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Phosphorylation of the $p34^{cdc2}$ target site on goldfish germinal vesicle lamin B3 before oocyte maturation

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Abstract

The nuclear membranes surrounding fish and frog oocyte germinal vesicles (GVs) are supported by the lamina, an internal, mesh-like structure that consists of the protein lamin B3. The mechanisms by which lamin B3 is transported into GVs and is assembled to form the nuclear lamina are not well understood. In this study, we developed a heterogeneous microinjection system in which wild-type or mutated goldfish GV lamin B3 (GFLB3) was expressed in *Escherichia coli*, biotinylated, and microinjected into *Xenopus* oocytes. The localization of the biotinylated GFLB3 was visualized by fluorescence confocal microscopy. The results of these experiments indicated that the N-terminal domain plays important roles in both nuclear transport and assembly of lamin B3 to form the nuclear lamina. The N-terminal domain includes a major consensus phosphoacceptor site for the $p34^{cdc2}$ kinase at amino acid residue Ser-28. To investigate nuclear lamin phosphorylation, we generated a monoclonal antibody (C7B8D) against Ser-28-phosphorylated GFLB3. Two-dimensional (2-D) electrophoresis of GV protein revealed two major spots of lamin B3 with different isoelectric points (5.9 and 6.1). The C7B8D antibody recognized the pI-5.9 spot but not the pI-6.1 spot. The former spot disappeared when the native lamina was incubated with lambda phage protein phosphatase (λ-PP), indicating that a portion of the lamin protein was already phosphorylated in the goldfish GV-stage oocytes. GFLB3 that had been microinjected into *Xenopus* oocytes was also phosphorylated in *Xenopus* GV lamina, as judged by Western blotting with C7B8D. Thus, lamin phosphorylation appears to occur prior to oocyte maturation in vivo in both these species. Taken together, our results suggest that the balance between phosphorylation by interphase lamin kinases and dephosphorylation by phosphatases regulates the conformational changes in the lamin B3 N-terminal head domain that in turn regulates the continual in vivo rearrangement and remodeling of the oocyte lamina.
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Keywords: Germinal vesicle; Intermediate filament; Nuclear matrix; Protein kinase; Protein phosphatase

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Introduction

The nucleus is a complex organelle in which a number of important functions, such as DNA replication, transcription, and RNA processing, are carried out in a temporally and spatially defined manner (Cox and Laskey, 1991; Ma et al., 1998; Mills et al., 1989; Misteli and Spector, 1998; Xing and Lawrence, 1991). The mechanisms by which cells achieve spatial ordering of their nuclear functions are unclear, however. Although recent observations suggest that the nuclear architecture plays an important role in establishing spatial organization in the nucleus, the molecules involved in this process have not been identified, with the exception of the nuclear lamins (Baskin, 1995; Berezney and Wei, 1998; Berezney et al., 1995; Hoffman, 1993; Nickerson et al., 1995; Spector, 1993; Van Driel et al., 1995). The lamins are the major constituents of the nuclear lamina (Aebi et al., 1986; Belmont et al., 1993; Gerace and Burke, 1988), a mesh-like structure that lies next to the inner nuclear membrane, and they are also present in the inner nuclear matrix (Bridger et al., 1993; Hozak et al., 1995; Moir et al., 1994).

Sequencing of lamin cDNA clones from different species has revealed that nuclear lamins are members of the intermediate filament (IF) protein family (e.g., McKeon et al., 1986). IFs are present in all animal cytoplasms and nuclei, and they have a self-polymerizing function (for reviews see Fuchs and Weber, 1994; Parry and Steinert, 1995; Stuurman et al., 1998). Site-specific phosphorylation and dephosphorylation of IF proteins alters the filament structure and organization (for a review, see Inagaki et al., 1996). Lamins also exhibit dynamic behavior by assembling and disassembling during the cell cycle, as is the case with other cytoplasmic IF proteins (for reviews, see Nigg, 1992; Skalli and Goldman, 1991).

Lamins contain several conserved phosphoacceptor sites that lie within the regions flanking either end of an α-helical-rod domain. Studies have revealed that specific phosphorylation of lamins at conserved Ser or Thr residues occurs only in M phase, and that it triggers lamina disassembly in vitro (Eggert et al., 1991; Peter et al., 1990; Ward and Kirschner, 1990). Furthermore, mutations within the phosphoacceptor sites interfere with M-phase-specific lamina disassembly in vivo (Heald and McKeon, 1990). The cdc2 kinase is a prominent candidate for the M-phase-specific kinase, since either purified $p34^{cdc2}$ kinase or maturation-promoting factor can induce lamin disassembly in isolated nuclei (Dessev et al., 1991; Peter et al., 1990), nuclear ghosts (Molloy and Little, 1992), and bacterially produced, assembled lamins (Heitlinger et al., 1991; Peter et al., 1991; Ward and Kirschner, 1990).

In addition to the cdc2 kinase phosphoacceptor sites, other M-phase-specific phosphorylation sites that regulate lamin assembly/disassembly have been identified in several species. For example, phosphorylation of a Ser that lies close to the cdc2 site inhibits head-to-tail polymerization of lamin, as does phosphorylation of the cdc2 kinase phosphoacceptor site itself (Stuurman, 1997), and M-phase disassembly of the nuclear lamina can be induced by protein kinase C (PKC)-mediated phosphorylation (Collas, 1999; Collas et al., 1997; Hocevar et al., 1993). These findings suggest that various kinases are involved in M-phase lamin disassembly, with distinct kinases functioning in different cell types.

In several species and cell lines, nuclear lamins are known to be phosphorylated in the interphase nucleus (Eggert et al., 1991; Ottaviano and Gerace, 1985; Schneider et al., 1999), but the function of interphase phosphorylation is not as clearly defined as that of mitotic phosphorylation. Interphase phosphorylation by PKC at a site close to the nuclear localization signal (NLS) of chick lamin B2 inhibits nuclear transport (Hennekes et al., 1993), suggesting that lamin phosphorylation regulates its spatial localization. Considerable evidence also exists for a structural role for lamins in DNA replication. An unidentified site in human lamin B2 is phosphorylated to a significant extent during S-phase (Kill and Hutchison, 1995), suggesting that S-phase phosphorylation of lamins may be involved in DNA replication. A few candidates for the interphase lamin kinase have been described. Ca^{2+}- and cAMP-independent lamin kinases have been shown to bind tightly to the lamin-enriched fraction of Ehrlich ascites tumor cells (Dessev et al., 1988). PKC phosphorylates nuclear lamin B2 during interphase, both in vivo and in vitro (Fields et al., 1988), and casein kinase II is associated with the nuclear matrix in rat liver nuclei (Tawfic and Ahmed, 1994). However, the biological significance of these observations is unclear.

An oocyte is a specialized cell that undergoes meiosis and passes on the maternal genetic material to the next generation. Oocytes differ from somatic cells in their appearance. Each oocyte contains a germinal vesicle (GV), a large, highly specialized nucleus that is arrested at the meiotic diplotene stage in almost all animals. The GV contains abundant nuclear material inside a nuclear membrane that is supported mechanically by a mesh-like structure called the nuclear lamina. In frogs and fish, the protein lamin B3 is the major component of the GV lamina (Benavente et al., 1985; Hofemeister et al., 2002; Krohne et al., 1981; Stick, 1988; Stick and Hausen, 1985; Yamaguchi and Nagahama, 2001; Yamaguchi et al., 2001), but the mechanism by which lamin B3 is transported into and assembled inside the GV is unclear. The fish and *Xenopus* GV lamin B3 proteins contain an N-terminal consensus site for phosphorylation by $p34^{cdc2}$ kinase, which induces lamina disassembly, in much the same manner as the somatic lamins (Hofemeister et al, 2002; Yamaguchi et al., 2001).

Recently, anti-phosphopeptide antibodies have been used to analyze the phosphorylation status of IFs in vivo (Inagaki, 1994; Sekimata et al., 1996). In the present study, we exploited the large nuclear *Xenopus* GV in examining

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