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The Role of Nucleoplasmin in Chromatin Assembly and Disassembly [and Discussion]

R. A. Laskey, A. D. Mills, A. Philpott, G. H. Leno, S. M. Dilworth, C. Dingwall, S. Lindquist, A. Horwich, N. M. Green, M.-J. Gething, W. Neupert and R. J. Ellis

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The role of nucleoplasmin in chromatin assembly and disassembly

R. A. LASKEY, A. D. MILLS, A. PHILPOTT†, G. H. LENO‡, S. M. DILWORTH§ AND C. DINGWALL

Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR, U.K.

SUMMARY

Nucleoplasmin is the most abundant nuclear protein in *Xenopus* oocytes and eggs. The term 'molecular chaperone' was coined to describe its role in the assembly of the nucleosome subunits of chromatin. Although histones and DNA can self-assemble into nucleosomes, nucleoplasmin can facilitate this process *in vitro* by competing against non-specific charge interactions. *In vivo* nucleoplasmin binds histones H2A and H2B and transfers them to DNA. Another acidic nuclear protein, N1, binds and transfers histones H3 and H4. Nucleoplasmin has at least one other role in modulating chromatin structure in *Xenopus* eggs. It is required for the first stage of sperm chromatin decondensation. It binds and removes sperm basic proteins and replaces them by histones H2A and H2B, again forming nucleosomes, and resulting in decondensation of the compacted sperm chromatin. In addition we propose that the properties of the nuclear localization signal of nucleoplasmin can be explained by a model in which heat shock cognate protein hsc70 has a chaperone role in signal presentation during nuclear transport.

1. INTRODUCTION

The molecular chaperone concept has found many applications in protein folding, transport and assembly processes, but it was introduced to account for the properties of one protein, nucleoplasmin (Laskey *et al.* 1978). This protein was discovered because of its ability to facilitate nucleosome core assembly from histones and DNA at physiological ionic strength. It is clear now that nucleoplasmin is required for one step of a more complex assembly process *in vivo*, and that it also performs a second modulation of chromatin structure in amphibian eggs. Nucleoplasmin decondenses the incoming sperm nucleus at fertilization (Philpott *et al.* 1991; Philpott & Leno 1992).

Nucleoplasmin has also been used widely to study the mechanism of selective protein import into the cell nucleus. Study of its nuclear localization signal has raised a puzzling paradox. However, a recent report that the heat shock protein hsp70 or its cognate hsc70 is a receptor for nuclear localization signals (Shi & Thomas 1992) raises an intriguing possible explanation of the paradox. We propose that hsc70 (or hsp70) has a chaperone role in signal presentation in nuclear protein import and thus 'chaperones the chaperone'.

† Present address: Massachusetts General Hospital Cancer Center, Building 149, 13th Street, Charlestown, Massachusetts 02144, U.S.A.

‡ Present address: Department of Biochemistry, School of Medicine, University of Mississippi Medical Center, 2500 North State Street, Jackson, Mississippi 39216, U.S.A.

§ Present address: Department of Chemical Pathology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London WC12 0NN, U.K.

2. NUCLEOPLASMIN AND THE CHAPERONE CONCEPT

Until the 1980s the dominant concept in biological assembly mechanisms was 'self-assembly'. There were several precedents including simple viruses and the nucleosome subunits of chromatin. Nucleosome cores could be assembled from purified histones and DNA by mixing them in 2 M salt and dialysing them slowly against a series of steps of decreasing salt concentrations. The resulting structures were indistinguishable from native nucleosome core particles, suggesting that the steric information for assembly of nucleosome cores resides in histones and DNA themselves (reviewed by Laskey & Earnshaw 1980).

Nevertheless the conditions which allowed assembly were very restricted. Mixing at physiological ionic strength resulted in insoluble precipitates. This was not surprising because nucleic acids are highly negatively charged and histones are amongst the most positively charged components in the cell. What then overcame this non-specific electrostatic interaction and allowed specific assembly to occur in the cell at physiological ionic strength?

Fractionation of a cell-free nucleosome assembly system from *Xenopus* eggs provided an explanation in the form of an apparent 'molecular chaperone'. *Xenopus* egg extracts assemble purified DNA into nucleosomes rapidly at physiological ionic strength using either a stored histone pool or exogenous histones. Fractionation identified an acidic protein which binds histones *in vitro* and transfers them to DNA with the formation of nucleosome cores (Laskey

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et al. 1978). This protein was subsequently named nucleoplasmin (Laskey & Earnshaw 1980) and its role in nucleosome assembly is discussed further below. The concept of a 'molecular chaperone' was introduced to explain how nucleoplasmin could facilitate an assembly reaction between components which can self-assemble. Nucleoplasmin was proposed to be an electrostatic filter which could filter out inappropriate interactions by masking the positive charges on histones and thus prevent precipitation. Thus the proposed role of nucleoplasmin was seen as analogous to that of a chaperone by filtering out inappropriate interactions, but allowing an ordered interaction to occur (Laskey *et al.* 1978; Laskey & Earnshaw 1980).

3. NUCLEOPLASMIN'S ROLE IN NUCLEOSOME ASSEMBLY

Although nucleoplasmin was isolated by assaying its ability to assemble all four core histones into nucleosome cores *in vitro* (Laskey *et al.* 1978; Earnshaw *et al.* 1980), it is now clear that it performs only one step in this process in the cell. Thus further fractionation and immunochemical studies of *Xenopus* eggs and oocytes (Kleinschmidt & Franke 1982; Kleinschmidt *et al.* 1985, 1990; Dilworth *et al.* 1987) have shown that nucleoplasmin binds only two of the four core histones *in vivo* (figure 1). These are histones H2A and H2B. The other core histones, H3 and H4, are bound to a second acidic chaperone protein called N1. Removal of either complex from *Xenopus* egg extracts by immunodepletion inhibits nucleosome assembly (Dilworth *et al.* 1987).

The large reserves of nucleoplasmin and N1 in *Xenopus* oocytes and eggs allow accumulation of large histone pools. Thus nucleoplasmin is the most abundant nuclear protein in *Xenopus* oocytes occurring at 5–8 mg ml^{-1} and forming 10% of the total nuclear proteins (Mills *et al.* 1980). N1 is the third most abundant nuclear protein (actin being the second most abundant). Together the nucleoplasmin and N1 in each mononucleate oocyte bind and store sufficient histones to assemble 12 000 diploid nuclei. Interestingly the accumulated histone pool appears to be expressed from that class of histone genes whose expression is uncoupled from DNA replication in somatic cells (Dilworth *et al.* 1987).

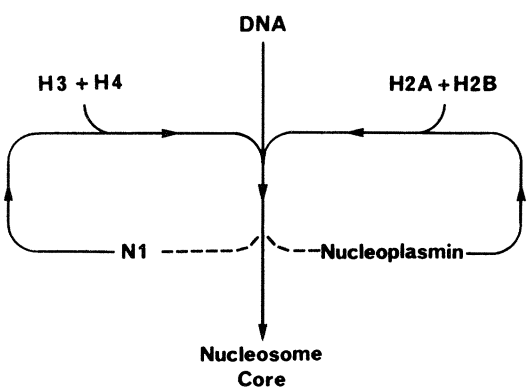

Figure 1. The role of nucleoplasmin in the nucleosome assembly pathway in *Xenopus* eggs. (Reproduced with permission from Dilworth *et al.* (1987); copyright held by Cell Press.)

Nucleoplasmin and N1 both have long polyglutamic acid tracts which are obvious candidates for histone binding sites (Kleinschmidt *et al.* 1986; Dingwall *et al.* 1987, Burglin *et al.* 1987), but, apart from that and a resemblance in their nuclear localization signals (see below), they are not obviously similar. Thus nucleoplasmin forms homopentamers of subunits of 22 000 Da where N1 exists as monomers, or perhaps dimers, of 66 000 Da. An unusual property they share is heat stability. They are not denatured by boiling, although the significance of this observation is not clear.

There is no evidence that either nucleoplasmin or N1 are also involved in nucleosome assembly in adult somatic cells. Instead, nucleosome assembly in cultured mammalian cells is replication dependent, occurring only on replicating DNA. A replication-dependent assembly factor CAF-1 has been identified which performs this action, transferring histones H3 and H4 to replicating DNA before histones H2A and H2B (Smith & Stillman 1989). Although DNA replication is not required for nucleosome assembly in *Xenopus* eggs, there is evidence that assembly is faster on replicating DNA and that this effect is mediated by a factor which resembles CAF-1 (Almouzni & Mechali 1988; Sapp & Worcell 1990). At the present time it appears likely that replication-dependent nucleosome assembly by CAF-1 is a general phenomenon. However, it remains possible that nucleoplasmin and N1 are only adaptations to allow the accumulation of large histone pools in amphibian embryos. An alternative possibility that deserves investigation is that nucleoplasmin and N1 chaperone the class of histones whose expression is not coupled to DNA synthesis, as this is the class of histones which is over-expressed in *Xenopus* oocytes (Dilworth *et al.* 1987).

4. NUCLEOPLASMIN DECONDENSES SPERM CHROMATIN AT FERTILIZATION

One of the most dramatic changes in chromatin structure occurs when a sperm nucleus enters egg cytoplasm. Chromatin is exceptionally densely packed in sperm nuclei to allow sperm motility. After fertilization it decondenses rapidly to produce the exceptionally dispersed chromatin of the male pronucleus. In *Xenopus*, decondensation occurs in two stages, an instantaneous limited expansion followed by a much slower, more extensive stage which requires the nuclear membrane and import of nuclear proteins.

Immunodepletion studies using monoclonal antibodies against nucleoplasmin have shown that the first, rapid stage of decondensation is mediated by nucleoplasmin (Philpott *et al.* 1991; Philpott & Leno, 1992). Removal of nucleoplasmin inhibits rapid sperm decondensation whereas re-addition fully restores it. Furthermore, purified nucleoplasmin performs the first stage of decondensation at the physiological rate in the absence of other egg components (figure 2).

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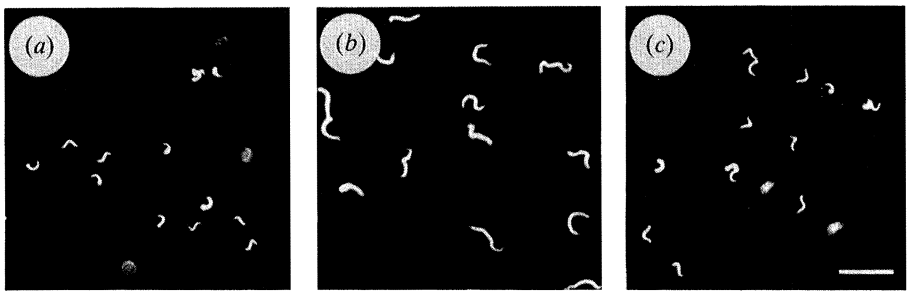

Figure 2. Purified nucleoplasmin decondenses *Xenopus* sperm nuclei. Sperm nuclei were incubated for 10 min in (*a*) buffer, (*b*) 700 μg ml^{-1} nucleoplasmin, and (*c*) 700 μg ml^{-1} polyglutamic acid. Scale bar is 50 μm. (Reproduced with permission from Philpott *et al.* (1991); copyright is held by Cell Press.)

Polyglutamic acid is much less effective than nucleoplasmin although it has a higher density of negative charges.

The mode of action of sperm decondensation by nucleoplasmin has been investigated by two-dimensional gel electrophoresis of sperm nuclear proteins during decondensation (Philpott & Leno 1992). By the use of triton-acid-urea gels as a first dimension followed by SDS in the second dimension, *Xenopus* sperm nuclei were found to contain histones H3 and H4 together with at least two sperm-specific basic proteins X and Y. Histones H2A and H2B were under-represented and present in only trace amounts (figure 3*a*).

After incubation of sperm nuclei in *Xenopus* egg cytoplasm for 3 min, most of the X and Y are removed and replaced by histones H2A and H2B (figure 3*b*). A mature nucleosome core particle can be detected in only 3 min using micrococcal nuclease digestion (Philpott & Leno 1992). Similarly purified nucleoplasmin can remove X and Y from sperm nuclei (figure 3*c*,*d*). Subsequent immunoprecipitation of the nucleoplasmin reveals that it has bound proteins X and Y, thus explaining its activity in decondensation. When purified histones H2A and H2B are also added, nucleoplasmin can assemble them on to the sperm chromatin in exchange for X and Y. We do not know if the same molecule of nucleoplasmin both donates H2A and H2B and removes X and Y, or if separate molecules perform these steps. A summary of nucleoplasmin's decondensation activity is shown in figure 4.

The sperm decondensation results described here have been partly corroborated by Ohsumi & Katagiri (1991*a*,*b*) who have shown a similar role for nucleoplasmin in sperm chromatin decondensation in *Xenopus* and *Bufo japonicus*. It will be interesting to determine if similar proteins mediate sperm decondensation in other species such as mammals.

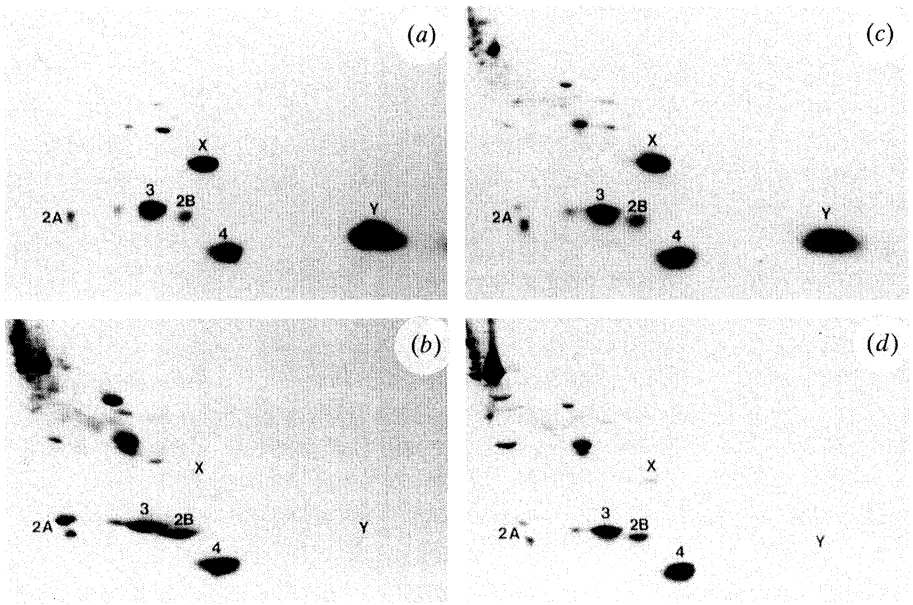

Figure 3. Two-dimensional poly-acrylamide gel electrophoresis (PAGE) analysis of sperm chromatin following incubation in *Xenopus* egg extract depleted of nucleoplasmin and in depleted extract reconstituted with nucleoplasmin. Sperm nuclei were incubated at 100 μg ml^{-1} for 3 min in (*a*) buffer, (*b*) egg extract, (*c*) egg extract depleted of nucleoplasmin, and (*d*) depleted egg extract with nucleoplasmin added back. Proteins were analysed by Triton-acid-urea two-dimensional SDS-PAGE gels. The positions of sperm basic proteins are labelled X and Y, and core histones are labelled 2A (H2A), 2B (H2B), 3 (H3) and 4 (H4). (Reproduced with permission from Philpott & Leno (1992); copyright is held by Cell Press.)

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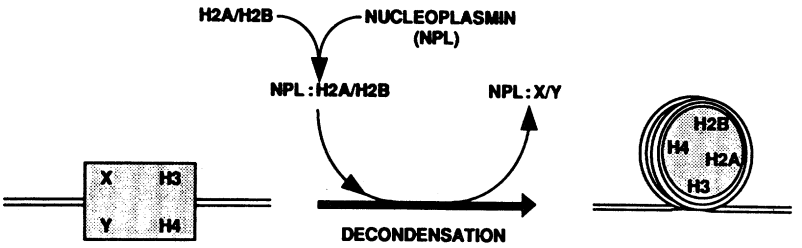

Figure 4. Model illustrating the modulation of sperm chromatin, mediated by nucleoplasmin during decondensation. (Reproduced with permission from Philpott & Leno (1992); copyright is held by Cell Press.)

5. A GENERAL NEED TO CHAPERONE CHARGE–CHARGE INTERACTIONS IN THE NUCLEUS

It is not clear yet if nucleoplasmin is confined to amphibian eggs or if it is more widely distributed. This apparently simple question has been unanswered for several reasons. First, an early study of its distribution was made using a polyclonal antiserum raised against whole nuclei (Krohne & Franke 1980*a*,*b*). Although this preferentially recognized nucleoplasmin, it is not safe to conclude that the widespread immunofluorescence signals arose only from nucleoplasmin. Second, the aminoterminal domain of nucleoplasmin is strikingly conserved with the aminoterminal domain of the nucleolar protein NO38, increasing the chance of cross-reaction (Schmidt-Zachmann *et al.* 1987). Third, nucleoplasmin varies greatly in its apparent size between eggs of various amphibian species (Krohne & Franke, 1980*a*,*b*). In addition, it is highly modified by phosphorylation, making it unusually disperse on two-dimensional gels (Laskey 1983; Cotten *et al.* 1986; Sealy *et al.* 1986). This problem is compounded by its tendency to remain pentameric under some SDS gel conditions. Therefore the distribution of this protein outside amphibians remains to be determined.

Studies of cultured *Xenopus* cells with a bank of monoclonal antibodies raised against nucleoplasmin from *Xenopus* eggs suggest that nucleoplasmin is either absent from these cells or present at only very low concentrations (Dilworth *et al.* 1987). Nevertheless the chaperone role identified for nucleoplasmin in filtering out non-specific charge–charge interactions could be generally important in the highly charged environment of the nucleus. Thus a high proportion of charged amino acids is a remarkably common feature of nuclear proteins. This is true of both positive and negative charges. In addition, charges are frequently clustered in nuclear proteins. Therefore the nucleus is an environment in which there must be a high probability of non-specific charge–charge interactions.

Nucleoplasmin clearly has the ability to edit out such non-specific interactions, allowing only selected specific interactions to occur. This property might be valuable in chaperoning many other protein–nucleic acid or protein–protein interactions in the nucleus. Therefore it will be interesting to see if other proteins provide similar functions in somatic cell nuclei. One possibility is that chromatin itself provides this chaperone function in somatic cell nuclei, by providing a high concentration of positive and negative charges to act as non-specific competitors. In this case, nucleoplasmin would substitute for chromatin in the greatly expanded nuclei of oocytes and early embryos in which the chromatin is so dilute. Nevertheless it would be surprising if analogous functions were not found elsewhere in modulating charge–charge interactions. Nucleolar protein NO38 provides an obvious candidate for a charge-masking role in ribosome assembly.

It was initially surprising to find that nucleoplasmin is pentameric (Earnshaw *et al.* 1980; Dingwall *et al.* 1982). Histones exist as dimers, tetramers and octamers which look unsuited to interact with a pentameric protein. We suggest that unusual symmetries such as pentameric nucleoplasmin or heptameric gro EL (or chaperonin 60) and its relatives, are ideal for the transient low affinities required for chaperone function. Thus inability to form tight stable complexes might greatly facilitate chaperone function and release so that pentameric or heptameric symmetry would be ideal.

6. CHAPERONING THE CHAPERONE: A ROLE FOR HSC70 IN IMPORT OF NUCLEAR PROTEINS

Nucleoplasmin has been used extensively to study the import of nuclear proteins into the cell nucleus (reviewed by Dingwall 1985; Dingwall & Laskey 1986, 1991). Its nuclear localization sequence has raised an interesting paradox which may be explained by the intriguing possibility of a chaperone role for hsc70 or hsp70 in nuclear import.

The nuclear localization signal of nucleoplasmin requires two clusters of basic amino acids separated by a gap of ten other amino acids (Robbins *et al.* 1991) (figure 5). Point mutations are tolerated in the gap of ten, suggesting that they function as a spacer rather than as a specific sequence (Robbins *et al.* 1991). A strikingly similar sequence motif was observed for the nuclear localization sequence of N1, the other histone-binding chaperone protein in *Xenopus* eggs (Kleinschmidt & Seiter 1988) and for nucleolar protein NO38 (Peculis & Gall 1992). Although NO38 shows strong local homology to the aminoterminal core region of nucleoplasmin, the homology does not extend to the region surrounding the nuclear localization signal (Schmidt-Zachman *et al.* 1987). The similarities seen in the nuclear localization signals of these three

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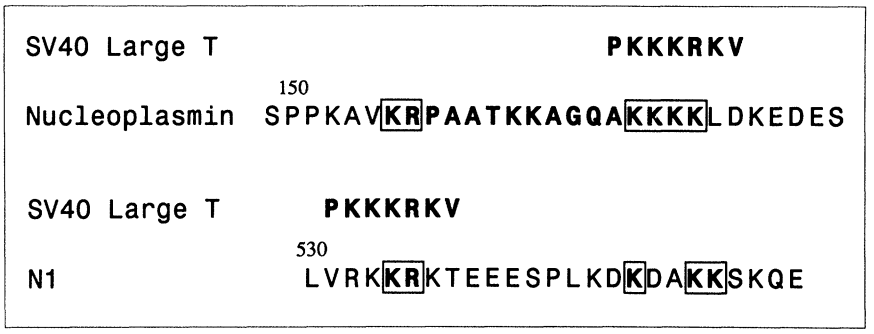

Figure 5. The nuclear targeting sequences from *Xenopus* nucleoplasmin (bold type) and N1 are aligned. The SV40 nuclear targeting sequence is shown above the homologous sequences in nucleoplasmin and N1. Amino acids important in nuclear targeting are boxed. (Reproduced with permission from Dingwall & Laskey 1991.)

proteins prompted Robbins *et al.* (1991) to search a protein database for a consensus motif consisting of two basic residues, a gap of any ten, followed by three more basic residues out of the next five. This motif was found in about half the nuclear proteins in the Swissprot database but in less than 5% of non-nuclear proteins. Furthermore, most of the few non-nuclear proteins containing this motif are known to be secreted or targeted to other organelles, suggesting that it is almost diagnostic for nuclear proteins (Robbins *et al.* 1991).

The significance of these observations was increased because the search identified several known nuclear localization regions, such as those of the steroid hormone receptor family. It has increased still further as a rapidly growing number of the sequences identified in the search have been assayed and shown directly to function as nuclear localization signals (reviewed by Dingwall & Laskey 1991). Therefore a motif in which ten spacer amino acids separate two parts of the signal appears to be important. This arrangement is puzzling. Proteins are not linear structures like DNA, in which spacers of defined length are common in sequence motifs. Proteins fold into complex three-dimensional structures. How then can ten amino acids be a significant feature?

A fascinating possible explanation arises from recent observations that one of the receptor proteins which recognizes nuclear localization sequences is the heat shock protein hsp70 or its cognate hsc70 (Shi & Thomas 1992; Y. Yoneda, personal communication). We propose that hsc70 (or hsp70) locally unfolds the signal, presenting it to a second receptor in an unfolded configuration; in this event the length of the unfolded peptide would be important. It is an interesting coincidence that the length of the peptide which gave greatest stimulation of ATPase activity in two members of the hsp70 family, hsc70 and BiP, is ten amino acids (Flynn *et al.* 1989). The role we propose for hsc70 in signal presentation is also analogous to that of the major histocompatibility complex (MHC) in antigen presentation. A further attraction of this model is its ability to explain the frequency of proline in the vicinity of nuclear localization signals, as this would increase the ease of signal unfolding.

This proposal has an obvious irony. The nuclear localization signal which prompted this proposal is that of nucleoplasmin, for which the term molecular chaperone was coined. The various heat shock proteins are the proteins most frequently called molecular chaperones. Just as 'big fleas have smaller fleas upon their back to bite them,' so, if this proposal is correct, then chaperones have chaperones and so *ad infinitum*...

We are grateful to the Cancer Research Campaign for support and to Ruth Dendy for help with the manuscript.

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Discussion

S. Lindquist (*Department of Molecular Genetics and Cell Biology, University of Chicago, U.S.A.*). Have the authors tested whether or not the nuclear localization signal becomes more susceptible to proteolytic attack when the proteins are associated with hsp70?

R. A. Laskey. No, but we intend to test this proposal.

A. Horwich (*Department of Molecular Genetics, Yale University School of Medicine, U.S.A.*). Nucleoplasmin has a pentameric ring structure; is phosphorylation of nucleoplasmin associated with any change in its appearance?

R. A. Laskey. Nucleoplasmin is smaller than groEL so it is not known if it undergoes any structural changes. It is surprising that nucleoplasmin has a pentameric structure as it recognizes histones which come in twos, fours and eights. One possible explanation is that the complex of histones and nucleoplasmin must be easily dissociated during nucleosome assembly, so having a mismatch of symmetry may facilitate this dissociation. Perhaps groEL has sevenfold symmetry for a similar reason.

N. M. Green (*National Institute for Medical Research, Mill Hill, London, U.K.*). The suggestion that hsp70 may be acting to present the recognition signal fits in well with the model we put forward for the peptide binding region of hsp70 which is based on the sequence of the carboxyterminal region of HLA. Is there any resemblance between those ten residues and peptides which bind to hsp70?

R. A. Laskey. We have not yet looked at this possibility. One problem is that there is an extraordinary diversity of sequences in this ten-residue region between different proteins which have nuclear localization signals. A common feature is the presence of proline, which may act as a signal for unfolding this region.

N. M. Green. How precise is the requirement for ten residues?

R. A. Laskey. This depends upon the peptide sequence tried. Shorter sequences will not work. However, signals with some insertions such as -SPGG- in one, two or three repeats will still act as nuclear localization signals.

M.-J. Gething (*Howard Hughes Medical Institute, University of Texas, Dallas, U.S.A.*). Are the lysines recognized by the hsp70 or is it the internal sequence that is recognized?

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R. A. Laskey. This is not known. Signals tend not to be recognized if some of the lysines are changed by mutation but binding has not been examined directly in this context.

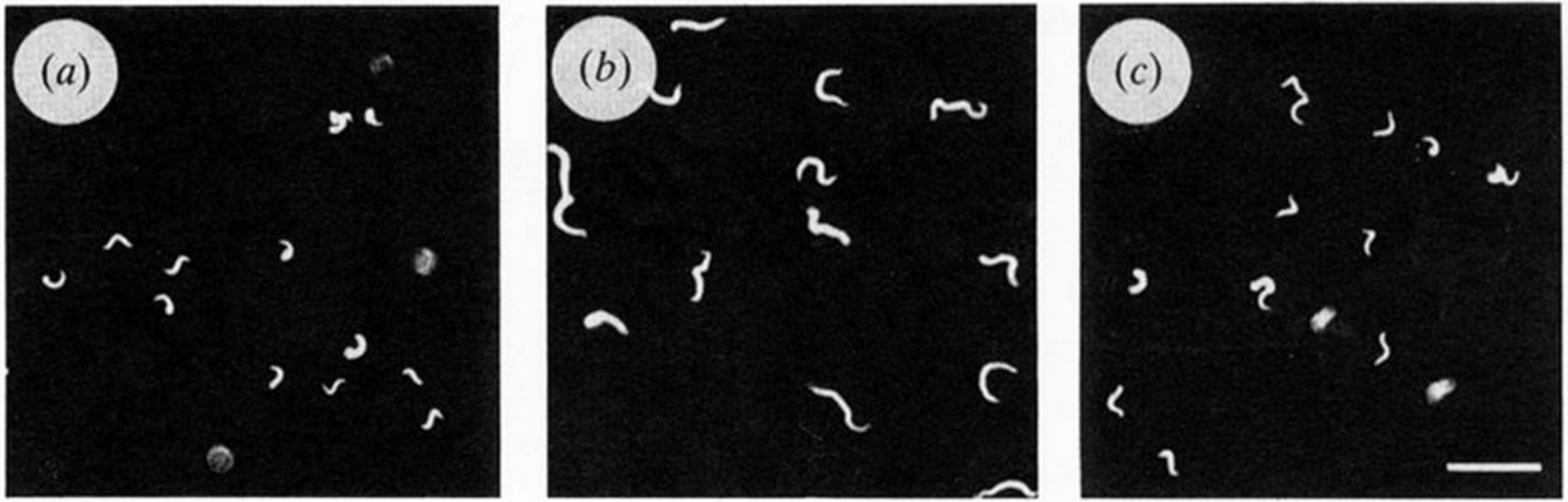
W. Neupert (*Institute for Physiological Chemistry, University of Munich, Germany*). When the authors suggest that the hsp70 locally unfolds the protein to be transported, are they implying that the hsp70 has an active unfolding role or that it binds to the unfolded conformer that is in equilibrium with the native protein?

R. A. Laskey. We are not implying a specific unfolding role for hsp70, as distinct from a stabilizing role.

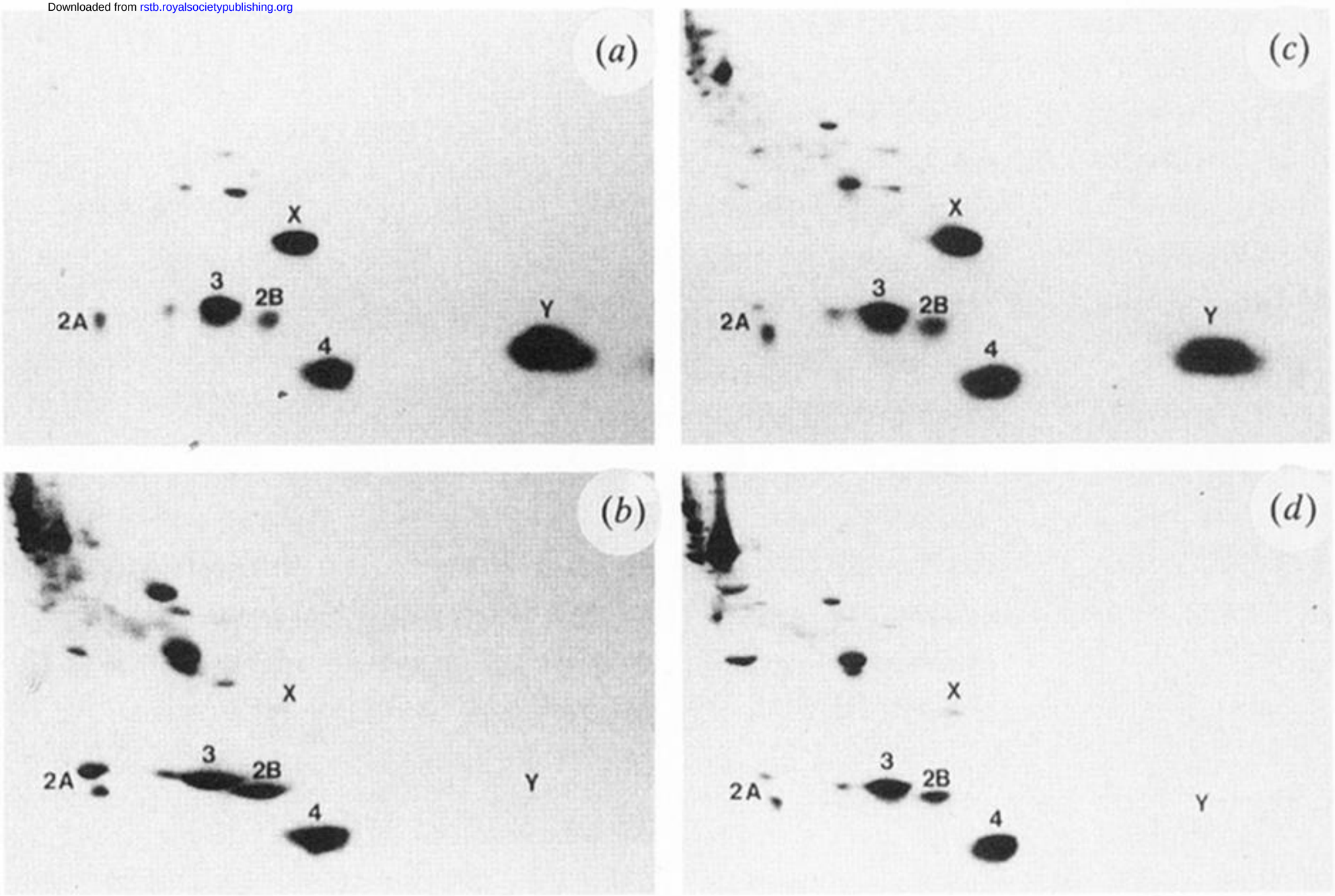
R. J. Ellis (*Department of Biological Sciences, University of Warwick, U.K.*). The authors have suggested that chaperones which bind to charged surfaces may be especially important in the nucleus because of the highly charged environment in that organelle. Is anything known about nuclear chaperones that may be involved in the assembly of ribosomes or ribonucleoprotein particles?

R. A. Laskey. Nucleoplasmin can be thought of as a molecular hand in that it is a pentameric protein with five exposed carboxyterminal domains and a highly compact aminoterminal domain. This aminoterminal domain is conserved in the nucleolar protein NO38, but as yet there is no evidence that this homologue to nucleoplasmin is involved in ribosome assembly.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



gure 2. Purified nucleoplasmin decondenses *Xenopus* sperm nuclei. Sperm nuclei were incubated for 10 min in (*a*) ıffer, (*b*) 700 μg ml^{-1} nucleoplasmin, and (*c*) 700 μg ml^{-1} polyglutamic acid. Scale bar is 50 μm. (Reproduced th permission from Philpott *et al.* (1991); copyright is held by Cell Press.)



gure 3. Two-dimensional poly-acrylamide gel electrophoresis (PAGE) analysis of sperm chromatin following cubation in *Xenopus* egg extract depleted of nucleoplasmin and in depleted extract reconstituted with cleoplasmin. Sperm nuclei were incubated at 100 μg ml^{-1} for 3 min in (*a*) buffer, (*b*) egg extract, (*c*) egg extract pleted of nucleoplasmin, and (*d*) depleted egg extract with nucleoplasmin added back. Proteins were analysed by iton-acid-urea two-dimensional SDS-PAGE gels. The positions of sperm basic proteins are labelled X and Y, and re histones are labelled 2A (H2A), 2B (H2B), 3 (H3) and 4 (H4). (Reproduced with permission from Philpott & no (1992); copyright is held by Cell Press.)