The Conserved Carboxy-Terminal Cysteine of Nuclear Lamins Is Essential for Lamin Association with the Nuclear Envelope

Georg Krohne, Irene Waizenegger, and Thomas H. Höger

Division of Membrane Biology and Biochemistry, Institute of Cell and Tumor Biology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany

Abstract. We have analyzed the interaction of soluble nuclear lamins with the nuclear envelope by microinjection of normal and mutated lamins into the cytoplasm of *Xenopus laevis* oocytes. Our results demonstrate that the conserved cysteine of the carboxy-terminal tetrapeptide Cys Ala/Ser Ile Met of lamins is essential for their association with the nuclear envelope. Removal of this sequence or replacement of the cysteine by serine resulted in *Xenopus* lamin L_I remaining in a soluble, non-envelope-associated state within the nucleus. Similar mutations of *Xenopus* lamin A resulted in only partial reduction of nuclear envelope association, indicating that lamin A contains additional signals that can partially compensate for the lack of the cysteine. Mammalian lamin C lacks this tetrapeptide and is not associated with the nuclear envelope in our experimental system. Cloning of the tetrapeptide Cys Ala Ile Met to the carboxy terminus of human lamin C resulted in lamin being found in a nuclear envelope–associated form in oocytes. Mutations at the amino terminus and in the α-helical region of lamin L_I revealed that the carboxy terminus mediates the association of lamins with the nuclear envelope; however, this alone is insufficient for maintenance of a stable association with the nuclear envelope.

THE nuclear envelope is the boundary between the nuclear and cytoplasmic compartments, which is involved in both the selective nucleo-cytoplasmic transport of macromolecules and particles and the structural organization of the nuclear interior (for reviews see Franke, 1974; Franke et al., 1981; Newport and Forbes, 1987; Gerace and Burke, 1988). Major components of the nuclear envelope are the outer and inner nuclear membrane, the nuclear pore complexes, and the nuclear lamina. Nuclear pore complexes are gateways in the nuclear envelope controlling transport between the nucleus and the cytoplasm (Newmeyer and Forbes, 1988; Dworetzky and Feldherr, 1988; for pore complex morphology see Franke and Scheer, 1974; Unwin and Milligan, 1982). The nuclear envelope displays an asymmetric organization. The outer nuclear membrane is in continuity with the endoplasmatic reticulum, whereas the inner nuclear membrane is in close contact with the nuclear lamina, a fibrillar meshwork that interconnects the pore complexes (Aaronson and Blobel, 1975; Scheer et al., 1976; Aebi et al., 1986).

The lamina is formed primarily by a polymer of one to three isotypes of nuclear lamins, which are a subfamily of the multigene family of the intermediate filament (IF)[1] proteins (McKeon et al., 1986; Fisher et al., 1986; Aebi et al., 1986; for reviews see Franke, 1987; McKeon, 1987). During mitosis in higher eucaryotic cells nuclear lamins are reversibly depolymerized and reassembled (Gerace and Blobel, 1980), concomitant with the disintegration and reformation of the nuclear envelope. Several experiments have shown that nuclear lamins play important roles both in nuclear envelope reformation after mitosis (Burke and Gerace, 1986), and in the postmitotic reorganization of chromatin and the intranuclear architecture (Benavente and Krohne, 1986).

Two major lamin types can be distinguished according to their behavior in mitotic cells. B-type lamins always remain associated with membranes, whereas A-type lamins are present in mitotic cells as soluble oligomers (Gerace and Blobel, 1980; Burke and Gerace, 1986; Stick et al., 1988). Analysis of cDNA-derived amino acid sequences revealed that lamins do not contain hydrophobic domains of sufficient length required for membrane insertion (Krohne et al., 1987; Höger et al., 1988; Stick, 1988). Direct biochemical experiments have shown that lamins have properties characteristic of peripheral membrane proteins (Gerace et al., 1982; Snow et al., 1987). It has been proposed that the close association of the lamina with the nuclear membrane is mediated by integral membrane proteins specific for the inner nuclear membrane (Senior and Gerace, 1988). One integral membrane protein has recently been reported to be a receptor for lamin B (Worman et al., 1988).

To gain insight into the molecular basis of the interactions between the inner nuclear membrane and the lamins in the living cell, we have analyzed the association of soluble lamin molecules with the nuclear envelope of *Xenopus* oocytes by microinjection experiments, comparing the behavior of nor-

1. *Abbreviations used in this paper*: IF, intermediate filament; NIM, nuclear isolation medium; XLKE, *Xenopus* kidney epithelial cells.

mal molecules with those modified by deletions or point mutations. Here we report that a conserved cysteine present at the carboxy-termini of all lamin molecules is crucial for the stable association of lamins with the nuclear envelope.

Materials and Methods

Generation of Mutated Lamins

Carboxy-terminal deletions were introduced by linearization of the "bluescript" expression vector (Stratagene Corp., San Diego, CA) containing the lamin L_I cDNA with restriction enzymes Hind III (M1) and Eco RI (M3). Amino-terminal deletions were obtained by digestion of lamin L_I cDNA with Bam HI (M2 and M3) or Bal I (M5) before subcloning into the expression vector. Point mutations (M4, M7-M-NL) and internal deletions (M6) were generated by oligonucleotide-directed mutagenesis using the Amersham system (Amersham Buchler, Braunschweig, FRG), which is based on the method of Taylor et al. (1985). Oligonucleotides had a length of 25 (mutants M4, M8–M10) and 39 (mutant M7) nucleotides. One step of the Amersham protocol was modified. Oligonucleotides, which had been purified on oligonucleotide purification cartridges (Applied Biosystems, Inc., Foster City, CA), were annealed to the single-stranded DNA template at 65°C for 60 min. Mutants were identified by nucleotide sequencing (Sanger et al., 1977). Mutated cDNA inserts were subcloned into the bluescript expression vector.

Expression of Cloned cDNA In Vitro

For in vitro synthesis of lamins, cDNAs of *Xenopus* lamins L_I and A (Krohne et al., 1987; Wolin et al., 1987) and of human lamin C (McKeon et al., 1986) were subcloned into the bluescript expression vector and transcribed by T7 or T3 polymerase according to the manufacturer's manual. The RNA was purified by phenol/chloroform extraction and ethanol precipitation. 0.1–0.5 μg RNA were translated in vitro using the rabbit reticulocyte lysate (Promega Biotec, Madison, WI) and 90 μCi [^{35}S]methionine. Afterwards, the in vitro translation mixture was dialyzed for 60 min at 4°C on a UM membrane (Millipore Corp., Bedford, MA) against the microinjection buffer (10 mM Pipes, pH 7.4; 40 mM KCl, 10 mM NaCl) and stored until use at −20°C.

Microinjection and Subfractionation of Oocytes

Dialyzed in vitro translation assays (50–70 nl/oocyte) were injected into the cytoplasm of growing oocytes (stage V; Dumont, 1972) of *Xenopus laevis* using a micromanipulator (Brinkmann Instrumentenbau, Mannheim, FRG). Oocytes were incubated in modified Barth's medium (Krohne and Franke, 1983) for 16–24 h at 18–20°C. In several experiments injected oocytes were incubated in Barth's medium containing cycloheximide (200 μg/ml) to inhibit incorporation of [^{35}S]methionine into endogeneous proteins, which were identical in mobility on SDS-PAGE with some of the mutated lamins. Cycloheximide did not influence nuclear transport of lamins and their association with the nuclear envelope.

All fractionation steps were performed at 4°C. Immediately before use, 0.2 mM PMSF was added to each solution. Oocytes were transferred into nuclear isolation medium (NIM; 83 mM KCl, 17 mM NaCl, 10 mM Tris-HCl, pH 7.2) and manually subfractionated under a dissecting microscope within 10–15 s into nuclei and cytoplasms. Nuclei were immediately collected in 96% ethanol and cytoplasm in NIM. Portions of six cytoplasms were homogenized by pipetting, centrifuged for 5 min at 10,000 *g*, and proteins in the supernatant were precipitated by adding 6 vol of acetone.

For subfractionation of nuclei, portions of three to five nuclei were transferred immediately after isolation into NIM containing 10 mM $MgCl_2$ in order to gelify the nuclear content. After 20–30 s, nuclear envelopes were separated from nuclear contents by sucking nuclei into a fine bored pipette (Krohne and Franke, 1983). Nuclear envelopes and nuclear contents were collected separately in NIM. Nuclear envelopes were pelleted by centrifugation (5 min, 10,000 *g*) and proteins of nuclear contents were precipitated with TCA (20% final concentration).

In parallel, 10–30 isolated nuclei were collected in 50–100 μl NIM and extracted by adding the same volume of NIM containing 2.0 M NaCl. Nuclei were homogenized by vortexing, incubated for 5 min, and then fractionated by centrifugation (10 min, 10,000 *g*). The supernatant was collected and the pellet washed once with 70 μl NIM containing 1.0 M NaCl. After centrifugation (10 min, 10,000 *g*) supernatants were combined and proteins precipitated with TCA (20% final concentration). The pellet containing nuclear envelopes and residues of amplified nucleoli was stored at −20°C. In parallel experiments, nuclei were extracted with NIM or with NIM containing 1% Triton X-100.

Lamins present in in vitro translation assays and in 10,000-*g* supernatants of nuclei and cytoplasm of injected oocytes were layered on top of 5–30% (wt/vol) sucrose gradients made up in NIM. For S-value determinations, catalase (11.3 S) and immunoglobulin (6.5 S) were analyzed in the same gradients. Gradients were centrifuged for 18 h at 36,000 rpm in a rotor (SW40; Beckmann Instruments, Munich, FRG) at 4°C. 0.4-ml fractions were collected and precipitated with TCA (20% final concentration).

Protein samples precipitated with acetone were stored for 1 h at −20°C and centrifuged (5 min, 10,000 *g*). Pellets were washed once with 90% acetone, then with 100% acetone and were finally air dried. Samples precipitated with 20% TCA were centrifuged (5 min, 10,000 *g*). Pellets were washed once with 10% TCA and then with acetone as described above. Nuclei fixed with ethanol were centrifuged (see above) and air dried. Proteins were boiled in sample buffer and separated by SDS-PAGE (Laemmli, 1970) containing 10% acrylamide. Gels were processed for fluorography. For quantitative analysis fluorographs were scanned using the computer program Elscript 400 (Hirschmann, Munich, FRG).

Results

A Model System for Studying Lamin Association with the Nuclear Envelope

Highly radioactively labeled nuclear lamins can be synthesized in vitro using lamin cDNAs cloned into expression vectors (Krohne et al., 1987). Because of their solubility, these molecules are ideal for analyzing in interphase cells the nucleo-cytoplasmic transport of lamins and their association with the nuclear envelope. *Xenopus* oocytes have been selected for in vivo studies because these large cells are easy to subfractionate manually (Fig. 1) and only few injected cells (6–30 oocytes) are required for biochemical analysis. Most experiments have been performed with *Xenopus* lamin L_I (Krohne et al., 1987), and as controls mouse lamin B (Höger et al., 1988), *Xenopus* lamin A (Wolin et al., 1987), and human lamin C (McKeon et al., 1986) were used. To test the validity of the system, oocytes were injected with lamin L_I, incubated overnight at 18–20°C and were then subfractionated according to Fig. 1. 16–18 h after injection, 50–60% of total lamin L_I had been transported into the nucleus (Fig. 2 *a*), whereas at earlier time points (9–10 h) only 20–30% of lamin L_I was detectable in the nucleus. Therefore, oocyte nuclei were routinely isolated and subfractionated not earlier than 16 h after injection of the cells.

Manual subfractionation of nuclei revealed that at least 95% of lamin L_I was present in the nuclear envelopes (Fig. 2 *b*, lane *NE*), whereas only trace amounts were detected in the nuclear contents (Fig. 2 *b*, lane *NC*). Extraction of isolated nuclei with NIM containing 1.0 M NaCl (Fig. 2 *c*) revealed that lamin L_I was salt insoluble, being recovered after centrifugal fractionation of nuclei in the pellet containing nuclear envelopes and residues of amplified nucleoli (Fig. 2 *c*, lane *P*; for nucleolar proteins see Benavente et al., 1984). Identical results were obtained by incubation of nuclei in NIM containing 1% Triton X-100 before centrifugal fractionation. These data indicate that lamin L_I is associated with a residual component of the nuclear pore complex-lamina fraction of the nuclear envelope.

To test whether other lamins also associate efficiently with the nuclear envelope, *Xenopus* oocytes were injected with mouse lamin B (not shown) *Xenopus* lamin A (Fig. 3, lanes *1* and *2*) and human lamin C (Fig. 3, lanes *3–6*). Nuclei were

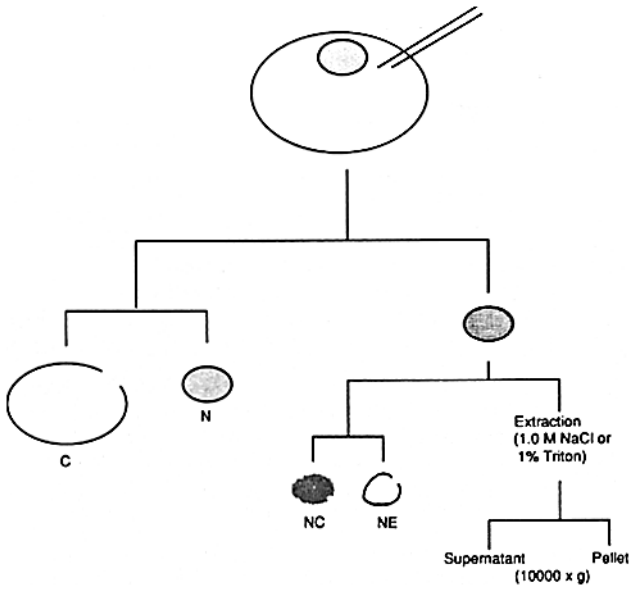

Figure 1. Scheme for the fractionation of an injected *Xenopus* oocyte into cytoplasm (*C*) and nucleus (*N*). Isolated nuclei were dissected into nuclear envelope (*NE*) and nuclear content (*NC*) or fractionated after extraction by centrifugation into pellet and supernatant.

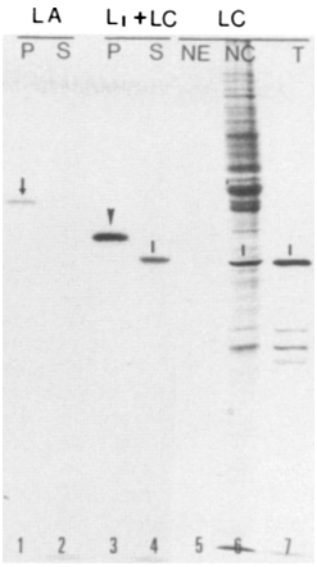

Figure 3. Behavior of *Xenopus* lamin A (*LA*) and human lamin C (*LC*) after injection into oocytes. Oocytes were incubated in the presence (lanes *1–4*) or absence (lanes *5* and *6*) of cycloheximide. Nuclei were salt extracted (lanes *1–4*) and fractionated by centrifugation into pellet (*P*) and supernatant (*S*) or were dissected into nuclear envelopes (*NE*, lane *5*) and nuclear contents (*NC*, lane *6*). Lane *7* (*T*), in vitro translation assay containing lamin C. As internal controls lamins L_I and C have been injected into the same cells (lanes *3* and *4*). Lamins are marked by *arrow* (*LA*), *arrowhead* (L_I) and *bars* (*LC*). Fluorographs of protein gels are shown.

manually subfractionated and fractionated by centrifugation according to the scheme in Fig. 1. For each lamin both methods yielded identical results. Lamins A (Fig. 3, lanes *1* and *2*; Table II) and B were nearly completely associated with the nuclear envelope, whereas only trace amounts of lamin C were detectable in the nuclear envelope fraction after manual subfraction of nuclei (Fig. 3, lanes *5* and *6*), or after extraction of nuclei with NIM containing 1.0 M NaCl (Fig. 3, lanes *3* and *4*; Table I). As an internal control lamins L_I and C were injected into the same cells (Fig. 3, lanes *3* and *4*). This shows clearly that lamin L_I was, as usual, highly enriched in the nuclear envelope pellet (Fig. 3, lane *3*), whereas lamin C was not stably associated with this structure.

In our experiments we never observed saturation of the nuclear envelope in its association capacity for injected radioactively labeled lamins even when the highest possible volume (100 nl) of in vitro translation assay was injected into the oocyte or when the translation assay was diluted (dilution 1:5) before injection. In addition, we did not detect a competition between lamins in their association with the nuclear envelope when oocytes were coinjected with two different lamin types or with mixtures containing wild-type lamins and mutant molecules (Fig. 3, and see below).

Identification of a Domain in Lamins Essential for Their Association with the Nuclear Envelope

Nuclear lamins contain a signal sequence at the beginning of the nonhelical carboxy-terminal tail (Fig. 4; *NL*) which is

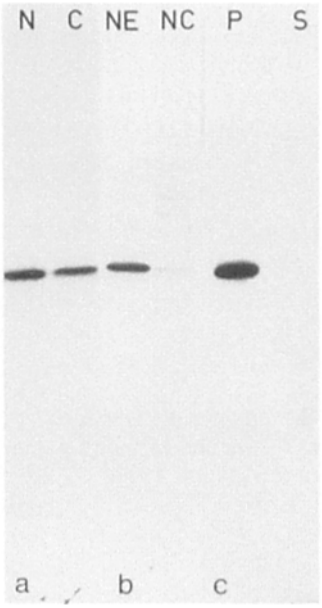

Figure 2. Subfractions of *Xenopus* oocytes after injection with lamin L_I. (*a*) Cells were manually fractionated into nuclei (*N*) and cytoplasm (*C*). (*b*) Isolated nuclei were dissected into nuclear envelopes (NE) and nuclear contents (*NC*). (*c*) Extraction of isolated nuclei with NIM containing 1.0 M NaCl and subsequent fractionation by centrifugation into pellet (*P*) and supernatant (*S*). Fluorographs of protein gels are shown.

Table I. The Influence of Carboxy-Terminal Mutations on Lamin Association with the Nuclear Envelope

		Nuclear envelope–associated lamins*	Extractable lamins
		%	
Human lamin C			
Wild-type	...VSGSR^{572}R	5.0	95.0
mutant M 7	...VSGSRRCAIM^{576}	98.0	2.0
Xenopus Lamin L_I			
Wild-type	..NKNCAIM^{583}	95.5	4.5
Mutant M 4	..N^{577}	7.2	92.8
Mutant M 8	..NKN^{579}	9.4	90.6
Mutant M 9	..NKNSAIM^{583}	13.5	86.5
Xenopus Lamin A			
Wild-type	...QNCSIM^{665}	98.0	2.0
Mutant M 10	...QNSSIM^{665}	40.4	59.6
Mutant M 11	...QN^{661}	38.2	61.8

* Results of isolated nuclei extracted with NIM containing 1.0 M NaCl are shown. Data are means of two to four experiments.
Numbers above the sequences denote the carboxy-terminal amino acid.

Table II. Influence of Amino-Terminal and Internal Mutations on Lamin Association with the Nuclear Envelope

	Nuclear envelope-associated lamins*	Extractable lamins
	%	
Lamin L_I		
Mutant 2 (Δaa 1–189)	40.5	59.5
Mutant 5 (Δaa 1–17)	77.5	22.5
Mutant 6 (Δaa 373–389)	64.2 (86.2)	35.8 (13.8)

*Results of isolated nuclei extracted with NIM containing 1.0 M NaCl (values without brackets) or with NIM containing 1.0% Triton X-100 (values in brackets) are shown.
Deleted sequences are indicated (Δaa). For further details see Figs. 4 and 6. Data are means of two to four experiments.

required for nuclear transport of lamins. The functional importance of this sequence has been demonstrated for human lamin A (Loewinger and McKeon, 1988), *Xenopus* lamin L_I (Chelsky et al., 1989) and could be confirmed by our own investigations for *Xenopus* lamin L_I (see below). Care was taken not to mutate this part of lamin L_I.

Lamin mutants have been analyzed by the two nuclear fractionation methods described in Fig. 1. For each mutant shown in Figs. 5 and 7, except for mutant M6, identical results have been obtained with both methods. Therefore, unless otherwise indicated, only data of one method (centrifugal fractionation) are shown. Lamin molecules not stably associated with the nuclear envelope were readily extractable from nuclei with buffer NIM. Identical results were obtained with NIM containing 1.0 M NaCl unless otherwise indicated.

A number of appropriate restriction sites allowed the generation of deletion mutants (Fig. 4) that lacked either a large part of the carboxy (mutant M1) or amino terminus (mutant M2), or contained only the central part of the lamin L_I molecule (mutant M3). The mutants were used for initial studies (Fig. 5, lanes *3–8*). The carboxy-terminal deletion (mutant M1) had the most dramatic effect on nuclear envelope association. In the experiment shown, the majority of mutated lamin L_I present in the oocyte nucleus was extractable (Fig. 5, lanes *3* and *4*).

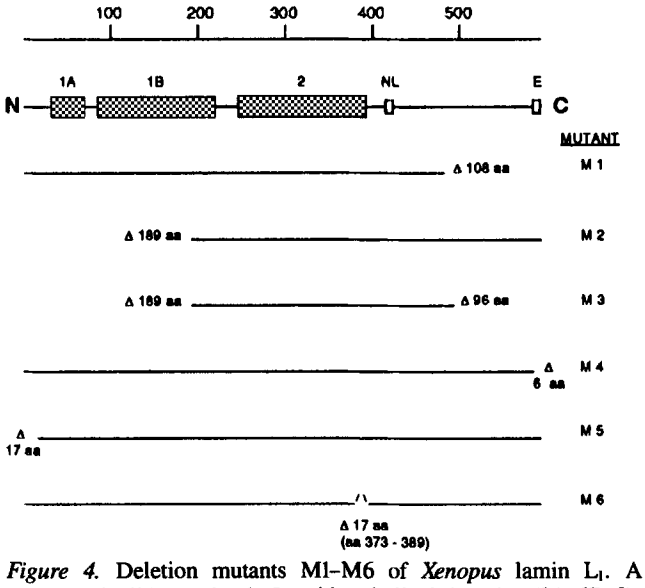

Figure 4. Deletion mutants M1–M6 of *Xenopus* lamin L_I. A scheme of wild-type lamin L_I with α-helical domains (*1A, 1B, 2*), nuclear localization signal (*NL*), and conserved tetrapeptide (*E*) at the carboxy terminus (*C*; *N*, amino terminus) is shown. The position of deleted amino acids (*Δaa*) in the molecule can be estimated by the scale.

In contrast, deletion of the first 189 amino acids of the amino terminus had less drastic effects (mutant M2). In this case, an average of 40% of mutant M2 protein remained associated with the nuclear envelope after extraction (Fig. 5, lanes *5* and *6*; Table II). Mutant M3 (Fig. 5, lanes *7* and *8*) behaved similar to mutant M1, with only minor amounts of this truncated lamin L_I being found in the nuclear envelope fraction. Our results for mutants M1–M3 indicate that salt-extractable mutant molecules were localized in the nuclear content and were not at all associated with the nuclear envelope.

Two further carboxy-terminal mutations of lamin L_I that deleted either the last 24 (linearization of lamin L_I cDNA with restriction enzyme Ssp I; data not shown) or the last 6 amino acids (mutant M4; Fig. 5, lanes *11* and *12*) were identical in their behavior with mutant M1. More than 90% of these mutated lamins were not stably associated with the nuclear envelope (Fig. 5, lanes *11* and *12*).

Mutant M4 indicates a dominant role for the last six amino acids in lamin L_I in nuclear membrane association. Only lamin C of human and mouse does not contain the conserved sequence Cys Ala/Ser Ile Met (McKeon et al., 1986; Fisher et al., 1986; Riedel and Werner, 1989) present in all other known lamins (Fig. 6). To test the functional significance of this sequence, the four amino acids Cys Ala Ile Met were cloned onto the carboxy terminus of lamin C (mutant M7) and deleted in parallel experiments from lamins L_I (mutant M8) and A (mutant M11). The behavior of all three lamins had entirely changed. Lamin C was now stably associated with the nuclear envelope (mutant M7, Fig. 7, lanes *3* and *4*; Table I), whereas the majority of lamins L_I (mutant M8, Fig. 7, lanes *7* and *8*; Table I) and an average of 60% of A (mutant M11, Fig. 7, lanes *13* and *14*; Table I) remained soluble in the nuclear content. These experiments demonstrate that the amino acid sequence Cys Ala Ile Met contains sufficient information to mediate the association of lamins with the nuclear envelope. It also indicates, however, that lamin A seems to contain an additional signal that can partly compensate for the absence of the conserved carboxy terminus (see Discussion).

The Conserved Carboxy-Terminal Cysteine Is Essential

The cysteine in the conserved carboxy-terminal domain of lamins is a candidate that may mediate binding to the nuclear envelope either by a disulfide bond or by a covalently attached lipid (Hancock et al., 1989; for review see Sefton and Buss, 1987; Olson, 1988).

The mutation of this cysteine into serine drastically reduced the association of lamins L_I (mutant M9) and A (mutant M10) with the nuclear envelope (Table I; Fig. 7). Only 13% of lamin L_I (Fig. 7, lanes *9* and *10*) and 40% of lamin A (Fig. 7, lanes *11–14*) were associated in a salt stable form with the nuclear envelope. As an internal control in some experiments mutated lamin A and wild-type lamin L_I were

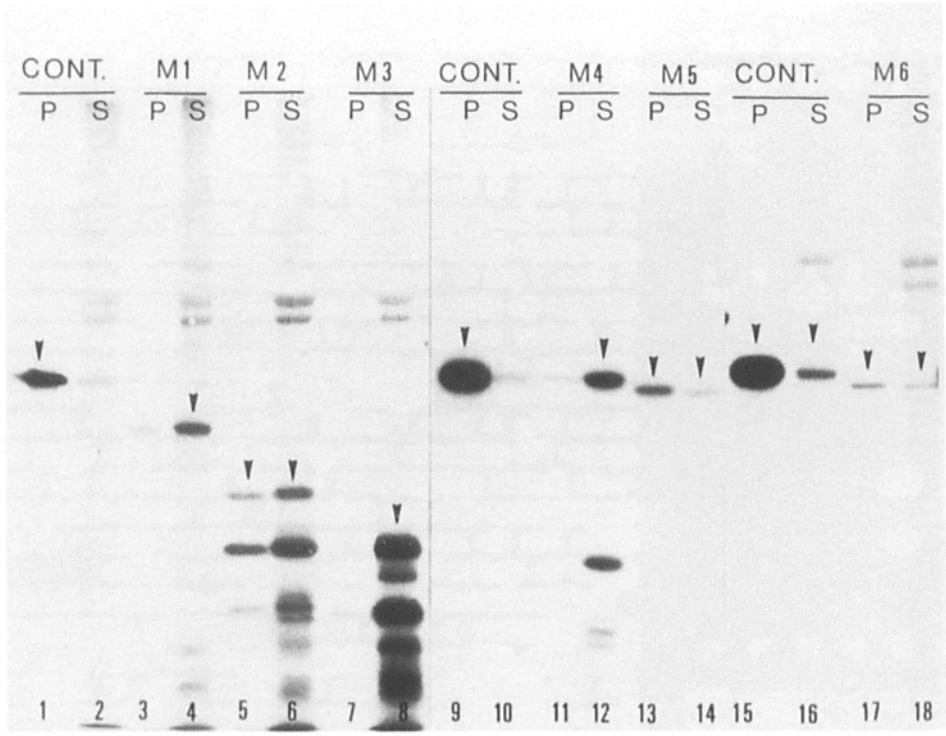

Figure 5. Behavior of deletion mutants M1–M6 in comparison with wild-type lamin L_I (CONT.). Fluorographs of protein gels are shown. After injection with radioactively labeled lamins *Xenopus* oocytes were incubated in the absence (lanes *1–8, 15–18*) or presence of cycloheximide (lanes *9–14*) before isolation of nuclei. Nuclei were extracted with NIM containing 1.0 M NaCl and fractionated by centrifugation into pellet (*P*) and supernatant (*S*). Lamin L_I is marked by arrowheads. Additional protein bands in the lower molecular weight range (lanes *5, 6, 8, 12*) resulted from starts of translation at internal AUG codons.

coinjected, lamin L_I being recovered in the nuclear envelope fraction as usual (Fig. 7, lane *11*). Deletion of the last six amino acids (mutant M4, Table I) always more efficiently inhibited association of lamin L_I with the nuclear envelope than mutation of the cysteine into serine (mutant M9; Table I). Therefore, minor influences of the other carboxy-terminal amino acids of lamin L_I cannot be excluded. In summary, our experiments clearly demonstrate the essential nature of the cysteine residue.

The Influence of Amino-Terminal and α-Helical Sequences on Lamin Association with the Nuclear Envelope

The association of lamin L_I with the nuclear envelope was significantly reduced when amino-terminal sequences were deleted from the molecule. Two mutants lacking 17 (mutant M5; Fig. 5) and 189 (mutant M2; Fig. 5) amino acids at the amino-terminus were tested (Table II). On average, only 77% of lamin molecules of mutant M5 were associated in isolated nuclei with the nuclear envelope indicating that at least some of the 17 amino-terminal amino acids are involved in the interaction of lamin L_I with the nuclear envelope. Interestingly, 5 of the 17 amino acids are conserved in the oocyte lamin L_{III} (Fig. 6). However, we do not know whether the amino termini of both lamins interact in the oocyte nuclear envelope. Additional sequences required for the stable association of lamin L_I with the nuclear envelope seem to be present in the 189 amino-terminal amino acids. This notion is supported by the results obtained with mutant M2 (Fig. 5, lanes *5* and *6*; Table II). On average, only 40% of mutated lamin molecules present in isolated nuclei were associated with the nuclear envelope. The reduced association of mutant M2 with the nuclear envelope was not due to a limited association capacity of the nuclear envelope. Dilution (1 vol in vitro translation assay plus 4 vol of injection buffer) of in vitro translation assays prior to injection and reduction of the injected volume to 50 nl gave the same relative proportions for mutant M2 (see Table II).

To determine the influence of highly conserved sequences in the α-helix on the association of lamins with the nuclear envelope a deletion mutant (M6; Figs. 4 and 6) lacking the last 17 amino acids of helix 2 was analyzed. Extraction of isolated nuclei with NIM containing 1% Triton X-100 revealed that on average, 86% of mutant M6 molecules were associated with the nuclear envelope (Table II), whereas extraction with NIM containing 1.0 M NaCl resulted in a reduction of nuclear envelope associated lamins to 64% (Fig. 5, lanes *17* and *18*; Table II). This behavior of mutants M2, M5, and M6 indicates that the amino terminus of lamin

Mutant M 4

	578					583
Xenopus L_I	. .	K	N	C	A	I M
Human C	. .	V	S	G	S	R R

Mutant M 5

	1															17
Xenopus L_I	M	A	T	A	T	P	S	G	P	R	S	S	G	R	R	S S . .

Mutant M 6

	373															389
Xenopus L_I	. .	E	I	S	A	Y	R	K	L	L	E	G	E	E	R	L K . .

Figure 6. Sequences of deleted amino acids of mutants M4, M5, and M6 of *Xenopus* lamin L_I. Numbers denote the first and last deleted amino acid. The bold printed amino acids are conserved in all sequenced lamins from *Drosophila* to man. Amino-terminal sequences conserved in *Xenopus* lamins L_I and L_{III} and in mouse lamin B are underlined. Only lamin C has a different carboxy terminus (for sequence comparisons see Stick, 1988; Höger et al., 1988; Krohne et al., 1987; Riedel and Werner, 1989).

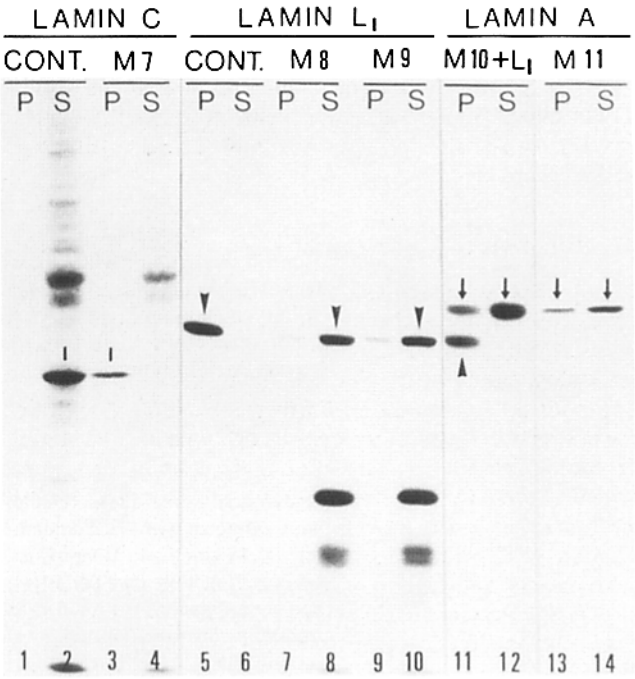

Figure 7. Carboxy-terminal mutations of lamins C (mutant M7), L_I (mutants M8 and M9), and A (mutants M10 and M11) compared with wild-type lamins (*CONT.*). Fluorographs of protein gels are shown. Injected oocytes have been incubated in the absence (lanes *1–4*) or presence (lanes *5–14*) of cycloheximide. Nuclei were extracted with NIM containing 1.0 M NaCl (lanes *1–12*) or with NIM alone (lanes *13* and *14*) and fractionated by centrifugation into pellet (*P*) and supernatant (*S*). In one experiment, lamins L_I and A were coinjected into the same cell (lanes *11* and *12*). Lamins are marked by *bars* (lamin C), *arrowheads* (lamin L_I), and *arrows* (lamin A).

L_I is involved in its association with the nuclear envelope. The reduced stability of mutant M6 to extraction of nuclei with NIM containing 1.0 M NaCl suggests that α-helical regions of lamins L_I and L_{III} interact in the nuclear lamina of the oocyte nuclear envelope.

Properties of Soluble Lamins

During our experiments, we questioned why ~50% of lamin L_I were 16–20 h after injection still present in the cytoplasm of oocytes (Fig. 8, lanes *1* and *2*). To control whether this is a general characteristic of lamins, we analyzed mutants of lamin L_I, including one containing two amino acid exchanges in the nuclear localization signal (mutant M-NL; Arg 415 → Thr and Lys 416 → Thr). As expected, molecules of lamin L_I mutant M-NL remained completely in the cytoplasm (Fig. 8, lanes *3* and *4*). Lamin L_I molecules deleted at the amino terminus behaved like wild-type lamin L_I. As an example, results obtained with mutant M2 are shown (Fig. 8, lanes *5* and *6*). In contrast, mutations that deleted or exchanged the conserved carboxy-terminal cysteine increased the nuclear accumulation of lamins. All carboxy-terminal mutations of lamin L_I analyzed accumulated to the same extent in the nucleus. As an example, results for mutant M9 are shown (Fig. 8, lanes *7* and *8*). On average, 75–80% of total mutant M9 molecules were present 16–20 h after injection in the nuclear compartment. Lamin C behaved like the carboxy-terminal deletions of lamin L_I. Our results clearly indicate that the conserved carboxy-terminal cysteine influenced the nucleo-cytoplasmic transport of lamins.

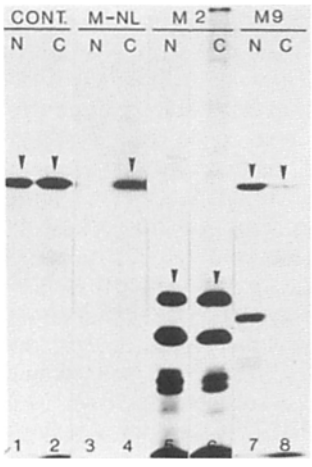

Figure 8. Nucleo-cytoplasmic transport of *Xenopus* lamin L_I mutants (lanes *3–8*) compared with wild-type lamin L_I (*CONT.*). For each experiment, five injected oocytes were dissected into nuclei (*N*) and cytoplasm (*C*). Mutant M-NL (lanes *3* and *4*) has been mutated at two positions in the nuclear localization signal (Arg 415 → Thr and Lys 416 → Thr). For details of mutants M2 and M9, see Tables I and II. Lamin L_I is marked by arrowheads. Fluorographs of protein gels are shown.

It cannot be excluded that this cysteine is involved in complex formation of lamin L_I with cytoplasmic components which may influence lamin accumulation in the nucleus. To test this possibility in vitro translation assays of wildtype lamin L_I and the cysteine mutant M9, and 10,000 *g* supernatants of injected oocytes were analyzed by sucrose gradient centrifugation. Lamin L_I of the 10,000 *g* supernatant of injected oocytes had sedimentation coefficients of 6–7 S (Fig. 9 *b*). Lamin L_I present in the in vitro translation assay had identical S-values (Fig. 9 *a*), indicating that lamin L_I forms oligomeric complexes which are indistinguishable from postinjected soluble lamin L_I, in respect to their sedimentation coefficients. To analyze whether soluble lamin L_I is associated with cytoplasmic membranes, injected oocytes were homogenized in NIM containing 1% Triton X-100 and analyzed in sucrose gradients containing 1% Triton. No change in the S value was observed. Whole oocytes have been used for the analysis of wild-type lamin L_I because neglectible amounts of soluble lamin L_I are present in the nuclear compartment.

Sucrose gradient centrifugation of lamin L_I mutant M9 also revealed sedimentation coefficients of 6–7 S for lamin molecules present in in vitro translation assays (Fig. 9 *c*). Mutant M9 molecules of manually isolated cytoplasm (Fig. 9 *d*) and nuclei were separately (Fig. 9 *e*) analyzed because soluble molecules were present in both compartments (see Fig. 7, lane *10* and Fig. 9, lane *8*). Molecules of mutant M9 present in the cytoplasm had S values of 6–7 (Fig. 9 *d*), whereas the nuclear form had significantly lower sedimentation coefficients of ~4 S (Fig. 9 *e*). Results similar to those shown in Fig. 9 *c–e* were obtained with mutant M2 of lamin L_I (data not shown). Analysis of soluble lamins revealed that they form defined oligomeric complexes after in vitro translation that seem to be unaltered in respect to their sedimentation coefficients in the cytoplasm of injected oocytes. In contrast, the soluble nuclear form had in the analyzed mutants significantly lower S values. Previously a sedimentation coefficient of 4.5 S for lamin dimers has been reported (Aebi et al., 1986).

Soluble lamins of dividing cells (Benavente et al., 1985; Smith and Fisher, 1989), and soluble cytoplasmic IF proteins (Söllner et al., 1985) are present in oligomeric complexes

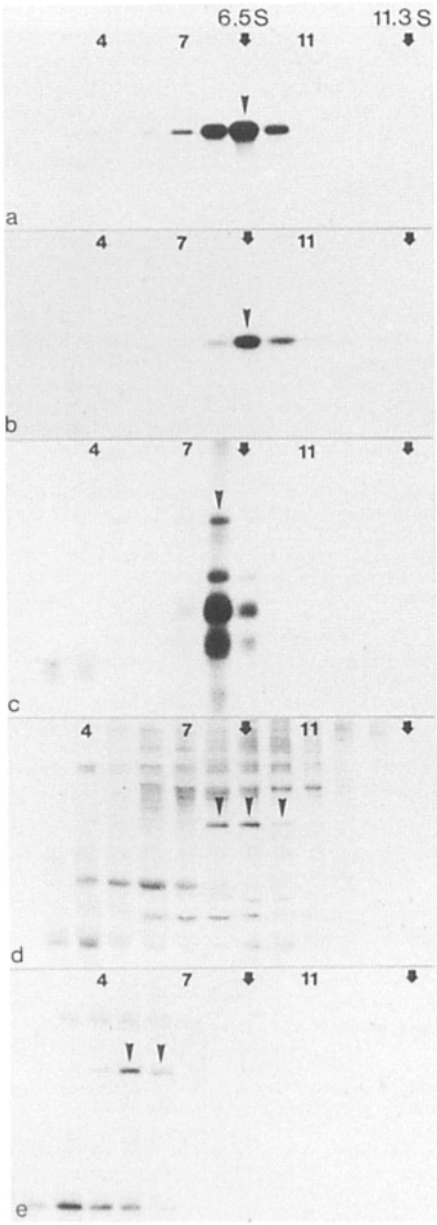

Figure 9. Sucrose gradient centrifugation of soluble wild-type lamin L_I (*a* and *b*) and of lamin L_I mutant M9 (*c–e*). Lamins present in in vitro translation assays (*a* and *c*) were compared with soluble lamin L_I of whole oocytes (*b*) and with soluble mutant M9 molecules from oocytes that have been manually subfractionated into cytoplasm (*d*) and nuclei (*e*) before sucrose gradient centrifugation. Injected oocytes have been incubated in the absence of cycloheximide. The position of lamin L_I is indicated by arrowheads. Positions of internal standards for S-value determination (6.5 *S*, immunoglobulin; 11.3 *S*, catalase) are marked as well as fraction numbers. Fluorographs of protein gels are shown.

similar to the soluble cytoplasmic lamins of injected oocytes. Soluble complexes of cytoplasmic IF-proteins have been identified as tetramers (Söllner et al., 1985).

Analysis of wildtype lamin L_I by two-dimensional IEF-gel electrophoresis revealed very small differences in charge between nuclear envelope–associated lamin L_I and soluble lamin L_I present in the cytoplasm of injected oocytes. For these determinations lamin L_I of *Xenopus* kidney epithelial (XLKE) cells was separated in the same gels and used as internal standard. Cytoplasmic lamin L_I was slightly more acidic than lamin L_I of XLKE cells, whereas the nuclear envelope–associated lamin L_I was indistinguishable from L_I of XLKE cells.

Discussion

Using a new approach we have shown that soluble synthesized in vitro lamins are able to associate with the nuclear envelope of an injected oocyte in a form that is, in respect to its biochemical properties, indistinguishable from the endogenous polymeric lamin. Furthermore, it is obvious that the oocyte nuclear envelope does incorporate foreign lamins. In contrast to the majority of somatic cells of vertebrates which express two or three lamins (Aaronson and Blobel, 1975; Krohne et al., 1981; Benavente et al., 1985; Stick and Hausen, 1985; Wolin et al., 1987; Lehner et al., 1986*b*), the *Xenopus* oocyte contains only one lamin (lamin L_{III}) which is different from other *Xenopus* lamins (Krohne et al., 1981; Stick, 1988). We do not presently know whether the injected lamins are directly associated with lamin L_{III} or with another salt- and detergent-resistant component of the nuclear envelope.

It is obvious from our data that the conserved cysteine of the carboxy-terminal tetrapeptide Cys Ala/Ser Ile Met is essential for the mediation of lamin association with the nuclear envelope. This tetrapeptide is the only signal that is required in our experimental system for lamins L_I and C. In contrast, lamin A seems to have additional domains which can partially compensate for the lack of this sequence. Sequence comparison of lamins A and C suggests that these domains are localized in the carboxy-terminal tail of *Xenopus* lamin A between amino acids 568 and 662. This is experimentally supported by transfection experiments of Loewinger and McKeon (1988) in which deletion mutants of human lamin A lacking the last 110 carboxy-terminal amino acids are not properly associated with the nuclear envelope. Experiments for the identification of these sequences are in progress.

At present it is not known how lamin C is associated with the nuclear envelope of mammalian cells. It is possible that heterodimers are formed during translation as it has been proposed for lamins A of human and hamster in transfection experiments (Loewinger and McKeon, 1988). However, cross-linking experiments detected only minor amounts, if any, of lamin C in complexes with lamin A (Lam and Kasper, 1979; Shelton et al., 1982).

One obvious possibility for the interaction of the carboxy-terminal cysteine with the nuclear envelope is a posttranslational modification by a lipid moiety that would allow a direct interaction of lamins with the lipid bilayer of the nuclear envelope (for modifications of cysteines in other proteins see Hancock et al., 1989; Olson, 1988; Sakagami et al., 1981). Recently, a modification of lamins B and A by an isoprenoid has been reported (Wolda and Glomset, 1988; Beck et al., 1988). This modification is absent from lamin C, indicating that it is most probably localized on the 93 amino acid carboxy-terminal extension of lamin A which is lacking in lamin C. Interestingly, *ras* proteins, which are associated with the cytoplasmic side of the plasma membrane, also have

an invariant cysteine at the fourth last position in their "lamin-like" carboxy-terminal tetrapeptide (for reviews see Clarke et al., 1988; Magee and Hanley, 1988). This cysteine is posttranslationally modified by covalent attachment of a polyisoprenoid (Hancock et al., 1989). The published data on lamins and on *ras* and our present results lead us to the suggestion that the conserved carboxy-terminal cysteine of lamins might be modified with this lipid moiety. Further, it is important to analyze whether the carboxy terminus of lamins can also be processed by proteolytic removal of the last three amino acids and carboxy methylation of the conserved cysteine as it has been demonstrated for *ras* proteins (Gutierrez et al., 1989; Hancock et al., 1989). Apparent precursors for lamins A of mammals and chicken and for lamin B_2 of chicken have been described (Ottaviano and Gerace, 1985; Lehner et al., 1986*a*). Of course, it cannot be presently decided whether the carboxy-terminal cysteine mediates the association of lamins with integral membrane proteins of the nuclear envelope via this possible modification or by a disulfide bond.

Our data indicate that several domains of lamin molecules are involved in their targeting to the nucleoplasmic surface of the nuclear envelope. The nuclear localization signal is required for translocation of newly synthesized lamins from the cytoplasm into the nucleus, and the carboxy-terminal cysteine is essential for the initiation of lamin interaction with a component of the nuclear envelope. We speculate that this component is either the lipid bilayer or an integral membrane protein specific for the inner nuclear membrane (Senior and Gerace, 1988; Worman et al., 1988). At present, however, we cannot exclude that the acceptor is part of the nuclear lamina, i.e., the endogenous lamin L_{III} polymer. In addition, these lamins interact with their amino termini and their α-helical regions with other lamins present in a polymeric form in the nuclear lamina, which also contribute to their stable association with the nuclear envelope. The importance of the amino terminus for lamin-lamin assembly has been shown previously by in vitro experiments (Georgatos et al., 1988). Our amino-terminal deletions and the homogeneous nuclear distribution of a neuronal protein containing the carboxy-terminal tetrapeptide (Miller et al., 1989) emphasize that the conserved sequence Cys Ala/Ser Ile Met is just one component, albeit essential, for lamin interaction with the nuclear envelope but that it is not sufficient for maintaining a stable association of lamins with the nuclear envelope. Our results further indicate that interactions of the amino-terminal and the α-helical regions contribute only to lamin association with the nuclear envelope if lamins contain the carboxy-terminal tetrapeptide.

In contrast to nuclear IF proteins (lamins), the carboxy-terminal tail of cytoplasmic IF proteins seems to be less essential for assembly of higher ordered structures (Albers and Fuchs, 1987; Bader et al., 1986; Kaufmann et al., 1985), whereas the nonhelical amino terminus (Kaufmann et al., 1985; Geisler and Weber, 1988) and the α-helical region (Loewinger and McKeon, 1988; Albers and Fuchs, 1989) are of similar importance in polymer formation in both filament systems. An obvious difference between lamins and cytoplasmic IF proteins is that lamins are always associated in interphase cells with the surface of a membrane. Additional studies are required to gain more insight into the interaction of lamins with nonlamin components of the nuclear envelope.

We thank Drs. W. W. Franke, T. Magin, H. Herrmann, J. Kleinschmidt, and M. Murray (German Cancer Research Center, Heidelberg, FRG) for valuable discussions and Dr. Doris Meyer (German Cancer Research Center) for help by the quantification of fluorographs. Dr. F. McKeon (Harvard Medical School, Boston, MA) kindly provided the human lamin C cDNA clone. We thank Dr. M. Murray for correcting and E. Gundel for typing the manuscript (German Cancer Research Center).

This work has been supported by the Deutsche Forschungsgemeinschaft (grants Kr 758/2-2 and Kr 758/3-2).

Received for publication 21 June 1989 and in revised form 22 August 1989.

References

Aaronson, R. P., and G. Blobel. 1975. Isolation of nuclear pore complexes in association with a lamina. *Proc. Natl. Acad. Sci. USA.* 72:1007–1011.

Aebi, U., J. Cohn, L. Buhle, and L. Gerace. 1986. The nuclear lamina is a meshwork of intermediate-type filaments. *Nature (Lond.).* 323:560–564.

Albers, K., and E. Fuchs. 1987. The expression of mutant epidermal keratin cDNAs transfected in simple epithelial and squamous cell carcinoma lines. *J. Cell Biol.* 105:791–806.

Albers, K., and E. Fuchs. 1989. Expression of mutant keratin cDNAs in epithelial cells reveals possible mechanisms for initiation and assembly of intermediate filaments. *J. Cell Biol.* 108:1477–1493.

Bader, B. L., T. M. Magin, M. Hatzfeld, and W. W. Franke. 1986. Amino acid sequence and gene organization of cytokeratin no. 19, an exceptional tail-less intermediate filament protein. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:1865–1875.

Beck, L. A., T. J. Hosick, and M. Sinensky. 1988. Incorporation of a product of mevalonic acid metabolism into proteins of chinese hamster ovary cell nuclei. *J. Cell Biol.* 107:1307–1316.

Benavente, R., and G. Krohne. 1986. Involvement of nuclear lamins in postmitotic reorganization of chromatin as demonstrated by microinjection of lamin antibodies. *J. Cell Biol.* 103:1847–1854.

Benavente, R., G. Krohne, and W. W. Franke. 1985. Cell type-specific expression of nuclear lamina proteins during development of *Xenopus laevis*. *Cell.* 41:177–190.

Benavente, R., G. Krohne, M. S. Schmidt-Zachmann, B. Hügle, and W. W. Franke. 1984. Karyoskeletal proteins and the organization of the amphibian oocyte nucleus. *J. Cell Sci.* (Suppl.) 1:161–186.

Burke, B., and L. Gerace. 1986. A cell free system to study reassembly of the nuclear envelope at the end of mitosis. *Cell.* 44:639–652.

Chelsky, D., R. Ralph, and G. Jonak. 1989. Sequence requirements for synthetic peptide-mediated translocation to the nucleus. *Mol. Cell. Biol.* 9:2487–2492.

Clarke, S., J. P. Vogel, R. J. Deschenes, and J. Stock. 1988. Posttranslational modification of the Ha-*ras* oncogene protein: evidence for a third class of protein carboxyl methyltransferases. *Proc. Natl. Acad. Sci. USA.* 85:4643–4647.

Dumont, J. N. 1972. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* 136:153–180.

Dworetzky, S. I., and C. M. Feldherr. 1988. Translocation of RNA-coated gold particles through the nuclear pores of oocytes. *J. Cell Biol.* 106:575–584.

Fisher, D. Z., N. Chaudhary, and G. Blobel. 1986. cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filaments. *Proc. Natl. Acad. Sci. USA.* 83:6450–6454.

Franke, W. W. 1974. Structure, biochemistry, and functions of the nuclear envelope. *Int. Rev. Cytol.* (Suppl.) 4:71–236.

Franke, W. W. 1987. Nuclear lamins and cytoplasmic intermediate filament proteins: a growing multigene family. *Cell.* 48:3–4.

Franke, W. W., and U. Scheer. 1974. Structure and function of the nuclear envelope. *In* The Cell Nucleus. Vol. I. H. Busch, editor. Academic Press, New York. 220–347.

Franke, W. W., U. Scheer, G. Krohne, and E.-D. Jarasch. 1981. The nuclear envelope and the architecture of the nuclear periphery. *J. Cell Biol.* 91:395–505.

Geisler, N., and K. Weber. 1988. Phosphorylation of desmin in vitro inhibits formation of intermediate filaments: identification of three kinase A sites in the aminoterminal head domain. *EMBO (Eur. Mol. Biol. Organ.) J.* 7: 15–20.

Georgatos, S. D., C. Stournaras, and G. Blobel. 1988. Heterotypic and homotypic associations between the nuclear lamins: site-specificity and control by phosphorylation. *Proc. Natl. Acad. Sci. USA.* 85:4325–4329.

Gerace, L., and G. Blobel. 1980. The nuclear envelope lamina is reversibly depolymerized during mitosis. *Cell.* 19:277–287.

Gerace, L., and B. Burke. 1988. Functional organization of the nuclear envelope. *Annu. Rev. Cell Biol.* 4:335–374.

Gerace, L., Y. Ottaviano, and C. Kondor-Koch. 1982. Identification of a major polypeptide of the nuclear pore complex. *J. Cell Biol.* 95:826–837.

Gutierrez, L., A. I. Magee, C. J. Marshall, and J. F. Hancock. 1989. Post-translational processing of $p21^{ras}$ is two-step and involves carboxyl-methylation and carboxy-terminal proteolysis. *EMBO (Eur. Mol. Biol. Or-*

gan.) J. 8:1093–1098.
Hancock, J. F., A. J. Magee, J. E. Childs, and C. J. Marshall. 1989. All *ras* proteins are polyisoprenylated but only some are palmitoylated. *Cell.* 57: 1167–1177.
Höger, T. H., G. Krohne, W. W. Franke. 1988. Amino acid sequence and molecular characterization of murine lamin B as deduced from cDNA clones. *Eur. J. Cell Biol.* 47:283–290.
Kaufmann, E., K. Weber, and N. Geisler. 1985. Intermediate filament forming ability of desmin derivatives lacking either the amino-terminal 67 or the carboxy-terminal 27 residues. *J. Mol. Biol.* 185:733–742.
Krohne, G., M.-C. Dabauvalle, and W. W. Franke. 1981. Cell type specific differences in protein composition of nuclear pore complex-lamina structures in oocytes and erythrocytes of *Xenopus laevis*. *J. Mol. Biol.* 151:121–141.
Krohne, G., and W. W. Franke. 1983. Proteins of pore complex-lamina structures from nuclei and nuclear membranes. *Methods Enzymol.* 96J:597–608.
Krohne, G., S. L. Wolin, F. D. McKeon, W. W. Franke, and M. W. Kirschner. 1987. Nuclear lamin L_1 of *Xenopus laevis*: cDNA cloning, amino acid sequence and binding specificity of a member of the lamin B subfamily. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3801–3808.
Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 277:680–685.
Lam, K. S., and C. B. Kasper. 1979. Electrophoretic analysis of three major nuclear envelope polypeptides. *J. Biol. Chem.* 254:11713–11720.
Lehner, C. F., G. Fürstenberger, H. M. Eppenberger, and E. A. Nigg. 1986*a*. Biogenesis of the nuclear lamina: in vivo synthesis and processing of nuclear protein precursors. *Proc. Natl. Acad. Sci. USA.* 83:2096–2099.
Lehner, C. F., V. Kurer, H. M. Eppenberger, and E. Nigg. 1986*b*. The nuclear lamin protein family in higher vertebrates. *J. Biol. Chem.* 261:13293–13301.
Loewinger, L., and F. McKeon. 1988. Mutations in the nuclear lamin proteins resulting in their aberrant assembly in the cytoplasm. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:2301–2309.
Magee, T., and M. Hanley. 1988. Sticky fingers and CAAX boxes. *Nature (Lond.).* 335:114–115.
McKeon, F. 1987. Nuclear lamin proteins and the structure of the nuclear envelope. Where is the function? *Bioessays.* 7:169–173.
McKeon, F. D., M. W. Kirschner, and D. Caput. 1986. Primary and secondary structural homology between the major nuclear envelope and cytoplasmic intermediate filament proteins. *Nature (Lond.).* 319:463–468.
Miller, M., M. Kloc, B. Reddy, E. Eastman, C. Dreyer, and L. Etkin. 1989. Xlgv7: a maternal gene product localized in nuclei of the central nervous system in *Xenopus laevis*. *Genes. & Dev.* 3:572–583.
Newmeyer, D. D., and D. J. Forbes. 1988. Nuclear import can be separated into distinct steps in vitro: nuclear pore binding and translocation. *Cell.* 52:641–653.
Newport, J. W., and D. J. Forbes. 1987. The nucleus: structure, function and dynamics. *Annu. Rev. Biochem.* 56:535–565.
Olson, E. N. 1988. Modification of proteins with covalent lipids. *Prog. Lipid. Res.* 27:177–197.
Ottaviano, Y., and L. Gerace. 1985. Phosphorylation of the nuclear lamins during interphase and mitosis. *J. Biol. Chem.* 260:624–632.
Riedel, W., and D. Werner. 1989. Nucleotide sequence of the full-length mouse lamin C cDNA and its deduced amino-acid sequence. *Biochim. Biophys. Acta.* 1008:119–122.
Sakagami, Y., M. Yoshida, A. Isogai, and A. Suzuki. 1981. Peptidal sex hormones inducing conjugation tube formation in compatible mating-type cells of *Tremella mesenterica*. *Science (Wash. DC).* 212:1525–1527.
Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-termination inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.
Scheer, U., J. Kartenbeck, M. F. Trendelenburg, J. Stadler, and W. W. Franke. 1976. Experimental disintegration of the nuclear envelope. Evidence for pore-connecting fibrils. *J. Cell Biol.* 69:1–18.
Sefton, B. M., and J. E. Buss. 1987. The covalent modification of eucaryotic proteins with lipid. *J. Cell Biol.* 104:1449–1453.
Senior, A., and L. Gerace. 1988. Integral membrane proteins specific to the inner nuclear membrane and associated with the nuclear lamina. *J. Cell Biol.* 107:2029–2036.
Shelton, K. R., V. H. Guthrie, and D. L. Cochran. 1982. Oligomeric structure of the major nuclear envelope protein lamin B. *J. Biol. Chem.* 257:4328–4332.
Smith, D. E., and P. A. Fisher. 1989. Interconversion of *Drosophila* nuclear lamin isoforms during oogenesis, early embryogenesis, and upon entry of cultured cells into mitosis. *J. Cell Biol.* 108:255–265.
Snow, C., A. Senior, and L. Gerace. 1987. Monoclonal antibodies identify a group of nuclear pore complex glycoproteins. *J. Cell Biol.* 104:1143–1156.
Söllner, P., R. A. Quinlan, and W. W. Franke. 1985. Identification of a distinct soluble subunit of an intermediate filament protein: tetrameric vimentin from living cells. *Proc. Natl. Acad. Sci. USA.* 82:7929–7933.
Stick, R. 1988. cDNA cloning of the developmentally regulated lamin L_{III} of *Xenopus laevis*. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3189–3197.
Stick, R., B. Angres, C. F. Lehner, and E. A. Nigg. 1988. The fates of chicken nuclear lamin proteins during mitosis: evidence for a reversible redistribution of lamin B_2 between inner nuclear membrane and elements of the endoplasmatic reticulum. *J. Cell Biol.* 107:397–406.
Stick, R., and P. Hausen. 1985. Changes in the nuclear lamina composition during early development of *Xenopus laevis*. *Cell.* 41:191–200.
Taylor, J. W., J. Ott, and F. Eckstein. 1985. The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA. *Nucleic Acids Res.* 13:8765–8785.
Unwin, P. N. T., and R. A. Milligan. 1982. A large particle associated with the perimeter of the nuclear pore complex. *J. Cell Biol.* 93:63–75.
Wolda, S. L., and J. A. Glomset. 1988. Evidence for modification of lamin B by a product of mevalonic acid. *J. Biol. Chem.* 263:5997–6000.
Wolin, S. L., G. Krohne, and M. W. Kirschner. 1987. A new lamin in *Xenopus* somatic tissues displays strong homology to human lamin A. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3809–3818.
Worman, H. J., J. Yuan, G. Blobel, and S. D. Georgatos. 1988. A lamin B receptor in the nuclear envelope. *Proc. Natl. Acad. Sci. USA.* 85:8531–8534.