Dynamics of *Tetrahymena* macronuclear lamina during cell division

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ABSTRACT

During mitosis, the nuclear lamina in higher eukaryotic cells undergoes a distinctly morphological change. It breaks down into lamin polymers or monomers at prophase. At telophase, the lamins reassemble around the condensed chromatin to form the layer of lamina. Using antiserum to mammalian lamins, we studied the dynamics of lamina during cell division in the macronucleus of *Tetrahymena shanghaiensis*, which divided in the way of amitosis. In contrast to those in higher animal cells, the typical perinuclear lamin distribution in the macronucleus persisted throughout the whole cell cycle. It was further found that in some synchronized cells, the lamin distribution displayed an unusual pattern consisting of a series of spots within the macronucleus. Using South-western hybridization, we found that the purified 66 KD lamin in *Tetrahymena* showed specific affinity with the telomere DNA sequence in the same species. Therefore, we propose that pattern of immunofluorescence may be due to the interaction of lamin protein with the nucleoli and the condensed chromatins in the macronucleus.

Key words: *lamina, mitosis, amitosis, telomere.*

INTRODUCTION

Generally the nuclear envelope (NE) in higher animal cells consists of 3 parts: inner and outer membranes, nuclear pore complexes and nuclear lamina[1, 2]. The nuclear lamina, a fibrillar meshwork lining the inner nuclear membrane, provides an attachment site at the NE for interphase chromatin[3-5]. In higher animal cells,

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the lamina consists mainly of one or more polypeptides called lamins, which are members of the intermediate filament protein superfamily[6].

The nuclear lamina displays a spectacularly morphological change during cell division period. With an onset of mitosis and meiosis, the lamina is disassembled into soluble subunits that are dispersed throughout the cytoplasm[7, 8, 9]. Subsequently, lamins are reassembled and become parts of the reforming daughter nuclei during telophase. It has been demonstrated that the disassembly-assembly is regulated by p34^{cdc2}-mediated phosphorylation-dephosphorylation of lamins[10].

Interactions between nuclear membranes, lamina and chromatin are likely to be fundamentally important for the higher level chromosome organization during interphase and for the dynamic of the NE during cell cycle[5, 11]. Lamins have been shown to bind directly to chromatin[4, 12, 13, 14] and to DNA[15, 16] *in vitro*, though the physiological significance of these interactions remains to be elucidated. The membrane association of certain lamins is partly due to an isoprenoid derivative that is covalently linked to the carboxyl terminus of vertebrate lamin B and newly synthesized lamin A[17].

In an earlier paper[18], we demonstrated the existence of lamina in the macronucleus of *Tetrahymena*. Although a hypothesis has emerged to explain the dynamics of the nuclear lamina during cell cycle[19], nothing is known about the fate of this structure during amitosis. It is important to make a further exploitation in this field for understanding the general existence of nuclear lamina in all eukaryotic cells as well as its function.

MATERIALS AND METHODS

Cell culture and synchrony

Cells of *Tetrahymena shanghaiensis* strain were cultured at 25°C- 26°C in a protose - peptone medium containing 2% protose peptone, 0.1% yeast extract, and 0.1% glucose.

For cell synchrony, the cells were alternatively exposed to sublethal temperature (37°C) for 30 min, and then to optimum temperature (26°C) for another 30 min. After 6 heat shock cycles, the cells were transferred to 26°C, and then sampled when most cells began to divide.

Differential fraction of the cells

Cells were fractionated as described in the earlier paper[18]. Briefly, cells were treated with 0.5% Triton X-100 in CSK buffer (10 mM PIPES, pH 6.8, 100 mM KCl, 300 mM sucrose, 10 mM MgCl₂, 1 mM EGTA, 10 mM PMSF) for 5 min at 0°C. The cytoskeleton framework was separated from the soluble proteins by centrifugation at 1,000 g for 5 min. Then the pellets were extracted in RSB-Magic solution (42.5 mM tris-HCl, pH 8.3, 8.5 mM NaCl, 1 mM PMSF, 1% Tween-40, 0.5% sodium deoxycholate) at 0°C for 10 min and pelleted as mentioned above. This step stripped away the microtubules and microfilaments. The remnant pellets were resuspended in CSK buffer and treated by DNase I (100 g/ml) at room temperature for 30 min followed by 0.25 M ammonium sulfate. Finally, the sample was centrifuged at 1,000 g for 5 min. This step removed the chromatin fraction, leaving finally the intermediate filaments - lamina - nuclear matrix system.

Preparation of lamins from rat liver

The livers were taken out from male Sprague-Dawley rats, washed with 0.9% NaCl, and stored at -20 °C.

The rat liver nuclear envelopes were isolated following Kauffmann's method[20]. The salt-washed nuclear envelopes were prepared as described by Gerace et al[8]. Then the envelopes were incubated for 30 min in TKE 1 solution (10% sucrose, 2% Triton X-100, 20m M MES-KOH, pH 6.0, 300 mM KCl, 2 mM EDTA, 1% -ME), and centrifuged for 30 min at 6,000 g. Then the pellets were incubated for 30 min in TKE 2 solution (2% Triton X-100, 20 mM tris-HCl, pH 9.0, 500 mM KCl, 2 mM EDTA, 1%-ME), and centrifuged for 40 min at 200,000 g. The supernatant contained enriched rat nuclear lamins[21]. The dissolved lamin was pelleted with acetone and dissolved in sample lysis buffer.

Production of antiserum to rat liver lamins

The enriched rat liver lamin samples were run on 8% polyacrylamide gels and stained with Coomassie brilliant blue R-250[22]. The bands of the lamins were excised from the gel and used to immunize a rabbit. The animal was administered about 2 mg proteins each injection, in a total volume of less than 2 ml. Antigens were injected for 3 times at 2 weeks interval and that rabbit was bled 10 days after the third immunization.

Characterization of the rabbit antiserum

Ouchterlony double diffusion analysis was used to titrate the antiserum. Specificity of the antiserum was examined using Western blotting analysis as described earlier[18].

Indirect immunofluorescent staining of T. shanghaiensis cells with the rabbit antiserum

Cells were fixed with cold methanol for 5 min, and then with cold acetone for 5 min. The cells were washed in PBS, then incubated with the rabbit antiserum for 24 h at 4 °C. After being washed in PBS, the sample was incubated with FITC conjugated goat anti-rabbit IgG, for 24 hours at 4 °C. The cells were washed in PBS and examined.

³²P-labeling DNA probe

Tetrahymena rDNA, rDNA 5'NTS, telomere DNA and pBR322 DNA were labeled with ³²P-dTTP by nick translation.

South-western hybridization

South-western hybridization was performed as described[23]. The proteins were transferred to nitrocellulose sheets after SDS-PAGE. The sheets were equilibrated in hybridization buffer (10 mM tris-HCl, pH 7.4, 1 mM EDTA, 0.5% tween-20) for 1 h, then blotted with ³²P-labeled probes for 4 h at room temperature. The sheets were washed in washing buffer (containing 0.5% lipid-depleted milk in the hybridization buffer) for 30 min at 60 °C. The sheets were dried and autoradiographed.

Electron microscopy

Samples were prefixed with 2.5% glutaraldehyde in 0.1 M PBS for 1 h, washed in PBS, and postfixed with 1% OsO₄ in 0.1 M PBS for 30 min. After washed in PBS, the samples were dehydrated through gradient ethanol series. Subsequently, the cells were embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate.

RESULTS

Production and characterization of rabbit antiserum to liver lamins

To study the behavior of macronuclear lamina during cell division, we prepared

Fig 1. SDS-PAGE of the enriched rat liver lamin proteins.

Fig 2. Characterization of the rabbit antiserum to rat liver lamins.

a: SDS-PAGE of the enriched rat liver lamin proteins. The two bands of lamins (*) were excised from the gel and used to immunize the rabbit. b: SDS-PAGE of the proteins of the extracted *Tetrahymena* cells. c: Western blotting analysis of the proteins extracted from carrot suspension culture cells. The antisera showed positive reaction with 66 KD lamin protein, but also some cross reaction with a 84 KD protein. d: Western blotting analysis of the extracted *Tetrahymena* cells. Only 66 KD lamin protein positively reacted with the antiserum e: Western blotting analysis of rat liver lamins with the antiserum.

Fig 3. Indirect immunofluorescent staining of the synchronized *Tetrahymena* cells with the antiserum. a - e: The positive reaction of the periphery of the macronucleus persisted through the cell division period. a) before cell division; b, c, d) during cell division; e) a newly-formed daughter cells. f- h) The unusual immunofluorescent staining in macronucleus of synchronized cells. f) before cell division; g) during cell division; h) in a newly-formed daughter cell.

antiserum that can react specifically with the 66 KD lamin in *Tetrahymena* cells. Because of the small amount of lamin proteins contained in *Tetrahymena* cells, we chose rat liver as the source of antigens for its abundant quantity and easy availability. The composition of the enriched nuclear lamin proteins from rat liver was shown in Fig 1. The lamins in rat liver consist of three kinds of proteins: laminA, B and C. The molecular weight of lamin B and C were very similar, and was difficult to distinguish them from each other on a SDS-PAGE gel. The enriched sample consisted mainly of three kinds of lamins with little other proteins. To make sure of the purity of the antigen, the two bands of lamins were excised from the gel, and used to immunize the rabbit.

The animal was bled 10 days after the third injection. By Ouchterlony double diffusion analysis, the titre of the antiserum was found to be 1:16. Furthermore, its specificity was studied by Western blot analysis, Fig 2 showed the result of SDS-PAGE (2-a, b) and the results of Western blot analysis (2-c, d, e). The antiserum reacted specifically with the lamin proteins in *Tetrahymena* cells (Fig 2-d) and in rat liver (Fig 2-e). Besides the lamin proteins, protein isolated from carrot suspension cells, with the molecular weight of 84 KD, also crossreacts with the antiserum (Fig 2-c).

Cell synchronization and dynamics of the macronuclear lamina during cell division

Cells were harvested in the division period through cell synchrony experiment. After being transferred to the optimum temperature following the last heat shock cycle for 60 min, 80% of the cells began to divide synchronously. The cells were then sampled and used for the immunofluorescence microscopy.

The results of indirect immunofluorescent staining of *Tetrahymena* cells were shown in Fig 3. When the cells were about to divide, their cell volume as well as the nuclear volume became enlarged, and the lamina structure was very significant at the periphery of the micronucleus (Fig 3-a). During cell division, the macronucleus became longer and the periphery of the macronucleus showed strong positive reaction with the antiserum (Fig 3-b, c). At the end of the cell division and before the complete separation of cytoplasm macronuclear lamina could be seen at the periphery of the daughter macronucleus (Fig 3-d). In a newly - formed cell, the antiserum gave positive reaction with its macronucleus periphery (Fig 3-e). It is noticed that in every phase of the cell cycle, some cells displayed an unusual pattern of immunofluorescence consisting of a series of fluorescent spots within the macronucleus (Fig 3-f, g, h).

The 66 KD lamin protein bound specifically with the telomere DNA sequence of Tetrahymena cells

It was found that the nucleoli in the macronucleus of *Tetrahymena* cells often

Fig 4. The nucleoli in the macronucleus of *Tetrahymena* cells lie under the inner nuclear membrane. (NE: nuclear envelope; CH: chromatin; NU: Nucleoli; macN: macronucleus. $\times 11,000$)

Fig 5. a, b: SDS-PAGE of the standard molecular weight proteins (a) and the purified 66 kD *Tetrahymena* lamin protein. c: Western blotting analysis of the purified 66 kD protein with the polyclonal antibodies to mammalian lamins. d, e: South-western analysis of the standard molecular weight proteins (d) and the lamin protein (e) with the ^{32}P -labelled telomere DNA sequence. f, g: South-western analysis of the standard molecular weight proteins (f) and the lamin protein (g) with the ^{32}P -labelled rDNA sequence. h, i: South-western analysis of the standard molecular weight proteins (h) and the lamin protein (i) with the ^{32}P -labelled rDNA 5-NTS sequence.

located under the nuclear envelope (Fig 4). In our earlier paper[18], we demonstrated that only lamina structure and nucleolar residue remained after the depletion of all inner nuclear matrix, chromosomes and the membranes. The nucleoli bound tightly with the macronuclear lamina, which seemed to imply that there was some affinity between the lamina and the nucleoli. In order to explain this phenomenon as well as the occurrence of the unusual immuno-fluorescent staining, we took some Western blotting and South-western hybridization. The result shown in Fig 5 demonstrated that *Tetrahymena* telomere DNA sequence bound tightly with the 66 KD lamin *in vitro* (Fig 5-e), and the affinity between rDNA sequence and lamin protein was lower (Fig 5-g), while rDNA 5'NTS did not bind to the protein at all (Fig 5-i). The molecular weight standard proteins did not bind to all three probes (Fig 5-d, f, h).

DISCUSSION

The macronucleus of *Tetrahymena* cells disappears during cell conjugation. During cell vegetative growth, the macronucleus undergoes amitosis and becomes elongated and separated into daughter nuclei. During the whole life cycle, the macronucleus of *Tetrahymena* can not proceed the karyokinetic division. We have demonstrated that there was a lamina structure in the macronucleus of *Tetrahymena* cells[18]. The dynamics of lamina during mitosis has been studied extensively, but nothing is known about this structure during amitosis. Using *Tetrahymena* cells, we gained some results about the dynamics of the macronuclear lamina during amitosis.

The volume of cells as well as that of the macronucleus enlarged after cell synchrony. The increase of cell volume is the prelude of cell division. From the beginning of cell division to the formation of daughter cells, there was consistently a region of a strongly positive staining reaction at the periphery of the macronucleus following the treatment with anti-lamin antiserum and FITC-labeled 2nd antibody (Fig 3-a to e). It seemed that either the macronuclear lamina did not disassemble at all during the macronucleus division, or the macronuclear lamina only disassembled partly. Why the behavior of nuclear lamina during amitosis is quite different from that during mitosis? Here we can only make some hypothetical speculations. Hyperphosphorylation of lamin proteins has long been considered to be causally related to the transient disassembly of the nuclear lamina during mitosis[18, 24]. Perhaps during amitosis of the macronucleus of *Tetrahymena*, its cellular environment may not be entirely favorable to the lamina disassembly. Some cells (including cells in division) displayed an unusual pattern of cytochemical distribution of lamin after cell synchrony. This unusual staining consisted of a series of spots within the macronucleus (Fig 3-f to h) which like that in synchronized fibroblast cells[25]. The positively reactive spots could have two possible sources. The first was the excess lamin protein synthesized during cell synchronization. In this period, cell division was blocked, whereas the synthesis of proteins went on under optimum temperature. When there were much more lamin proteins than actually needed for the synthesis of nuclear

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envelope, the unassembled lamin proteins would deposit in the macronucleus and cause the staining by anti-lamin protein antiserum. The second may be the lamin proteins derived from the partial disassembly of lamina structure. During cell division in higher animal cells, the disassembly of lamina was closely correlated with nuclear envelope breakdown, but there were two separate processes. The nuclear envelope could remain intact, while the lamina had already disassembled[26]. The disassembly of lamina led to nuclear envelope breakdown, but the former did not necessarily cause the latter. Using immunofluorescent analysis, we have demonstrated that there was a region of positive reaction at the periphery of the macronucleus during the whole process of cell division. But we could not exclude the possibility that the lamina structure had partly disassembled.

Why does the lamin proteins not distribute evenly in the macronucleus but gave an pattern of immunofluorescence consisting of a series of spots within the nuclei? There are 20,000 copies of rDNA molecules forming about 8,000 nucleoli in the macronucleus of a *Tetrahymena* cell. There are also a large amount of chromatins in the macronucleus. During cell division, they form about 80 chromosome aggregates. The telomere sequences exist at the ends of these rDNA molecules and chromosomes. Earlier studies have shown that lamin proteins had specific affinity to mitotic chromosomes [4,12-14]. Using the method of South-western hybridization, we demonstrated that the telomere DNA sequence could bind tightly to lamin proteins associated with nucleoli and chromosomes. Therefore, the lamin proteins were shown as spot pattern of distribution in the immunostained macronucleus. With the redistribution of the nucleoli and chromosomes between the daughter cells, the intra-macronuclear lamin proteins were distributed into the lamina structure of the newly-formed cells, or they immediately took part in the next cell division process.

REFERENCES

- [1] Dessev GN. Nuclear envelope structure. *Curr Opin Cell Biol* 1992; **4**:403-35.
- [2] Gerace L. Molecular trafficking across the nuclear pore complex. *Curr Opin Cell Biol* 1992; **4**:637-45.
- [3] Gerace L, Burke B. Functional organization of the nuclear envelope. *Annu Rev Cell Biol* 1988; **4**:335-74.
- [4] Glass JR, Gerace L. Lamin A and C bind and assemble at the surface of mitotic chromosomes. *J Cell Biol* 1990; **111**:1047-57.
- [5] Paddy MR, Belmont AS, Saumweber H, Agard DA, Sedat JW. Interphase nuclear envelope lamins form a discontinuous network that interacts with only a fraction of the chromatin in the nuclear periphery. *Cell* 1990; **62**:89-106.
- [6] Frank WW. Nuclear lamins and cytoplasmic intermediate filament proteins: a growing multigene family. *Cell* 1987; **48**:3-4.
- [7] Gerace L, Blum A, Blobel G. Immunocytochemical localization of the major polypeptides of the pore complex-lamina fraction. Interphase and mitotic distribution. *J Cell Biol* 1978; **79**:546-66.
- [8] Gerace L, Comeau C, Benson M. Organization and modulation of the nuclear lamina structure. *J Cell Sci* 1984; **1 (Suppl)**:137-60.
- [9] Benavente R, Krohne G: Change of karyoskeleton during spermatogenesis of *Xenopus*: expres-

- sion of lamin LIV, a nuclear lamina protein specific for the male germ line. Proc Natl Acad USA 1985; **82**:6176-80.
- [10] McKeon F. Nuclear lamin proteins: domains required for nuclear targeting, assembly, and cell-cycle-regulated dynamics. Curr Opin Cell Biol 1991; **3**:82-6.
- [11] Burke B, Gerace L. A cell free system to study reassembly of the nuclear envelope at the end of mitosis. Cell 1986; **44**:639-52.
- [12] Burke B. On the cell-free association of lamins A and C with metaphase chromosomes. Exp Cell Res 1990; **166**:169-76.
- [13] Hoger T, Krohne G, Kleinschmidt J. Interaction of *Xenopus* lamins A and L II with chromatin *in vitro* mediated by a sequence element in the carboxyl terminal domain. Exp Cell Res 1991; **197**:280-9.
- [14] Yuan J, Simos G, Globe1 G, Georgatos SD. Binding of lamin A to polynucleosomes. J Biol Chem 1991; **266**:9211-5.
- [15] Shoeman R, Traub P. The *in vitro* DNA-binding properties of purified nuclear lamin proteins and vimentin. J Biol Chem 1990; **265**:9055-61.
- [16] Luderus MME, Jong L de, Driel R Van. Binding of matrix attachment regions to lamin B1. Cell 1992; **70**:949-59.
- [17] Burke B. The nuclear envelope and nuclear transport. Curr Opin Cell Biol 1990; **2**:514-20.
- [18] CHEN B, CAI ST, ZHAI ZH. Investigation of nuclear lamina in Tetrahymena thermophila. Acta Biologicae Experimentalis Sinica 1994; **27**(2): 153-64.
- [19] Nigg EA. Assembly-disassembly of the nuclear lamina. Curr Opin Cell Biol 1992; **4**:105-9
- [20] Kaufmann SH, Gibson W, Shaper JH . Characterization of the major polypeptides of the rat liver nuclear envelope. J Biol Chem 1983; **258**:2710-9.
- [21] Aebersold U, Cohn J, Gerace L. The nuclear lamina is a meshwork of intermediate filaments. Nature 1986; **323**:560-4.
- [22] Laemmli UX. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; **227**:680-5.
- [23] Wang GS , Luo WJ , Pan WJ, Ding MX , Zhai ZH . Association of chromosomal telomere DNA with nuclear matrix in HeLa cell. Science in China (Scientia Sinica) Series B 1994; **37**(6):691-700.
- [24] McKeon F. Nuclear lamin proteins: domains required for nuclear targeting, assembly, and cell-cycle-regulated dynamics. Curr Opin Cell Biol 1991; **3**:82-6.
- [25] Bridger JM, Kill IR, O'Farrell M, Hutchison CJ. Internal lamin structures with G1 nuclei of human dermal fibroblasts . J Cell Sci 1993; **104**(2):297-306.
- [26] Newport JW, Spann T. Disassembly of the nucleus in mitotic extracts : membrane vesicularization, lamin disassembly, and chromosome condensation are independent processes. Cell 1987; **48**:219-30.

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