milligan, R. A. (1982) *J. Cell Biol.* **93**, 63-75
6. Feldherr, C. M. (1965) *J. Cell Biol.* **25**, 43-51
7. Paine, P. L. & Feldherr, C. M. (1972) *Exp. Cell Res.* **74**, 81-98
8. Bonner, W. M. (1975) *J. Cell Biol.* **64**, 421-430
9. Bonner, W. M. (1975) *J. Cell Biol.* **64**, 431-437
10. Paine, P. L., Moore, L. C. & Horowitz, S. B. (1975) *Nature (London)* **254**, 109-114
11. Horowitz, S. B. (1972) *J. Cell Biol.* **54**, 609-625
12. Paine, P. L. (1975) *J. Cell Biol.* **66**, 652-657
13. Feldherr, C. M., Cohen, R. J. & Ogburn, J. A. (1983) *J. Cell Biol.* **96**, 1486-1490
14. Dingwall, C. S., Sharnick, V. & Laskey, R. A. (1982) *Cell* **30**, 449-458
15. Dabauvalle, M.-C. & Franke, W. W. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5302-5306
16. Kalderon, D., Roberts, B. L., Richardson, W. D. & Smith, A. E. (1984) *Cell* **39**, 499-509

17. Richardson, W. D., Roberts, B. L. & Smith, A. (1986) *Cell* **44**, 77–85
18. Kondor-Koch, C., Riedel, N., Valentin, R., Fasold, H. & Fischer, H. (1982) *Eur. J. Biochem.* **127**, 285–289
- 18a. Riedel, N. & Fasold, H. (1987) *Biochem. J.* **241**, 203–212
19. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
20. Klingenberg, M. & Pfaff, E. (1967) *Methods Enzymol.* **10**, 680
21. Johns, E. W. (1964) *Biochem. J.* **92**, 55–59
22. Sanders, C. (1977) *Biochem. Biophys. Res. Commun.* **78**, 1034–1042
23. Spelsberg, T. C. & Hnilica, L. S. (1971) *Biochim. Biophys. Acta* **228**, 202–211
24. Wilson, E. M. & Spelsberg, T. C. (1975) *Methods Enzymol.* **40**, 171–176
25. Gurdon, J. B. (1970) *Proc. R. Soc. London Ser. B* **176**, 303–314
26. Elgin, S. C. R. & Weintraub, H. (1975) *Annu. Rev. Biochem.* **44**, 725–774
27. Cox, G. S. (1982) *J. Cell Sci.* **58**, 363–384
28. Dwyer, N. & Blobel, G. (1976) *J. Cell Biol.* **70**, 581–591
29. Bendtzen, K. & Rocklin, R. E. (1980) *J. Immunol.* **125**, 1775–1781
30. Rabbani, A., Goodwin, G. H. & Johns, E. W. (1978) *Biochem. Biophys. Res. Commun.* **81**, 351–358
31. Reeves, R. & Chang, D. (1983) *J. Biol. Chem.* **258**, 679–687
32. Weisbrod, S. T. (1982) *Nucleic Acids Res.* **10**, 2017–2042
33. Goodwin, G. H. & Johns, E. W. (1978) *Biochim. Biophys. Acta* **519**, 279–284
34. Mardian, J. K. W., Paton, A. E., Bunick, G. J. & Olins, D. E. (1980) *Science* **209**, 1534–1536
35. Uberbacher, E. C., Mardian, J. K. W., Rossi, R. M., Olins, D. E. & Bunick, G. J. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5258–5262
36. De Robertis, E. M., Longthorne, R. F. & Gurdon, J. B. (1978) *Nature (London)* **272**, 254–256
37. Goldstein, L. & Ko, C. (1981) *J. Cell Biol.* **88**, 516–525
38. Sanger, J. W., Sanger, J. M., Kreisl, T. E. & Jockusch, B. M. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5268–5272
39. Hall, M. N., Hereford, L. & Herskowitz, I. (1984) *Cell* **36**, 1057–1065
40. Silver, P. A., Keegan, L. P. & Ptashne, M. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5951–5955
41. Mattaj, I. W. & De Robertis, E. M. (1985) *Cell* **40**, 111–118
42. Clawson, G. A. & Smuckler, E. A. (1982) *Theor. Biol.* **95**, 607–613
43. Clawson, G. A., James, J., Woo, C. H., Friend, D. S., Moody, D. & Smuckler, E. A. (1980) *Biochemistry* **19**, 2748–2756
44. Kletzien, R. F. (1980) *Biochem. J.* **192**, 753–759
45. Moffet, R. B. & Webb, T. E. (1981) *Biochemistry* **20**, 3253–3262
46. Palayoor, T., Schumm, D. E. & Webb, T. E. (1981) *Biochim. Biophys. Acta* **654**, 201–210
47. Schumm, D. E. & Webb, T. E. (1981) *Arch. Biochem. Biophys.* **210**, 275–279
48. Agutter, P. S. (1982) in *The Nuclear Envelope and the Nuclear Matrix* (Maul, G. G., ed), vol. 2, pp. 91–109, Liss, New York
49. Riedel, N., Bachmann, M., Prochnow, D., Richter, H. P. & Fasold, H. (1986) *Proc. Natl. Acad. Sci. U.S.A.*, in the press

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