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The role of keratin filaments during nuclear envelope reassembly in *Xenopus* egg extracts

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Abstract We report here a new structure, named 'strings-of-pearls', which are seen to form in *Xenopus* egg extracts after incubation, as 200 nm membrane vesicles attach to 10 nm filaments. These membrane vesicles fuse together along the filaments to form annulate lamellae (AL) or attach to demembranated sperm chromatin to initiate assembly of a nuclear envelope. Immunoassay with anti-keratin antibodies AE3 showed that the filaments were mainly composed of a 56 kDa keratin-like protein. Addition of AE3 to the extracts resulted in inhibition of AL formation and defective assembly of NE. These results suggest a function of keratins in the assembly of nuclear envelopes during *Xenopus* development.

Key words: Membrane vesicle; Keratin; Nuclear assembly; Nuclear envelope; Annulate lamellae; *Xenopus* egg extract

1. Introduction

Since 1983 [1,2] it has been known that egg extracts from amphibia such as *Xenopus laevis* are a wonderful cell-free system to explore mechanisms of nuclear envelope (NE) assembly, which in eukaryotic cells occurs normally at the end of mitosis. By adding exogenous DNA or chromatin such as demembranated *Xenopus* sperm to the extracts, nuclei surrounded by an NE are formed, which are indistinguishable from those assembled in vivo. Annulate lamellae (AL), stacks of folded NE studded with nuclear pore complexes (NPC) on the other hand can form spontaneously in the extracts alone, or after addition of DNA or chromatin [3,4]. By ultracentrifugation the egg extracts can be fractionated into soluble cytosol and a membraneous vesicle fraction. Both fractions are required for the assembly of NE or AL [5]. Many previous studies have described round, smooth membrane vesicles with diameter of about 200 nm as precursors of both NE and AL [6–11]. How these vesicles get involved in the process of NE assembly however remains unclear. In this report, we present evidence that the 200 nm membrane vesicles participate in cell-free NE and AL formation by attaching themselves to a keratin-like 10 nm filament network which assembles in *Xenopus* egg extracts.

Intermediate filaments (IFs) are 8–10 nm protein fibers, which form part of the cytoskeleton in the cytoplasm of eukaryotic cells. Usually they compose a fibrous network surrounding the nuclei and radiating towards the cell surface. Particularly, IFs are resistant to nonionic detergents such as Triton X-100 and high salt solutions. IFs are thought to play important roles in maintaining cellular integrity and the mechanical properties of cytoplasm, though their functions should not be restricted simply to this aspect. Recent research suggests involvement of IFs in signal transduction and nuclear transport, as well as in nuclear envelope assembly [11–13].

The most diverse family of IFs are the keratins. While they occur primarily in epithelial cells and have been shown to be essential for the mechanical stability to those cells, keratins are usually the only IFs expressed in many vertebrate oocytes [14]. In *Xenopus* oocytes, keratin filaments consist of one type II keratin which is the homologue of human keratin K8 and two type I keratins which are homologues of K18 and K19, respectively. During oocyte maturation, keratin filaments disassemble into soluble oligomers accompanied by hyperphosphorylation of the type II keratin [14]. Upon activation of the eggs, the keratins gradually reorganize into a filament network. *Xenopus* egg extracts are derived from eggs activated spontaneously during centrifugation or by calcium ionophore A23187 and are arrested at interphase by cycloheximide inhibition of protein synthesis [5]. No attention has been paid to the dynamics and function of keratins in this cell-free system. We report here that type II keratins assemble into 10 nm filament networks which appear to link 200 nm membrane vesicles together and play an important role in NE formation in vitro.

2. Materials and methods

Xenopus egg extracts were prepared and fractionated according to Hartl et al. [15] with some modifications. Briefly, a mature *Xenopus laevis* female was induced to lay eggs by two injections of HCG (Human Chorionic Gonadotrophin) the night before use. The eggs were dejellied with 2% Cysteine (pH 7.8) and activated in 0.2 μg/ml calcium ionophore A23187. After washing in a lysis buffer (250 mM sucrose, 50 mM KCl, 3 mM $MgCl_2$, 10 mM HEPES, pH 7.5, 1 mM DTT, 100 ug/ml cycloheximide), they were fractured and sedimented into layers by centrifugation at $10\,000\times g$ for 15 min at 4°C. The middle layer was collected and used freshly as 'crude extract' or further fractionated at $200\,000\times g$ into soluble cytosol and membrane vesicles which could be frozen in liquid nitrogen and stored at −70°C separately for later use.

Demembranated *Xenopus* sperm chromatin was prepared following Newmeyer and Wilson [5] with some modifications. Sperm obtained from a mature *Xenopus* male was resuspended in SB (sperm buffer: 200 mM sucrose, 15 mM NaCl, 80 mM KCl, 7 mM $MgCl_2$, 5 mM EDTA, 15 mM PIPES-NaOH, pH 7.4, 0.5 mM spermidine, 0.2 mM

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Abbreviations: NE, nuclear envelope; AL, annulate lamellae; IF, intermediate filament; NPC, nuclear pore complex; CPD, critical point drying; TEM, transmission electron microscope; SEM, scanning electron microscope

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spermine) and pelleted repeatedly to wash off residual blood cells. They were then were demembranated in 0.05% lysolycithin for 5–10 min at room temperature. Demembranated sperm were pelleted and resuspended in SB at a concentration of 4×10^7/ml, frozen and stored like the egg extracts.

To carry out nuclear envelope assembly (or AL assembly), crude extracts were mixed with (or without) demembranated sperm (1000 sperm/μl extracts), and an ATP regenerating system (2 mM ATP, 20 mM phosphocreatine, 100 μg/ml creatine phosphokinase), and then incubated at 22–24°C. The crude extracts could be substituted by a mixture of cytosol and membrane vesicle fractions (v/v = 10:1) which would give identical results concerning NE and AL assembly. To test the effect of anti-keratin antibodies on NE and AL assembly, cytosol and membrane vesicle fractions were mixed with AE3 (an anti-type II keratin monoclonal antibody (mAb), a gift from Prof. T.T. Sun of New York University) at a final concentration in excess of keratins and incubated for 1 h at 22–24°C. Demembranated sperm and the ATP regenerating system were then added and the mixture was further incubated. The process of sperm nuclear assembly was observed routinely by fluorescence microscopy after staining the DNA with DAPI.

To prepare whole mounts of specimen for imaging by scanning electron microscopy (SEM), coverslips were made sticky with 3-aminopropyltriethoxysilane (Aldrich) as described by Ris and Malecki [16]. The specimens were dropped on the coverslips and left to attach for a few minutes. The coverslips were then immersed into 1% glutaraldehyde, rinsed with the lysis buffer, postfixed in 1% osmium tetraoxide (OsO_4), dehydrated in a series of ethanol and critical point dried (CPD) [17]. After CPD, the coverslips were coated with a thin layer of Pt by argon ion sputter coating and observed with a high resolution field-emission SEM (HR-FESEM) at 1.5 kV, the Hitachi S-900 at the Madison Integrated Microscopy Resource (IMR). To observe NE assembly around demembranated sperm, we generally attached the sperm to the sticky coverslips first and then put them in the mixture of extracts and the ATP regenerating system.

Immunogold labeling was carried out according to Jiao et al. [18] with modifications. The specimens attached to sticky grids were treated with 1% Triton X-100-CSK (300 mM sucrose, 100 mM KCl, 3 mM $MgCl_2$, 100 mM PIPES, pH 6.8, 1 mM EGTA, 1 mM PMSF), then prefixed in 2% paraformaldehyde-2.5% glutaraldehyde for 10 min, rinsed in CSK, incubated in 3% BSA for 30 min and then with the primary antibodies (AE3, or AE1: an anti-type I keratin mAb, also a gift from Prof. T.T. Sun) for 1 h. The preparation was then treated with the secondary antibodies, conjugated to 10 nm gold. After that they were postfixed with 0.25% glutaraldehyde and 1% OsO_4 respectively, dehydrated in ethanol and CPD, and observed by TEM (JEM-1010). For immunofluorescence staining, sticky coverslips were used instead of the grids.

Epon 812 embedment and ultrathin sectioning for TEM were performed following conventional procedures after glutaraldehyde and OsO_4 fixation. The sections were stained with uranyl acetate and lead citrate and then observed with the JEM-1010.

For electrophoresis and Western blots, 10% SDS-PAGE gel was used to analyze the proteins in the extracts after treatments. The proteins were transferred onto nitrocellulose paper and characterized with antibodies (AE3, AE1, 14h7: an anti-vimentin mAb, a gift from Prof. M.W. Klymkowsky, University of Colorado, USA; X223: a gift from Prof. G. Krohne, University of Würzburg, Germany) according to conventional methods.

3. Results

3.1. Formation of 'strings-of-pearls' in Xenopus egg extracts

When cytosol and membrane vesicle fractions were mixed with demembranated sperm and incubated in the presence of an ATP regenerating system, a new complex structure was observed by HR-FESEM, which is involved in the assembly of NE and AL (Fig. 1). It consists of small vesicles attached to a fine filament network. The vesicles are all of similar size, about 200 nm in diameter, attached along a 10 nm thick fiber. We therefore called this new structure 'strings-of-pearls' (Fig. 1a, b). In fact, we had reported in a previous publication

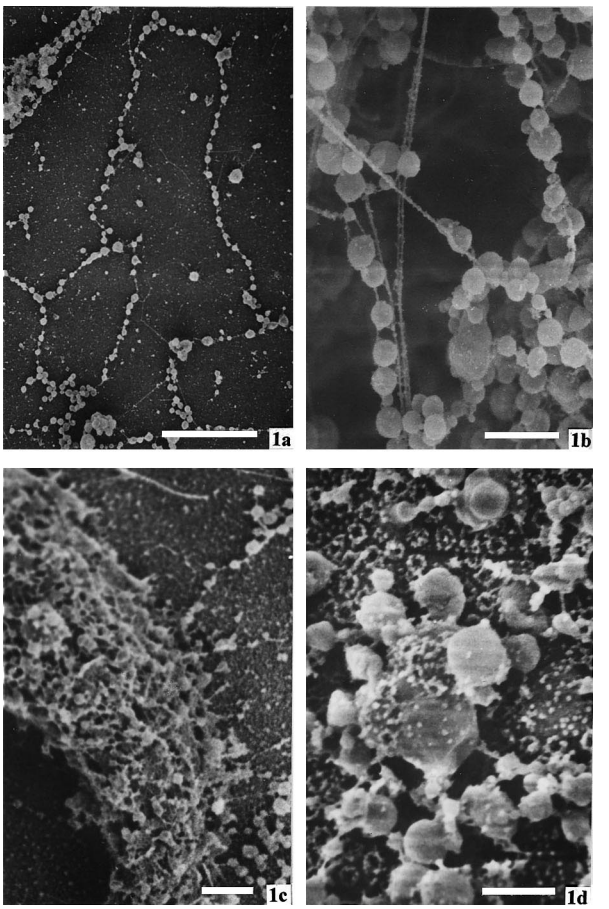

Fig. 1. Electron micrographs obtained with the Hitachi S-900 HR-FESEM showing 'strings-of-pearls' assembled in *Xenopus* egg extracts. a: 'Strings-of-pearls' observed at low magnification. Bar = 3 μm. b: 'Strings-of-pearls' observed at higher magnification. Bar = 0.5 μm. c: Early stage of NE assembly on demembranated sperm. Demembranated sperm were attached to sticky glass, then incubated with cytosol and membrane fraction for 2 h. 'Strings-of-pearls' are seen to attach to the sperm chromatin. The 200 nm vesicles then fuse to form the inner membrane for the new NE. Bar = 1 μm. d: Late stage of NE assembly on demembranated sperm. A continuous NE has been formed and NPCs are being inserted. Large ER vesicles with attached ribosomes attach to the nuclear surface and get incorporated into the enlarging NE. Bar = 300 nm.

similar structures which formed in the process of NE assembly induced by bacteriophage Lambda DNA in crude extracts and observed in whole mount preparations by TEM [19]. Before incubation, we observed free vesicles of various sizes and only few irregular filaments, indicating that the 'strings-of-pearls' were assembled de novo after mixing cytosol and membrane fraction. If the cytosol or membrane fraction was incubated alone, no 10 nm filaments or structures resembling 'strings-of-pearls' could be found, suggesting that the formation of these new structures requires the participation of both fractions. The vesicles in 'strings-of-pearls' may gradually fuse together into small membrane fragments. Normal looking NPCs appeared in these fragments at the same time, resulting in the formation of AL. The 'strings-of-pearls' could also attach to the surface of chromatin leading to the assembly of a nuclear envelope with NPCs (Fig. 1c, d). These observations indicated that 'strings-of-pearls' may play a role in the assembly of NE and AL in the *Xenopus* egg extract system.

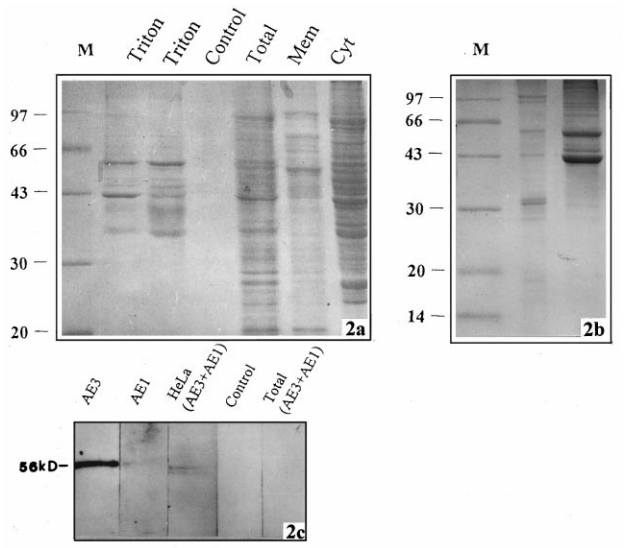

Fig. 2. Protein components revealed by SDS-PAGE and Western blot in the extracts. a: Electrophoretic results from fractionated extracts. M: Markers. Triton: Triton extraction of the mixture of cytosol and membrane fractions after incubation for 2 h. There were two major proteins (asterisks) in the Triton insoluble fraction, corresponding to the components of 10 nm filaments in 'strings-of-pearls'. Control: Cytosol and membrane fractions were incubated separately for 2 h and then mixed together for Triton extraction. No residual proteins were left after the extraction. Total: Total proteins of crude extracts. Mem: Protein components of membrane fraction. Cyt: Protein components of cytosol fraction. b: Residual proteins after Triton extraction of crude extracts incubated for 2 h. c: Western blots probed with anti-keratin antibodies (AE3, AE1) of Triton insoluble proteins. HeLa: HeLa cell cytoskeletons used as positive control, probe with AE3 + AE1. Control: Negative control, no primary antibodies were used. Total: Crude extracts probed with AE3+AE1.

3.2. Characterization of the 10 nm filaments

The diameter of the 'strings-of-pearls' fibers suggested that they might be some kind of IF. To characterize the nature of these filaments, we applied various antibodies to different kinds of IF proteins both for biochemical and ultrastructural analyses. A special feature of IFs is their resistance to non-ionic detergents. Cytosol and membrane fractions were mixed with an ATP regenerating system and incubated for 2 h allowing the 'strings-of-pearls' to assemble and then treated with 1% Triton X-100 for 5 min. Whole mount observation with TEM showed a 10 nm filament network remaining while the vesicles had been dissolved (see Fig. 3b, c). SDS-PAGE electrophoresis showed there were two major protein components in the Triton insoluble fraction with molecular weights of about 56 kDa and 43 kDa respectively (Fig. 2a). Using crude extracts instead of cytosol and membrane fractions gave similar results (Fig. 2b). Similar results were also obtained in repeated experiments (Fig. 2a, b). If the cytosol or membrane fraction was incubated alone for 2 h, and then mixed and Triton extracted, no proteins could be found in the Triton insoluble fraction (Fig. 2a). This was consistent with our EM observation that no filaments were assembled when cytosol or membrane fractions were incubated alone. Western blot analysis on Triton insoluble proteins using various antibodies showed that only anti-keratin antibodies (AE3 and AE1) gave positive results. No positive signal could be detected when anti-vimentin antibody (14h7) or anti-lamin antibodies (X223) were applied. Moreover, the 56 kDa protein cross-reacted with AE3 (specific for type II keratins) strongly while it showed only very weak reaction with AE1 (specific for type I keratins in mammalian cells) (Fig. 2c). In contrast, the 43 kDa protein gave no detectable signal to any of the antibodies tested. It is worthy being mentioned that no positive signal was shown in total proteins of the extracts without Triton extraction. It might be that the concentration of keratins in the whole extracts was too low to be detected.

All above results suggest that the 56 kDa protein, which is one of the major components of the 10 nm filaments, is a type II (basic) keratin, and that the filaments are keratin-like IFs. Immunofluorescence staining and immunogold labeling give further support to it. Fig. 3 shows the AE3 positive filament network formed in mixed extracts of cytosol and membrane vesicles, while no positive structure was observed with AE1 or in negative controls when neither AE3 nor AE1 were used (not shown). Immunogold labeling with 10 nm colloidal gold showed gold particles specifically bound to the filaments (Fig. 3c), which is in sharp contrast to the negative control where there is no labeling at all (Fig. 3b). To summarize, the 10 nm filament in 'strings-of-pearls' is a kind of keratin-like IF with the 56 kDa Triton insoluble protein as a basic component. The 43 kDa protein may either be the acidic keratin counterpart of the 56 kDa protein or an IF associated protein.

3.3. Effects of AE3 on 10 nm filament network formation and cell-free AL and NE assembly

According to the above results, the keratin-like 10 nm filaments act as an organizer for 200 nm vesicles. Since these vesicles are important for NE and AL assembly, we may expect that if we prevent assembly of keratin-like proteins, then AL and NE assembly should be inhibited. To test this hypothesis, we introduced AE3 antibodies, which should bind the 56 kDa proteins of the 10 nm filaments, into the extracts. We found that 56 kDa proteins disappeared from the Triton insoluble fraction, and no 'strings-of-pearls' or 10 nm fila-

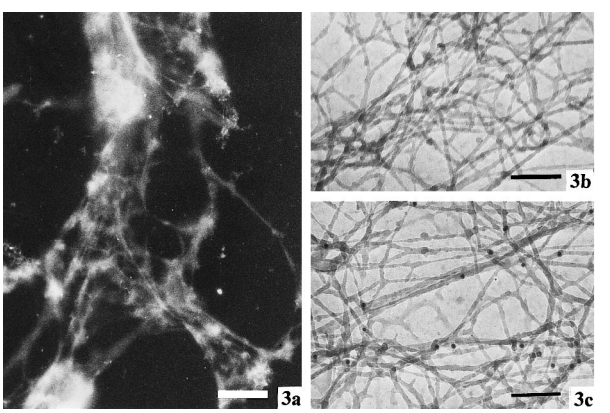

Fig. 3. a: Immunofluorescence staining with AE3 showing positive filament network formed in the extracts after incubation. Bar = 10 μm. b: TEM micrograph of whole mount showing 10 nm filament network assembled in the extracts, immunogold labeling without addition of primary antibody (AE3), used as a negative control for c. Bar = 200 nm. c: Immunogold labeling with AE3 of 10 nm filaments from 'strings-of-pearls' after Triton extraction, showing that 10 nm gold particles bind specifically to the filaments. Bar = 200 nm.

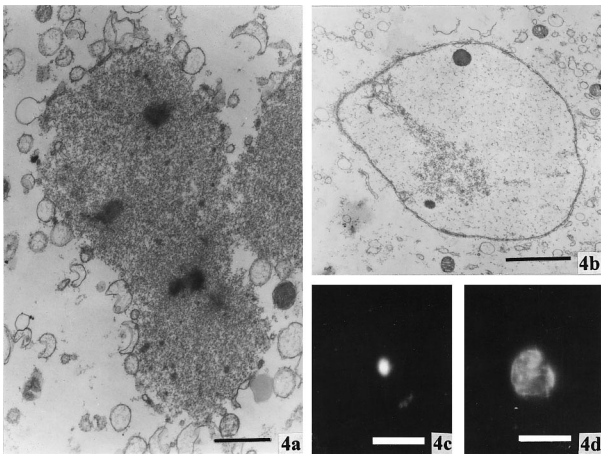

Fig. 4. Effects of AE3 on NE assembly in vitro. a: TEM micrograph of ultrathin section showing defective NE assembly around demembranated sperm chromatin when egg extracts were treated with AE3. The chromatin decondensed to a limited extent, no double nuclear envelope was formed though some small vesicles are visible around chromatin. Bar = 1 μm. b: Ultrathin section of a typical sperm nucleus assembled from condensed sperm chromatin in cell-free extracts. Note the typical parallel double nuclear envelope with NPCs and well-decondensed chromatin inside. Bar = 2 μm. c: DAPI staining of demembrananted sperm incubated in AE3 extracts. Bar = 10 μm. d: DAPI staining of a mature sperm nucleus in normal extracts. Bar = 10 μm.

ments were observed. Furthermore, we examined NE assembly on demembranated sperm and AL assembly in AE3 extracts in which there were no 10 nm filaments to bind the 200 nm vesicles. Normally, After DAPI staining, the demembranated sperm first appeared as long, thin curved lines (stage 1). With increasing incubation time, they swelled and decondensed (stage 2) and became small round dots (stage 3, Fig. 4c). Then the sperm chromatin continued to swell and decondense (stage 4, Fig. 4d). During this process they acquire an NE [1,2]. In contrast, in AE3 extracts, demembranated sperm could swell and round up (Fig. 4c), but they could not develop into mature nuclei. Observed in ultrathin sections by TEM, a typical sperm nucleus in AE3 extracts showed only limited chromatin decondensation (Fig. 4a) in sharp contrast to the full decondensation of sperm chromatin in control extracts (Fig. 4b). No typical intact NE formed around the limited decondensed chromatin in AE3 extracts, though small vesicles are seen along the chromatin surface (Fig. 4a). In normal extracts, a typical double membraned NE encircles the decondensed chromatinan (Fig. 4b). Correspondingly, no AL could be found in AE3 extracts, while large numbers of AL are present in normal incubations where NEs are formed (not shown). All the above results are consistent with our conclusion that inhibition of 10 nm filament assembly prevents the assembly of AL and NE.

In summary, we conclude that: (1) 'Strings-of-pearls' assemble in *Xenopus* egg extracts as 200 nm vesicles attach to newly assembled 10 nm filaments. (2) The 10 nm filaments are composed of keratin-like IF proteins. (3) Ten nm filament formation and cell-free AL and NE assembly are inhibited by addition of anti-keratin antibody AE3 to the extracts. Therefor, the 'strings-of-pearls' may play an important role in the assembly of AL and NE in *Xenopus* egg extracts.

4. Discussion

We report here a new structure, named 'strings-of-pearls', which appears during AL and NE assembly in *Xenopus* cell-free egg extracts. It consists of 200 nm vesicles bound to a keratin-like IF. We also provide evidence that this structure is involved in AL and NE assembly. These 'strings-of-pearls' were imaged as whole mounts by HR-FESEM and by TEM. They develop in crude extracts and in combinations of cytosol and membrane fractions. Similar structures are found in egg extracts incubated alone or with the addition of either demembranated sperm or purified protein-free Lambda DNA.

In *Xenopus* egg extracts, AL are always found to form simultaneously and competitively with NE adjacent to or even continuous with the NE [3,4], suggesting they share common membrane precursors. Here we extend the similarity between AL and NE in that they also share a common way in their assembly process.

The source of membrane precursors for NE has long been an attractive topic in the research of nuclear reassembly at the end of mitosis. The NE formation can be divided into two steps: binding followed fusion of membrane vesicle precursor at the surface of chromatin. Many attempts have been made in understanding the signals and mechanisms involved in sorting of membrane vesicles to the chromatin, though many questions still remain to be elucidated [6,7]. Different vesicle populations with the size ranging from 40–500 nm or even larger have been reported by different research groups [2,6–11]. However, a common agreement has led to the recognition of a kind of 200 nm membrane vesicles which may have certain relations with lamins. In *Xenopus* egg extracts, these vesicles appear to bind lamin L_{II}, a B-type lamin in *Xenopus* [9], while in cultured CHO cells, they are shown to be associated with lamin B [10]. These vesicles bind to the surface of chromatin and fuse to form the inner nuclear membrane. An interesting question is how do they reach the chromatin, by random assortment or a specific targeting process? Our data are in favor of the latter: these vesicles are delivered to the chromatin attached to specific 10 nm filaments. A similar result has been previously reported in CHO cells that lamin B-containing vesicles are docked on vimentin filaments during prometaphase and released from the filaments to assemble around chromosomes at the end of mitosis [11]. Yet we do not know at present whether the 200 nm membrane vesicles attached to the 10 nm filaments in our experiments contain any lamins. At least part of them should be devoid of lamins for no lamins have been detected in AL. Unlike CHO cells, *Xenopus* eggs are rich in keratins and little, if any, vimentin could be found.

IFs have long been believed to function in providing mechanical stability to cells. Early researches showed that they can be disrupted in muscle cells, fibroblasts and epithelial cells in culture without detectable effects on cell behavior [14]. In recent years, however, IFs were shown to participate also in other cellular activities such as signal transduction and nuclear transport [12,13]. However, controversial results were presented regarding the importance of IFs in embryonic development. Emerson reported that inhibition of cytokeratin assembly by antibody injection into 2-cell mouse embryos had no effect on blastocyst formation [20]. In contrast, knock out of the type II keratin K8 gene in mice resulted in a severe

embryonic lethal phenotype [12]. The latter would agree with our finding that keratins play an important role in NE assembly. In *Xenopus*, Klymkowsky et al. found that anti-keratin antibodies AE1 and AE3 induced abnormal gastrulation injected into fertilized eggs, which could be explained by our results. Unfortunately, they provide no data whether the embryonic nuclei were also abnormal [21].

The keratin system in *Xenopus* eggs is composed of one type II keratin and two type I keratins. So far we have only identified one 56 kDa protein which is most likely a type II keratin. Since keratin filaments are usually heteropolymers of type I and type II keratins, there remains one type I keratin to be identified in the 10 nm filaments of 'strings-of-pearls'. The 43 kDa unknown protein coexisting with the 56 kDa protein in the Triton insoluble fraction may be a possible candidate.

Our results about the formation of 'strings-of-pearls' and their possible role in AL and NE assembly provide an opportunity to reconsider the function of keratin IFs. Probably the most interesting question concerns the signals for membrane vesicles to recognize the 10 nm filaments or factors that mediate the interaction between the vesicles and the filaments. We are also further investigating the effects of keratins on AL and NE assembly after immunodepletion by antibodies, and shall isolate and sequence the 56 kDa protein to analyze its molecular structure related to its function.

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References

[1] Lohka, M.J. and Masui, Y. (1983) Science 220, 719–721.
[2] Lohka, M.J. and Masui, Y. (1984) J. Cell Biol. 98, 1222–1230.
[3] Dabauvalle, M.C., Loos, K., Merkert, H. and Scheer, U. (1991) J. Cell Biol. 112, 1073–1082.
[4] Zhang, B. and Zhai, Z. (1993) J. Chin. Electron Microsc. Soc. 12, 451–456.
[5] Newmeyer, D.D. and Wilson, K.L. (1991) Methods Cell Biol. 36, 607–634.
[6] Wilson, K.L. and Newport, J. (1988) J. Cell Biol. 107, 57–68.
[7] Boman, A.L., Delannoy, M.R. and Wilson, K.L. (1992) J. Cell Biol. 116, 281–294.
[8] Zhang, B. and Zhai, Z. (1995) Acta Biol. Exp. Sin. 28, 41–53.
[9] Lourim, D. and Krohne, G. (1993) J. Cell Biol. 123, 501–512.
[10] Burke, B. and Gerace, L. (1986) Cell 44, 639–652.
[11] Maison, C., Pyrpasopoulou, A. and Georgatos, D. (1995) EMBO J. 14, 3311–3324.
[12] Klymkowsky, M.W. (1995) Curr. Opin. Cell Biol. 7, 46–54.
[13] Chou, Y.-H., Skalli, O. and Goldman, R.D. (1997) Curr. Opin. Cell Biol. 9, 49–53.
[14] Klymkowsky, M.W. (1995) Curr. Top. Dev. Biol. 31, 455–485.
[15] Hartl, P., Olson, E., Dang, T. and Forbes, D.J. (1994) J. Cell Biol. 124, 235–248.
[16] Ris, H. and Malecki, M. (1993) J. Struct. Biol. 111, 148–157.
[17] Ris, H. (1985) J. Cell Biol. 100, 1474–1487.
[18] Jiao, R., Wu, D., Zhang, B., Cai, S. and Zhai, Z. (1991) J. Electron Microsc. Tech. 18, 126–134.
[19] Zhang, B. and Zhai, Z. (1994) J. Chin. Electron Microsc. Soc. 13, 157–162.
[20] Emerson, J.A. (1988) Development 104, 219–234.
[21] Klymkowsky, M.W., Shook, D.R. and Maynell, L.A. (1992) Proc. Natl. Acad. Sci. USA 89, 8736–8740.