Identification of specific polypeptides of the nuclear envelope by iodination of mouse liver nuclei

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A sensitive technique is described for the rapid identification of nuclear-envelope proteins. Mouse liver nuclei (purified on sucrose gradients) were iodinated with Na^{125}I by the immobilized water-insoluble reagent Iodogen. Iodinated nuclei were digested with RNAase A and DNAase I and then salt-extracted to obtain labelled nuclear envelopes. Nuclear envelopes were characterized by morphological and biochemical criteria and by SDS/polyacrylamide-gel electrophoresis. In all, 13 polypeptides of molecular masses 145, 115, 98, 85, 75, 70, 65, 54, 50, 45, 40, 38 and 36 kDa were identified in the labelled nuclear envelopes. The labelled polypeptides were localized to the nuclear envelope by extraction of the envelope with Triton X-100 and different concentrations of salt. Iodination of intact nuclei was shown to be specific for the nuclear envelope by the absence of labelling of histones and cytoplasmic contaminants.

INTRODUCTION

The nucleus in a eukaryotic cell is partitioned from the cytoplasm by the double membrane of the nuclear envelope. The outer membrane is closely associated with the endoplasmic reticulum, whereas the inner membrane is embedded in a network of highly insoluble proteins, the lamins, which in turn surround the peripheral chromatin [1,2]. The inner and outer membranes are spanned by the nuclear-pore complexes, which are thought to be the route of most nucleocytoplasmic traffic [3,4].

The transport of macromolecules across the nuclear envelope is a highly selective process, and for some known nuclear proteins transport depends on the presence of a basic nuclear location signal sequence in the mature protein [5,6]. However, the pathway by which proteins specifically enter the nucleus is not known. Recent studies suggest that nuclear proteins may initially bind to the nuclear envelope and then translocate through the pore complex [7,8]. Thus it is possible that, in addition to the pore-complex proteins, other proteins involved in nuclear transport may be associated with the nuclear envelope. As a first step, therefore, a detailed characterization of the nuclear envelope should give more insight into the mechanics of nuclear transport. Nuclear envelopes have been isolated by different methods and characterized by one-dimensional and two-dimensional gel electrophoresis [10,11], as well as by monoclonal antibodies raised against enriched nuclear-pore-complex preparations [12–14]. By gel analysis the only clearly identifiable proteins are the lamins, 60–68 kDa, which comprise 25–50% of the total envelope proteins, and a set of proteins of molecular mass ~ 50 kDa which are reportedly different forms of cytochrome *P*-450 associated with nuclear and endoplasmic-reticulum membranes [1,10,15]. On the basis of their interaction with monoclonal antibodies, two proteins, 62 kDa and 190 kDa, have been proposed to be part of

the pore structure [12,13]. More recently, a number of proteins (210, 180, 145, 100, 63, 58 and 45 kDa) have been tentatively identified as part of the pore complex by using monoclonal antibodies [14]. Considering the effort involved in raising monoclonal antibodies to nuclear-envelope proteins from a particular source, it would be useful to have a sensitive technique to identify nuclear-envelope proteins rapidly and also to detect other proteins associated with the envelope in addition to the pore-complex proteins.

In the present paper we describe a method for the specific radiolabelling of nuclear-envelope proteins. Since iodination of several cell types with the immobilized water-insoluble reagent Iodogen (1,3,4,6-tetrachloro-3 α ,6 α -diphenylglycouril) [16,17], has been shown to specifically iodinate the cell surface, we have used Iodogen to label the surface of mouse liver nuclei. These nuclei have been processed to obtain nuclear envelopes in good yield and purity by modifications of previous procedures [11,18]. We report the identification of 13 polypeptides of the nuclear envelope and demonstrate that radiolabelling of nuclear-envelope polypeptides is highly specific as compared with that described in an earlier report [19].

MATERIALS AND METHODS

Chemicals

DNAase I was obtained from Pharmacia Biotechnology International, Uppsala, Sweden. Sucrose (grade I), RNAase A and Hoescht 33258 were from Sigma; phenylmethanesulphonyl fluoride (PMSF) was from Boehringer-Mannheim. All electrophoresis reagents, urea and β -mercaptoethanol were from Bio-Rad. Iodogen was obtained from Pierce Chemicals. Na^{125}I (carrier-free) was obtained from Bhabha Atomic Research Centre, Bombay, India.

Abbreviations used: PMSF, phenylmethanesulphonyl fluoride; DTT, dithiothreitol; phosphate-buffered saline, 0.01 M-sodium phosphate/0.15 M-NaCl, pH 7.5

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Isolation of nuclei and nuclear envelopes

Nuclei and nuclear envelopes were isolated from the livers of Balb/c mice as described by Kaufmann *et al.* [11,18], except for the following minor modifications. During the homogenization of the tissue, 25 mM-KCl and 2 mM-DTT were included in the homogenization buffer, STM/PMSF [250 mM-sucrose/50 mM-Tris/HCl (pH 7.4 at 4 °C)/5 mM-MgSO₄/1 mM-EGTA/0.5 mM PMSF]; digestion of nuclei with nucleases was carried out for 30 min at 37 °C with DNAase 1 (30 µg/ml) and heat-treated RNAase A (20 µg/ml) per $(2-4) \times 10^8$ nuclei.

Samples were analysed for protein by a modified Lowry method [20]; for phospholipid by complex-formation with ammonium ferrothiocyanate [21]; for DNA by fluorescence enhancement upon binding of Hoescht 33258 [22]; and for RNA by the orcinol method [23].

Isolation of subcellular fractions

The postnuclear supernatant obtained after sedimentation of nuclei at 800 *g* for 10 min was processed as follows to isolate different fractions. To obtain partially purified mitochondria, the postnuclear supernatant was centrifuged at 10 000 *g* for 10 min and the mitochondrial pellet was washed once with STM/PMSF. One aliquot of the postmitochondrial supernatant was layered over a discontinuous gradient of 1.3 M- and 2.25 M-sucrose and centrifuged at 105 000 *g* for 1 h to collect endoplasmic reticulum, which banded at the 1.3 M/2.25 M-sucrose interface [24]. Another aliquot of the postmitochondrial supernatant was centrifuged at 100 000 *g* for 1 h to pellet plasma membranes. The supernatant remaining after sedimenting membranes was used as a source for cytosol markers. Samples were assayed for mitochondrial succinate dehydrogenase [25], plasma-membrane-bound 5'-nucleotidase [26] and cytosolic pyrophosphatase [27].

Electron microscopy

Nuclei and nuclear envelopes were processed for electron microscopy as follows. Purified nuclei were sedimented at 800 *g* for 10 min, and nuclear envelopes obtained after the second salt extraction were centrifuged at 1600 *g* for 30 min. The pellets were fixed in 30% glutaraldehyde and post-fixed in 1% OsO₄. Samples were embedded and ultrathin sections were stained in 2% uranyl acetate for 2 h, followed by 0.2% lead citrate for 5 min. Sections were examined in a JEOL 100 CX electron microscope.

Gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed by the method of Laemmli [28].

Iodination of nuclei

Iodination of nuclei using Iodogen was carried out by modification of methods used for labelling red blood cells with the same reagent [16,17]. Scintillation vials were coated with 40 µg of reagent in 100 µl of acetone and stored desiccated before use. Nuclei were labelled within 1 h of ultracentrifugation on sucrose cushions and one wash in STM/PMSF. A 500 µl portion of purified nuclei in STM/PMSF [generally $(1-2) \times 10^8$ nuclei/ml for optimum yield of envelopes, but lower concentrations could be used] was added to the coated vial in ice. About 300 µCi of Na¹²⁵I solution in phosphate-buffered saline

(50 µCi/µl) was added to the nuclei and incubation was carried out for 10 min on ice with occasional shaking. The sample was immediately added to 10 ml of STM/PMSF and centrifuged at 800 *g* for 10 min at 4 °C. Nuclei were washed three more times in a similar manner to remove Na¹²⁵I that had not reacted. Nuclei were then processed for isolation of nuclear envelopes as described above. As controls, nuclei after treatment with RNAase and DNAase and lysis in 1% SDS, and an aliquot of the postmitochondrial supernatant (4 µg of protein), were iodinated exactly as described above. Aliquots of lysed, iodinated nuclei and postmitochondrial supernatant were directly loaded on SDS/polyacrylamide gels.

Fractionation of nuclear envelopes

Iodinated, salt-extracted nuclear envelopes in 10 mM-Tris/HCl, pH 7.4/20% glycerol were centrifuged at 1600 *g* for 30 min (4 °C) and the pellets were resuspended at a concentration of 0.8 mg/ml protein in the following buffers.

Method 1. The buffer comprised 2% Triton X-100 in 10 mM-Tris/HCl (pH 7.4)/0.2 mM-MgSO₄.

Method 2A. The buffer comprised 10% sucrose, 2% Triton X-100, 20 mM-triethanolamine/HCl, pH 7.4, 20 mM-KCl, 5 mM-MgCl₂ and 1 mM-DTT.

Method 2B. The buffer comprised 10% sucrose, 2% Triton X-100, 20 mM-Mes/KOH, pH 6.0, 300 mM-KCl, 2 mM-EDTA and 1 mM-DTT.

Method 3. The buffer comprised 3% Triton X-100, 10 mM-triethanolamine/HCl, pH 7.5, 0.28 M-sucrose and 0.1 mM-MgCl₂.

After 30 min in ice, samples were centrifuged at 1600 *g* for 30 min (4 °C). For Method 3, the pellet obtained was further extracted with 1 M-NaCl/10 mM-triethanolamine/HCl (pH 7.5)/0.28 M-sucrose/0.1 mM-MgCl₂ for 20 min in ice and centrifuged at 1600 *g* for 30 min (4 °C). All the above supernatants were directly analysed by SDS/polyacrylamide-gel electrophoresis.

RESULTS AND DISCUSSION

Characterization of isolated nuclear envelopes

The procedure adopted for the isolation of nuclei and nuclear envelopes was a minor modification of that of Kaufmann *et al.* [11,18]. Firstly, homogenization conditions to obtain crude nuclei were changed to include K⁺ ions and DTT, and this gave a better yield of nuclei and nuclear envelopes (~50% of nuclei). Secondly, a larger amount of DNAase 1 was used at 37 °C instead of 4 °C, and this resulted in a more efficient removal of histones. Nuclear envelopes were assessed for purity by morphological criteria, biochemical properties and analysis by SDS/polyacrylamide-gel electrophoresis. The phase-contrast micrographs in Figs. 1(a) and 1(b) indicate that the nuclear envelopes are obtained as intact empty vesicles with dimensions close to those of nuclei (internal diameter ~9 µm). The electron micrograph in Fig. 1(c) shows the integrity of the nuclei and absence of contamination with cytoplasm and other organelles. In Fig. 1(d) the outer and inner membranes of the nuclear envelopes and the absence of intranuclear contents are clearly seen. Biochemical analysis of envelopes gave a protein/phospholipid ratio (mg/mg) of 2.3 and RNA and DNA contents [mg/(mg of total protein + phospholipid)] of 0.01 and 0.02. This agrees very well with values

Fig. 1. Morphology of nuclei and nuclear envelopes

Nuclei and nuclear envelopes were isolated as described in the Materials and methods section. (a, b) Phase-contrast optical microscopy of (a) nuclei and (b) nuclear envelopes. (c, d) Electron microscopy of (c) nuclei and (d) nuclear envelopes. The bar denotes 5 μm in (a) and (b), 2 μm in (c) and 1 μm in (d). Arrows indicate the double membrane of the nuclear envelope.

reported elsewhere [10,18]. As there is no characteristic marker enzyme for the nuclear envelope, its purity was judged by the absence of markers for mitochondria, plasma membranes and cytosol (succinic dehydrogenase, 5'-nucleotidase and pyrophosphatase respectively). The data shown in Table 1 clearly indicate an absence of contamination of nuclear envelopes with mitochondria and a negligible contamination with cytosol and plasma membranes. Since this analysis compares purified nuclear envelopes with partially purified cell organelles, it tends to overestimate the contamination in the nuclear-envelope fraction. Hence less than 3–4% of the protein in the nuclear-envelope fraction probably results from contamination by cytoplasmic organelles. These results compare very well with those reported elsewhere [18]. As marker enzymes for the endoplasmic reticulum are also known to be integral components of nuclear membranes [10], in order to estimate endoplasmic-reticulum contamination, we have compared its RNA content [0.41 mg/(mg of total protein + phospholipid)] with that of nuclear envelopes [0.01 mg/(mg of total protein + phospholipid)], which indicates a contamination of less than 2.5%. This is also a considerable overestimate, since any RNA contamination in nuclear envelopes would be derived mostly from intranuclear contents.

The SDS/polyacrylamide-gel analysis of the different

steps of nuclear-envelope isolation is shown in Fig. 2. When nuclei are digested with RNAase A and DNAase 1 in STM/PMSF, the nuclear proteins are largely present in the nuclear pellet (lane 3) and are removed only after extraction of nuclei with 1.6 M-NaCl and β -mercaptoethanol, as was also observed by Kaufmann *et al.* [18].

Table 1. Analysis of marker enzymes

Cell fractions were isolated and assayed as described in the Materials and methods section. Enzyme activities are expressed as μmol of product formed/h per mg of protein. Similar data were obtained in three additional experiments.

Fraction	Enzyme activity		
	Succinate dehydrogenase	5'-Nucleotidase	Pyrophosphatase
Nuclear envelopes	0.0	0.1	1.6
Mitochondria	1.2	0.6	11.0
Plasma membranes	0.1	2.3	12.3
Cytosol	0.1	0.2	52.0

Fig. 2. Characterization of nuclear envelopes by SDS/polyacrylamide-gel electrophoresis

Purified mouse liver nuclei were fractionated to obtain nuclear envelopes as described in the Materials and methods section. Samples were separated on SDS/10%-polyacrylamide gels and the gel was stained with Coomassie Blue. Lane 2, intact nuclei (5×10^6 nuclei); lanes 3 and 4, pellet and supernatant of nuclei treated with RNAase and DNAase (5×10^6 nuclei); lanes 5 and 6, pellet and supernatant after first salt extraction of digested nuclei (5×10^6 nuclei); lanes 7 and 8, pellet and supernatant after second salt extraction of nuclear envelopes (1×10^7 nuclei); lane 1, molecular-mass (M) markers: myosin, 200 kDa; phosphorylase *b*, 97 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; α -chymotrypsinogen, 26 kDa; β -lactoglobulin, 18 kDa; and lysozyme, 14 kDa.

Histones (28, 26, 25 and ≤ 22 kDa) are mostly removed by the first salt extraction (lane 6), and any remaining histones are removed by the second salt extraction (lane 8). The purified nuclear envelopes (lane 7) are devoid of histone contamination and are highly enriched in the lamins (60–68 kDa) and cytochrome *P*-450 proteins (~ 50 kDa).

Iodination of purified nuclei and identification of iodinated polypeptides

Mouse liver nuclei were purified by sedimentation of crude nuclei through a cushion of 2.1 M-sucrose. Purified nuclei were iodinated by using Na^{125}I and immobilized water-insoluble reagent Iodogen as described in the Materials and methods section. A control iodination reaction with lysed nuclei was carried out to determine the specificity of labelling of the nuclear surface. Incorporation of label into intact nuclei was $(2\text{--}5) \times 10^6$ c.p.m. per 8×10^7 nuclei (average for five experiments). This corresponds to an incorporation of 0.4–1 % of the total label used. Incorporation of label into lysed nuclei was about 2.5×10^8 c.p.m. per 8×10^7 nuclei (lysed) or 40 % of the total label. The routinely observed efficiency of labelling of pure proteins using Iodogen is about 60 % [16]. Our observation that 40 % of the label is incorporated into lysed nuclei, but only 0.4–1 % of the label is incorporated into intact nuclei, suggests that only

the surface of intact nuclei is accessible to the labelling reagent.

In order to determine the specificity of labelling of the nuclear surface, iodinated nuclei were processed to isolate nuclear envelopes as described in the Materials and methods section. The pellets and supernatants at various stages of nuclear-envelope preparation from iodinated nuclei and iodinated lysed nuclei were analysed by SDS/polyacrylamide-gel electrophoresis and autoradiographed as shown in Fig. 3(a). The results shown are representative of five separate experiments. When the gel of the iodinated samples was stained with Coomassie Blue (results not shown), it showed a pattern identical with that in Fig. 2, indicating the absence of artefacts due to the iodination procedure. The specificity of labelling of the nuclear surface is clearly seen by: (1) comparison of labelled lysed nuclei (lane 9) and intact nuclei (lane 1) for the presence of labelled histones, and (2) analysis of the supernatants during processing of nuclei and envelopes (lanes 3, 5 and 7) for the presence of labelled histones or other proteins. Since histones constitute the major protein fraction of nuclei and contain several tyrosine residues for iodination, we have considered the absence of histone labelling as an appropriate measure of the specificity of labelling of the nuclear surface in intact nuclei. For lysed nuclei (lane 9), more than 90 % of the label is in material of molecular mass ≤ 22 kDa (histones H2, H3 and H4) and 25, 26 and 28 kDa (histones H1, H1 $^{\circ}$). In intact nuclei (lane 1) there is no labelling of histones and in the supernatants after extraction of nuclear envelopes, no labelled bands are seen (lanes, 3, 5 and 7). On the other hand, a set of bands are strongly labelled in intact nuclei and are clearly visible in purified nuclear envelopes (lane 6). These prominent bands have molecular masses of 145, 115, 85, 75, 70, 65, 54, 50, 38 and 36 kDa. Bands of molecular masses 98, 45 and 40 kDa are labelled to a lesser extent. Labelled material is also present at a molecular mass ≥ 180 kDa in lane 6 and may represent insufficiently solubilized envelopes or proteins of higher molecular mass. Since the marker-enzyme analysis of the nuclear envelopes clearly showed a negligible contamination of envelopes with cytosol or membranes, it is highly unlikely that the iodinated polypeptides could correspond to proteins artefactually adsorbed on to the envelopes. However, to clearly rule out this possibility, an aliquot of the postmitochondrial supernatant (protein content $\sim 5\%$ of nuclear envelopes) was iodinated under conditions identical with those used for labelling of nuclei. Under these conditions the postmitochondrial supernatant proteins were not labelled (Fig. 3a, lane 8).

In order to localize the labelled proteins, iodinated nuclear envelopes were extracted with Triton X-100 and different concentrations of salt by the three procedures described in the Materials and methods section. Fractionation of envelopes with 2 % Triton X-100 in low-salt buffers (Methods 1 and 2A) solubilizes the nuclear membranes and proteins of the outer membrane, but keeps the pore complex intact [11,14]. In the presence of 3 % Triton X-100 and 1 M-NaCl (Method 3), the nuclear membranes and integral membrane proteins are completely solubilized, whereas the pore complex is mostly insoluble [18]. To solubilize the pore complex without disrupting the lamina, a combination of 2 % Triton X-100, 0.3 M-KCl, EDTA and low-pH (6.0) buffer was found to be most effective ([14]; Method 2B). When

Fig. 3. Analysis of iodinated nuclei, nuclear envelopes and fractionated nuclear envelopes by SDS/polyacrylamide-gel electrophoresis

(a) Intact nuclei, nuclei after lysis and postmitochondrial supernatant were iodinated as described in the Materials and methods section; nuclear envelopes were isolated from iodinated nuclei, and samples (~ 50000 c.p.m. each) were separated on SDS/10%-polyacrylamide gels. Gels were fixed, dried and subjected to autoradiography for 2–5 days at -70°C , with intensifying screens. Lane 1, intact nuclei; lanes 2 and 3, pellet and supernatant of nuclei treated with RNAase and DNAase; lanes 4 and 5, pellet and supernatant after first salt extraction of digested nuclei; lanes 6 and 7, pellet and supernatant after second salt extraction of nuclear envelopes; lane 8, postmitochondrial supernatant; and lane 9, lysed nuclei. The positions of histones are indicated by bold dots (●). (b) Iodinated nuclear envelopes were fractionated by the three methods described in the Materials and methods section. The supernatants containing the extracted polypeptides were separated on SDS/10%-polyacrylamide gels as in (a). Lane 1, Triton X-100/low-salt extract (Method 1); lane 2, Triton X-100/low-salt extract (Method 2A); lane 3, Triton X-100/high-salt extract (Method 2B); lane 4, Triton X-100 extract (Method 3); and lane 5, high-salt extract (Method 3). Abbreviation: *M*, molecular mass.

iodinated nuclear envelopes were fractionated with Triton X-100/low salt by Method 1 (Fig. 3b, lane 1) or Method 2A (Fig. 3b, lane 2), the labelled extracted proteins had molecular masses of 75 (partly extracted) and 54 kDa. Upon extraction with Triton X-100/high salt (pH 6.0) by Method 2B (Fig. 3b, lane 3) almost all the remaining iodinated polypeptides were extracted out (145, 115, 85, 75, 70, 65, 54, 50, 38 and 36 kDa). By Method 3, which employed extraction by 3% Triton X-100 (lane 4) followed by 1 M-NaCl (lane 5), the extracted polypeptides have molecular masses of 54 kDa (partly extracted, lane 4) and 85, 75, 54, 38 and 36 kDa (lane 5).

Although there are some differences in the efficiency of extraction by the three different methods, and the clarity of the iodinated bands after Triton X-100 treatment is not as good as in the intact nuclear envelopes, it is evident that nearly all the iodinated polypeptides are extracted from the nuclear envelope and can thus be localized on the envelope. The ease of extraction of the 54 kDa protein suggests that it may correspond to a cytochrome *P*-450-like protein localized on the outer membrane [10,15], although a 54 kDa protein may also be associated with the pore complex [14]. All the other polypeptides appear to be pore-complex or integral membrane proteins. Four polypeptides (145, 98, 65 and 45 kDa) are likely to be components of the pore complex, since proteins of a similar size have been identified as components of the pore complex in other studies [12,14]. Proteins in the range ≥ 180 kDa were difficult to analyse in our gels owing to overlap of labelled bands with some

insufficiently solubilized envelopes. One other protein (58 kDa) identified as part of the pore complex in [14] is not labelled, possibly because it may not be exposed in the intact pore complex in the nucleus. Polypeptides of molecular masses 85, 50 and 40 kDa are probably integral membrane proteins not associated with the pore complex [14]. Polypeptides of molecular masses 115, 75, 70, 38 and 36 kDa do not correspond to previously identified envelope proteins and may be integral membrane proteins or presently unidentified pore-complex proteins. In a previous report on the iodination of rat liver nuclei with immobilized lactoperoxidase [19], the pattern of labelled polypeptides in nuclear envelopes is similar to ours; however, in nuclei there is a significant labelling of histones ($\sim 30\%$ of total label), suggesting damage to nuclei or non-specific labelling of nuclear contents. Our results demonstrate that labelling of nuclear envelope proteins by Iodogen-mediated radioiodination of nuclei is a sensitive technique for the rapid and specific identification of nuclear-envelope proteins.

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REFERENCES

1. Franke, W. W., Scheer, U., Krohne, G. & Jarasch, E. (1981) *J. Cell Biol.* **91**, 30s–50s
2. Gerace, L., Blum, A. & Blobel, G. (1978) *J. Cell Biol.* **79**, 546–566

3. Unwin, P. N. T. & Milligan, R. (1982) *J. Cell Biol.* **99**, 63–75
4. Feldherr, C. M., Kallenbach, E. & Schultz, N. (1984) *J. Cell Biol.* **99**, 2216–2222
5. Bonner, W. M. (1978) in *The Cell Nucleus* (Busch, H., ed.), vol. 6, part C, pp. 97–148, Academic Press, New York
6. Dingwall, C. & Laskey, R. A. (1986) *Annu. Rev. Cell Biol.* **2**, 367–390
7. Newmeyer, D. D. & Forbes, D. J. (1988) *Cell* (Cambridge, Mass.) **52**, 641–653
8. Richardson, W. D., Mills, A. D., Dilworth, S. M., Laskey, R. A. & Dingwall, C. (1988) *Cell* (Cambridge, Mass.) **52**, 655–664
9. Rine, J. & Barnes, E. (1985) *Bioessays* **2**, 158–161
10. Kasper, C. B. (1974) in *The Cell Nucleus* (Busch, H., ed.), vol. 1, pp. 349–384, Academic Press, New York
11. Agutter, P. S. (1986) in *Nuclear Structures* (MacGillivray, A. J. & Birnie, G. D., eds.), pp. 34–46, Butterworths, London
12. Davis, L. & Blobel, G. (1986) *Cell* (Cambridge, Mass.) **45**, 699–709
13. Gerace, L., Ottaviano, Y. & Kondor-Koch, C. (1982) *J. Cell Biol.* **95**, 826–837
14. Snow, C. M., Senior, A. & Gerace, L. (1987) *J. Cell Biol.* **104**, 1143–1156
15. Lebel, S. & Raymond, Y. (1984) *J. Biol. Chem.* **259**, 2693–2696
16. Fraker, P. J. & Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80**, 849–857
17. Markwell, M. A. K. & Fox, C. F. (1978) *Biochemistry* **17**, 4807–4817
18. Kaufmann, S. H., Gibson, W. & Shaper, J. H. (1983) *J. Biol. Chem.* **258**, 2710–2719
19. Richardson, J. C. W. & Maddy, A. H. (1980) *J. Cell Sci.* **43**, 253–267
20. Parnaik, V. K., Srivastava, P. K. & Das, M. R. (1983) *J. Biosci.* **5**, 107–114
21. Stewart, J. C. M. (1980) *Anal. Biochem.* **104**, 10–14
22. Labarca, C. & Paigen, K. (1980) *Anal. Biochem.* **102**, 344–352
23. Schneider, W. C. (1957) *Methods Enzymol.* **3**, 680–684
24. Scheele, G., Jacoby, R. & Carne, T. (1980) *J. Cell Biol.* **87**, 611–628
25. Earl, D. C. N. & Korner, A. (1965) *Biochem. J.* **94**, 721–734
26. Aronson, N. N. & Touster, O. (1974) *Methods Enzymol.* **31**, 90–102
27. Shatton, J. B., Shah, H., Williams, A., Morris, H. P. & Weinhouse, S. (1981) *Cancer Res.* **41**, 1866–1872
28. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685

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