Evolution of the lamin protein family
What introns can tell

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Abbreviations: CaXX, C-terminal isoprenylation motif; CDK1, Cyclin-dependent kinase 1; IF, intermediate filament; LMNA, human lamin A gene; Lmnb2, murine lamin B2 gene; NLS, nuclear localization signal; Rce1, Ras converting enzyme 1; ZMPSTE24, zinc metalloprotease STE24 homolog

Lamins are the major components of the nuclear lamina, a filamentous layer found at the interface between chromatin and the inner nuclear membrane. The lamina supports the nuclear envelope and provides anchorage sites for chromatin. Lamins and their associated proteins are required for most nuclear activities, mitosis, and for linking the nucleoskeleton to the network of cytoskeletal filaments. Mutations in lamins and their associated proteins give rise to a wide range of diseases, collectively called laminopathies. This review focuses on the evolution of the lamin protein family. Evolution from basal metazoans to man will be described on the basis of protein sequence comparisons and analyses of their gene structure. Lamins are the founding members of the family of intermediate filament proteins. How genes encoding cytoplasmic IF proteins could have arisen from the archetypal lamin gene progenitor, can be inferred from a comparison of the respective gene structures. The lamin/IF protein family seems to be restricted to the metazoans. In general, vertebrate genomes harbor only a single lamin gene encoding a B-type lamin. The archetypal lamin gene structure found in basal metazoans is conserved up to the vertebrate lineage. The completely different structure of lamin genes in Caenorhabditis and Drosophila are exceptions rather than the rule within their systematic groups. However, variation in the length of the coiled-coil forming central domain might be more common than previously anticipated. The increase in the number of lamin genes in vertebrates can be explained by two rounds of genome duplication. The origin of lamin A by exon shuffling might explain the processing of prelamin A to the mature non-isoprenylated form of lamin A. By alternative splicing the number of vertebrate lamin proteins has increased even further. Lamin C, an alternative splice form of the LMNA gene, is restricted to mammals. Amphibians and mammals express germline-specific lamins that differ in their protein structure from that of somatic lamins. Evidence is provided that there exist lamin-like proteins outside the metazoan lineage.

Introduction
In eukaryotic cells the nucleoplasmic and the cytoplasmic compartments are separated by the nuclear envelope. The nuclear envelope consists of two lipid bilayers, the inner and outer nuclear membranes. The two nuclear membranes are fused at sites where nuclear pore complexes are embedded. The nucleoplasmic side of the inner nuclear membrane of metazoans is closely associated with a fibrous protein network, the nuclear lamina. A “complex supporting layer of fine filaments closely applied to the inner aspect of the nuclear envelope” has been described in the fifties of the last century. Notably, the first observations of such a filamentous layer were not found in metazoans but in two unicellular eukaryotes followed by reports on nerve cells of an invertebrate and from different vertebrates including humans. These early electron microscopy reports are noteworthy because they show how prominent (30–200 nm thick) this layer might be in certain cells. The isolation of a pore complex-lamina fraction from rat liver nuclei and sequencing data from human lamins demonstrated that lamins are members of the multigene family of intermediate filament (IF) proteins. The lamina is the main components of the lamina. Finally, ultrastructural analysis of amphibian oocyte nuclei and sequencing of the respective gene structures. The lamin/IF protein family seems to be restricted to the metazoans. In general, vertebrate genomes harbor only a single lamin gene encoding a B-type lamin. The archetypal lamin gene structure found in basal metazoans is conserved up to the vertebrate lineage. The completely different structure of lamin genes in Caenorhabditis and Drosophila are exceptions rather than the rule within their systematic groups. However, variation in the length of the coiled-coil forming central domain might be more common than previously anticipated. The increase in the number of lamin genes in vertebrates can be explained by two rounds of genome duplication. The origin of lamin A by exon shuffling might explain the processing of prelamin A to the mature non-isoprenylated form of lamin A. By alternative splicing the number of vertebrate lamin proteins has increased even further. Lamin C, an alternative splice form of the LMNA gene, is restricted to mammals. Amphibians and mammals express germline-specific lamins that differ in their protein structure from that of somatic lamins. Evidence is provided that there exist lamin-like proteins outside the metazoan lineage.

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length of the lamin rod domain is conserved in most organisms. Moreover, the end segments on either sides of the rod are highly conserved. These so-called IF consensus motifs are present in every IF protein in nearly identical form.25-27 Head and tail domains of lamins are predicted to be very flexible without exhibiting pronounced secondary structures except for a globular domain within the tail, which resembles the immunoglobulin (Ig) structure. This Ig-domain consists of about 110 residues folded into a β-sandwich of nine β-strands.22,23 The core of this domain is formed by highly conserved residues, while most charged residues occur at the surface of the domain, allowing interactions with lamins, other proteins, and DNA.24

The designation of lamins as lamin A, B, and C refers back to the work with rat liver lamins and simply reflects their increasing gel electrophoretic mobility.24 Later, lamins were classified as A-type or B-type lamins according to their expression patterns, behavior during mitosis and biochemical characteristics.25 While this classification fits in for most of the cases, from an evolutionary point of view it is not justified for all lamins, as will be described in this review.

Lamin assembly differs from that of cytoplasmic IFs in certain aspects. The first assembly step involves formation of lamin dimers, which is followed by formation of polar head to tail polymers. It is suggested that the head to tail interaction is mediated by the IF consensus motifs.25 Next, antiparallel lateral assembly of two head to tail polymers forms a non-polar protofilament and three to four protofilaments can then assemble into 10 nm filaments.26 The exact mechanism of assembly is not fully understood, however, transmission and scanning electron microscopical analyses have revealed that lamins form long filaments in vivo having a diameter of about 10 to 15 nm.25,27

In addition to the general IF features, three sequence motifs are characteristic for lamins: A basic nuclear localization signal (NLS) within the C-terminus, a CaaX motif at the very C-terminus, and a cyclin-dependent kinase 1 (CDK1) phosphorylation site in front of the rod domain. While the NLS avails import into the nucleus, the CaaX motif serves as a target for a series of posttranslational modifications, which increase the hydrophobicity of the lamins and thereby enable interactions with the inner nuclear membrane. The motif contains a cysteine residue (C) followed by two aliphatic residues (aa) and a fourth residue (X) determines the type of isoprenylation, which is added to the cysteine. In case of lamins, a farnesyl group is transferred to the cysteine, forming a thioether bond. After addition of the isoprenoid moiety the last three amino acid residues (aaX) are cleaved off by either of the two endoproteinases Rec11 or ZMPSTE24, followed by carboxymethylation of the newly formed C-terminus.28 All lamins that carry a CaaX motif undergo this modification, but it must be noted that A-type lamins, which exhibit an additional ZMPSTE24 protease cleavage site in their tail region, loose the lipid modification upon processing by this protease.28 Therefore, B-type lamins, which do not undergo this second proprotein processing step, remain permanently isoprenylated while fully processed A-type lamins are recruited to the nuclear envelope via interactions with lamins and/or lamin-associated proteins.28,29

The highly conserved CDK1 recognition site in front of the rod domain, is of crucial importance for the reversible disassembly of the nuclear lamina24 in the course of nuclear envelope breakdown during M-phase of the cell cycle. Disassembly of lamin filaments is triggered by CDK1 phosphorylation at this site25-27 and reassembly starts upon dephosphorylation.28

The nuclear lamina is an essential component of the nuclear skeleton and contributes to mechanical stability of the nuclear envelope and is involved in anchoring nuclear pore complexes.25-27 Lamins interact with a wealth of proteins to fulfill a similar wealth of cellular functions.25 Lamins take part in regulation of chromatin organization,24 DNA-replication and RNA-transcription,22 nuclear size determination45 and cell cycle regulation.44,46 Furthermore, through interaction with SUN-domain proteins lamins interact with nucleoporins, which bind microtubules, actin- and cytoplasmic IFs, linking the cytoskeleton to the nucleoskeleton.47-49 The importance of lamin function is highlighted by mutations in genes encoding lamins, which cause a wide range of heritable human diseases, collectively termed laminopathies. Mutations in the human LMNA gene affect muscle, adipose, bone, nerve and skin cells. One group of laminopathies which exhibit particularly severe phenotypes comprises progeroid syndromes such as Hutchinson-Gilford progeria syndrome, atypical Werner syndrome and mandibuloacral dysplasia.45-48

Structure, assembly as well as the multitude of cell biological aspects of lamins and particularly their role in laminopathies is currently being described in a growing number of excellent reviews.25,48-57 In this review we will focus on the evolution of the lamin protein family. Here we review relevant literature and present new data obtained by sequence data bank searches.

An Interjection at the Beginning

A comparison of intron patterns might seem to be very dry matter for those who devote all their attention to the biological functions of lamins. But, with the evolution of species, nature has done experiments for us, which we probably would neither plan nor for which grant agencies would give us the money, but they can teach us a lot.

Lamins are the Founding Members of the IF Multigene Family

With the complete cDNA sequence of human lamin A the relationship between nuclear lamins and cytoplasmic IF proteins was discovered.14,15 It was speculated, that lamins are the founding members of the IF multigene family.15 First direct support for this speculation came from protein sequence comparisons, which showed that the coiled-coil rod domains of lamins and vertebrate IF proteins have the same length, while those of vertebrate IF proteins are shorter by six heptads. Moreover, within their tail domains, lamins show significant sequence similarity to invertebrate but not to vertebrate IF proteins. Cytoplasmic IF proteins of invertebrates are therefore closer to nuclear lamins than to the cytoplasmic IFs of vertebrates.50,51 Analysis of the
The exon/intron pattern of a vertebrate nuclear lamin gene, the LIII gene of *Xenopus laevis*, and an invertebrate gene of the gastropod *Helix aspersa* encoding a cytoplasmic IF protein has revealed a remarkable similarity in the structural organization of these genes. Eight out of ten introns present in both genes are located at homologous positions. This striking similarity substantiated the common ancestry of lamins and IF proteins. The few differences in intron/exon patterns also instantly suggested how the archetype IF protein gene might have arisen from a lamin-like progenitor (Fig. 1). Deletion of two signal sequences found in lamins but not in IF proteins convert a nuclear lamin into a cytoplasmic IF protein. These two signals are the NLS and the C-terminal CaaX motif. During evolution removal of the NLS could have been achieved by creation of splice recognition sites flanking the NLS. This would result in the removal of this signal during RNA maturation and consequently, in the generation of a new intron in the gene. Sequence alignments of lamins and invertebrate IF proteins necessitate the introduction of a gap in the IF sequence, which coincides with the position of the NLS in lamins. The Helix IF gene has an intron in this region, which has no counterpart in the lamin gene. The last intron, which delineates a short exon and encodes the CaaX motif in lamin genes, is absent in the IF gene. Introduction of a stop codon in the preceding exon of the lamin gene would delete the CaaX encoding exon and would shorten the protein chain of the resulting IF protein. The elimination of the two signal sequences freed the lamin-like archetype IF protein from nuclear compartmentalization and membrane interactions and provided the possibility to form cytoplasmic IFs.

The evolution of vertebrate IFs was accompanied by the loss of six heptads in coil1b. The site of deletion coincides with the position of an archetypal intron. It is therefore likely that this deletion was caused by the formation of a new splice acceptor site.

The analysis of lamin genes outside the vertebrate group was initially focused on genetically tractable model organisms like *Caenorhabditis elegans* and *Drosophila melanogaster*. These two species, however, show a strong genome drift and their lamin genes have entirely different organization (see below).

The search for lamin genes in different taxonomic groups was initially done by mapping of exon/intron boundaries of cloned genomic fragments. The emergence of a constantly growing number of genome assemblies made sequence information available for a broad spectrum of species and data bank mining became the main source for this type of evolutionary studies. However, successful identification of new (lamin) genes critically depends on the quality of the genome data. Gaps in the assemblies may prevent discovery or unambiguous assignment of individual (lamin) genes. The gene encoding chicken lamin A, for example, is still not found in the published genome sequence although its cDNA sequence has been published two decades ago. It would therefore be highly desirable to fill existing gaps in published genomes even if this would slow down the genome sequencing of further species.

The results presented here are mainly based on literature reports and, in addition, on our own database searches. We have screened publicly available databases with lamin sequences from a large variety of species. In many cases, annotated gene sequences had to be manually re-edited or had to be extracted from scratch. When available, exon/intron boundaries were verified by comparing genomic sequences with the corresponding cDNA or EST sequences. Whenever these were not available, intron positions were inferred from protein sequence alignments and the presence of suitable splice donor and acceptor consensus motifs. This

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**Figure 1.** A model of IF evolution from an ancestral lamin progenitor. Intron positions (triangles, numbered with Roman numerals) are shown with respect to protein structure. Large boxes represent coiled-coil segments. The oval represents the lamin Ig-domain. Archetypal intron positions are labeled in red, introns gained in the course of cytoplasmic IF evolution are in blue. Deletion of the region containing the NLS is indicated by gray lines, the resulting new intron is marked by a star. Invertebrate IF proteins lack the last CaaX-encoding exon. Coil 1b in vertebrate IF proteins is shorter by six heptads. The deletion of the six heptads can be explained by generation of a new splice acceptor site of intron 1. The heptads, which become deleted, are marked by dashed lines in coil 1b of the invertebrate IF. The tail domains of vertebrate IFs do not show resemblance to their invertebrate progenitors, marked by a hatched line. NLS, nuclear localization signal; CaaX, isoprenylation motif; CDK1, CDK1 phosphorylation site.
method provides reliable results for large parts of the lamin sequences, particularly for regions with high or moderately high sequence similarity. In some cases, e.g., in parts of the lamin tail that show little sequence conservation, this method might fail or produce ambiguous results. This applies particularly for the very short last exon, which encodes the CaaX sequence. Results solely based on genomic sequence information must therefore be considered with some caution.

From these analyses the following conclusions can be drawn: (1) Lamins seem to be restricted to metazoans, (2) invertebrate genomes possess a single lamin gene encoding a B-type lamin (for exceptions in certain insect groups see below), (3) the archetypal lamin gene structure, found in basic metazoans, is conserved in all vertebrate lamins, (4) within the protostome lineage, gene structures differ most from that of the archetypal lamin gene, (5) the increased number of lamin genes in vertebrates can be explained by two rounds of whole genome duplications at the onset of vertebrate radiation, 67,68 (6) lamin A is restricted to vertebrates. Its origin by exon shuffling might explain the processing of prelamin A to the mature non-isoprenylated lamin A. These points will be discussed in detail below.

**The Metazoans**

Metazoans constitute a monophyletic taxon, which includes all multicellular animals. The sister group of metazoans is formed by the choanoflagellates (Fig. 2). The most basal metazoans are the sponges, which form the phylum Porifera. Although sponges have different specialized cell types, these cells do not form tissues, as they are characteristic for all other metazoans. Sponges already differentiate oocytes and spermatozoa as generative cells. All other metazoans belong to the eumetazoan group. The most basal eumetazoans are the placozoans with the single genus Trichoplax. They are small disc-shaped animals, which consist of two epithelial layers separated by multinucleated fiber cells. 71 Phylogenetic analyses placed them as the basal eumetazoan
Lamins of the Protostome Lineage

Protostomes comprise two major groups, the lophotrochozoans and the ecdysozoans. The tunic Lophotrochozoa was discovered based on small-subunit rRNA sequence comparison. It includes the flatworms (plathelminths), annelids and mollusks (Fig. 2). The ecdysozoan group covers the priapulids, nematodes, and arthropods. The ecdysozoan group houses two model organisms widely used in classical and molecular genetics and developmental biology, i.e., Caenorhabditis elegans and Drosophila melanogaster. Drosophila belongs to the phylum Arthropoda while Caenorhabditis is grouped to the more basal group of nematodes. The gene structures of lamin Dmo of Drosophila and the Ce-lamin of Caenorhabditis were the first to be reported for invertebrates.

Noteworthy for protostome lamin genes is the presence of an intron in coil 1b, which is present in three of the lophotrochozoans and several ecdysozoans, i.e., all nematodes and several arthropods (marked green in Figs. 2, 4 and 5, Figs. S1-S4). This intron is therefore specific for the protostome lineage.

The Lophotrochozoan Lamin Genes

Not all of the archetypal introns have been retained in all members of the lophotrochozoans, but referred to the group as a whole, all are present. An alignment of lamin protein sequences of two plathelminths, the trematode Schistosoma mansoni and the nematode Schistostrongylus mansoni, the annelid Capitella teleta and the mollusk Aplysia californica and human lamin B1 is shown in Figure S1. Gain in introns unique for particular species is low.

A hallmark of lamins is the length conservation of the four α-helical sections in the central domain. These α-helical sections show the heptad repeat pattern characteristic for coiled-coil forming proteins. Three of the analyzed lophotrochozoan lamins show a remarkable deviation from this conserved feature. Coil 1b of the Schmidtea, Schistostrongylus and Aplysia lamins is shorter by 11 and 9 amino acid residues, respectively (Fig. S1A and B). Lack of these residues has been confirmed in all three cases by cDNA sequence data. Alignment to other lamins reveals that the gap is located approximately mid way in coil 1b. The lack of these residues disrupts the heptad repeat pattern and might result in an additional short linker region in coil 1b.

Lamin Gene Organization is Conserved Between the Most Basal Metazoans and Mammals

After completion of the first sponge genome project, lamin gene sequences are now available for at least one representative of each metazoan phylum. All invertebrate lamins show the same overall sequence organization typical for vertebrate lamins and resemble in size to the vertebrate B-type lamins. Comparison of the lamin genes of the basal metazoans, e.g., the poriferan Amphimedon queenslandica (a sponge), the plathelminth Trichobilharzia adhaerens and the cnidarians Hydra magnipapillata, a hydrozoan, Nematostella vectensis, an anemone, and vertebrate lamins has been particularly informative. Figure 3 shows an alignment of these four basic metazoan lamins with human lamin B1. Lamin B1 was chosen as reference since it is thought to be the vertebrate ortholog of the invertebrate lamin. This assumption is based on microsynteny. The single lamin gene in Nematoscelis is flanked by the gene encoding the membrane-associated Ring finger protein 2/3. The same gene flanks the lamin B1 genes in Xenopus and man. The sequence alignment of lamins from these distantly related species is nearly perfect (Fig. 3). It requires the introduction of a single gap within linker 12 of the Hydra rod domain to compensate for length heterogeneity of this region. The same applies to the conserved part of the tail, i.e., the Ig-domain, where small gaps have to be introduced. As seen for nearly all lamins, the remaining parts of the tail domain and the head domain vary in size between individual lamins.

Particularly remarkable is the conservation of the intron pattern between the basal metazoans and the human lamin B1 gene, the archetype of the vertebrate lamins. The positions of nine introns, which are identical in the lamin genes of basal metazoans and in those of vertebrates, (I–IX in Fig. 3) highlight the architecture of the lamin gene structure and hence that of all IF genes. Eight of these introns are also present in genes of cytoplasmic IFs of invertebrates and five (introns I to V) are present in nearly all type I–III vertebrate IF genes. It will become clear in the following description that the archetypal pattern of introns has been retained to very different extent in the different phyla of metazoans.
The Ecdysozoan Lamin Genes

Similar to the lophotrochozoans, all archetypal introns are present in the ecdysozoans, when this group is considered as a whole. The same holds for one of its subgroups, namely the arthropods. However, the ecdysozoans house those species, whose lamin gene structures differ most from that of the archetypal lamin gene. Drosophila and Caenorhabditis are among them. This will become clear when insects and nematodes are considered in detail (see below).

Lamin Genes of Arthropods

The phylum Arthropoda includes several subphyla such as the Chelicerata (including the Xiphosura (horseshoe crabs)) as well as the Arachnida (spiders, mites), the Crustacea, and the Insecta. The latter is the most species-rich class of the entire animal kingdom. In most arthropods the number of the archetypal introns is larger than in the nematodes (Fig S2, Figs. 4 and 5). The protostome specific intron in coil 1b (Figs. 2, 4 and 5; see also Figs. S1–S3) is retained in the mite lamin B1 (Homo B1) was performed with the MultAlin software using the Blosum62 matrix. The sequence for the sponge was manually extracted from the Amphimedon genome project. The Trichoplax, Hydra and Nematostella lamin sequences were taken from published data. Trichoplax intron positions were re-inspected and a putative CaaX motif-encoding exon was identified and added to the Trichoplax sequence. The gene structure of human lamin B1 was taken from. The position of all nine introns present in the lamin genes of the four basal metazoans is conserved in the human lamin. These intron positions are marked by white letters on red background and are numbered with roman numbers I to IX. Introns, which have no match in one of the other four lamin genes, are marked white on black. Where the exon/intron boundary is between two codons, the two adjacent amino acid residues are marked. In the other two cases the exon / intron boundary is between the second and third base of the codon of the marked amino acid residue. The short CaaX motif-encoding exon is heterogeneous in size. Consequently, intron positions of intron IX appear out of register in this alignment.

Lamin Genes of Insects

In the course of insect diversification more profound changes have taken place. A comparison of eight species from different orders,
Figure 4. Lamin gene evolution in insects. (A) Phylogenetic tree of insects. The tree and the time scale were taken from reference 81. Loss of phylogenetically relevant introns (archetypal in red, arthropod-specific in blue) is indicated below the lines of the tree. For the dipterans only the branches of the Cuculimorpha and the Ephydroidea are shown. (B) Intron patterns of insect lamins. Positions of archetypal introns (red triangles) are shown with respect to lamin protein structure in the top row. Large boxes represent coiled-coil segments. The oval represents the lamin Ig-domain. Head and tail domains are represented by gray bars. Color coding of triangles, green, intron restricted to the protostome lineage; blue, introns restricted to arthropods; gray, introns restricted to hymenopterans (Apis and Nasonia); black, introns unique to individual species. The split blue triangles indicate a deletion of seven heptads in coil 2b of the Anopheles and Aedes lamins.
which include the pea aphid (*Acyrthosiphon pisum*), two mosquitoes (*Aedes aegypti* and *Anopheles gambiae*), the honey bee (*Apis mellifera*), a parasitoid wasp (*Nasonia vitripennis*), the red flour beetle (*Tribolium castaneum*), a fruit fly (*Drosophila melanogaster*), and a butterfly (*Bombyx mori*). This reveals that the last common ancestor of the insects had lost the archetypal introns II, IV, and VII (*Fig. 4A*). In the arthropod lineage two new introns were gained (blue in *Fig. 4*; *Figs. S2 and S3*). The latter were lost again in the course of insect evolution in some of the lineages. Further loss of archetypal lamin introns differs greatly in the individual lineages. The majority of species retained at least two and up to five of the archetypal introns (*Fig. 4*; see also *Fig. S3*). Some species gained one or more new introns that are unique for these species. Strong genetic drift is seen in the lamin gene of *Tribolium* and the gene encoding lamin Dmo of *Drosophila*. These two genes have lost all archetypal lamin introns and in *Tribolium* only one intron matches a position found in another insect species.

The gene structure of the lamin Dmo differs entirely from that of all other species (*Fig. 4*; see also *Fig. S3*). It shows the strongest genetic drift of all known lamin genes. The coding region is interrupted by two introns, none of which matches an intron position outside the drosophilids. Within the drosophilids the first intron is conserved in all of the 11 species that were analyzed (data available on request). The second intron is present in all drosophilids except the two members of the obscura group, i.e., *D. pseudoobscura* and *D. persimilis*. Remarkably, *Drosophila* has a second lamin called LamC. LamC lacks a CaaX motif. Its expression is developmentally regulated. Since it shares these two properties with mammalian lamin C, it has been classified as an A-type lamin, while lamin Dmo was classified as a B-type lamin due to its ubiquitous expression. From an evolutionary point of view this classification is not justified. Mammalian lamin C is an alternative splicing product of the lamin A gene. The lamin A gene, however, arose only in the course of the vertebrate evolution (see below) and the protein structure of both *Drosophila* lamins, Dmo and LamC, is that of a B-type lamin. Moreover, the LamC gene shows a greater resemblance to the archetypal lamin gene than the Dmo gene. It houses three introns, two of which correspond to the archetypal introns I and VIII, respectively (*Fig. 4*; see also *Fig. S3*). The analogy to the A-type lamins of vertebrates is valid with respect to the lack of CaaX box (mammalian lamin C) and the absence of a (permanent) isoprenylation, in the case of lamin A. In terms of differential expression, it applies only limited because vertebrate lamin B1 is also differentially expressed. While lamin C is conserved within the drosophilids (data available on request) no evidence is found that it is present outside the drosophilids. It thus represents a special feature of the fruit flies, which is difficult to put in relation to the occurrence of lamin A in all vertebrates lineages.

Two lamin genes have also been found in two mosquitoes, i.e., *Aedes* and *Anopheles*. In contrast to *Drosophila*, both mosquito lamins, L1 and L2, carry a CaaX motif at their C-termini. Extent of identity between L1 and L2 within the same species is between 53% and 55%; that between the two species is 69% for L1 and 80% for L2. Particularly noteworthy is that all four mosquito proteins have a significantly shorter rod domain. They lack the first seven heptads of coil 2b. The L2 genes contain four of the archetypal lamin introns and an additional new intron. This intron is located at the beginning of coil 2b, at the site where the seven heptads have been deleted (*Fig. 4*; see also *Fig. S3*), suggesting that this deletion was caused by the formation of a new splice acceptor site. An EST clone of *Aedes* (Accession EB008934), which spans the deleted region, proves that this short lamin is indeed expressed. The L1 genes have no introns in their coding regions and might have originated by retro-transcription of processed mRNAs and subsequent insertion into the genome.

Lamin gene duplication in mosquitoes must have occurred after the deletion of the seven heptads since both genes, L1 and L2, have a short coil 2b. This also shows that the gene duplications...
have occurred independently in the mosquito and the drosophilid lineages. Gene duplications allow functional specializations (neofunctionalizations). The Drosophila LamC gene acquired an altered membrane targeting due to the lack of lipidation and a restricted, cell type-specific expression.

The Archetypal Pattern of Lamin Introns Has Been Retained to Varying Degrees in the Nematode Lineage

Similarly to the insects, genome drift in the nematode lineage is larger than in basal metazoans or in vertebrates. Within the nematodes intron loss and gain is pronounced (Fig. 5, see also Fig. S4). Of the archetypal lamin introns, three (Loa and Pristionchus), one (Heterodera) or none (Caenorhabditis) have been retained. Two introns (marked in yellow in the Figs.) have been gained in the nematode lineage and all four nematodes have retained the protostome specific intron (marked in green). In addition, all species gained between three and ten (Pristionchus!) new introns, which are unique for the respective species.

Two of the nematode lamins show length variation of their rods. Coil 2 of C. elegans is by two heptads shorter and coil 2 of Loa lacks four amino acid residues. The rod domains of Heterodera and Pristionchus are of normal length. The major mitotic phosphorylation site in front of coil 1a, which is one of the hallmarks of lamins, is conserved only in the Loa lamin. The functional impact of this loss in the other nematodes is not known.

The sequence alignment (Fig. S4) includes sequences of four nematodes and the lamin sequence of the priapulid Priapulus caudatus. Priapulids are marine worms, which together with the nematodes are members of the Cycloneuralia. Priapulus has retained two of the nine archetypal introns and did not gain any new ones. The intron in coil 1b common to the lophotrochozoans and nematodes is not present in Priapulus.
Taken together, now that several genomes of species of the respective taxonomic groups have been sequenced, it has become clear that the genome drift in the protostome lineage and particularly in insects and nematodes is larger than in basal metazoans or in vertebrates. Moreover, length variation in the coiled-coil segments of the rod domain is restricted to protostome lamins. But it has also become evident that neither the Drosophila Dmo gene nor the Ce-lam gene, which were the first invertebrate lamin genes to be analyzed, can be taken as representatives for their taxonomic groups.

Lamin Genes of the Deuterostomes

The group of deuterostomes comprises the echinoderms (among them the starfish and the sea urchin), hemichordates, and chordates. The latter includes the subphyla cephalochordates (e.g., lancelets), tunicates (e.g., sea squirts) and the vertebrates (Fig. 6A). The lamin gene structure of all these groups largely corresponds to that of the archetypal lamin (Fig. 6B; see also Fig. S5). Those introns, which have been gained, are in positions that are not found in any other lamin gene. Strongylocentrotus and Saccoglossus have lost the archetypal intron VII. The tunicate genes underwent a functionally relevant change. They lack the Ig-domain in the tail. This loss can be explained by deletion of three exons (Fig. 6; see also Fig. S5) and was confirmed by the analysis of two Ciona species, e.g. Ciona intestinalis and Ciona savignyi. The functional significance of this loss, however, is unclear. The genomes of both species encode a single lamin gene.

Invertebrates Have Only One Lamin Gene

Database searches have identified only a single lamin gene per invertebrate genome so far. We can therefore assume that the additional lamin genes found in the mosquitoes and fruit flies are exceptions rather than the rule for invertebrates.

As representatives of most metazoan phyla have been analyzed, it can be stated that in most phyla the evolution of lamin genes has much more uniformly proceeded as it was initially assumed on the basis of the gene analyses of Caenorhabditis elegans and Drosophila melanogaster. Moreover, the strong genomic drift, which is seen in some nematodes and insects, is also not representative for the entire group of nematodes and insects, respectively.

Figure 7. Protein structure of vertebrate lamins. (A) A model for the evolution of lamin A from a B-type progenitor. The lamin A specific domain is shown as a blue bar. The ZMPSTE24 cleavage site is indicated by an upward pointing blue arrow. (B) Lamin C, an alternative splice product of the LMNA gene. Lamin C is identical to lamin A up to residue 566 followed by six unique amino acid residues encoded by the 5′-end of intron II (marked in black). (C) Protein structure of the two mammalian germ-line-specific lamins, C2 and B3. In lamin C2 the head, coil 1a, and part of coil 1b of lamin C are replaced by a short stretch of amino acid residues (green bar), which serve as myristoylation site. In lamin B3 the head, coil 1a and 1b of lamin B2 are replaced by a new non-α-helical head. The length of the new head differs between rat, mouse, and human between 50 to 81 residues. Intron positions (triangle, and Roman numerals) are shown with respect to protein structure. Large boxes represent coiled-coil segments. The oval represents the lamin Ig-domain. Archetypal intron positions in red, newly gained intron positions in lamin A in red/blue. The vertebrate-specific intron position is marked by an open triangle. NLS, nuclear localization signal; CaaX, isoprenylation motif; CDK1, CDK1 phosphorylation site.
Lamin Evolution in Vertebrates

It is hypothesized that two rounds of whole genome duplications have occurred in the ancestral vertebrate.\(^2\) The timing of these duplications is constrained to after the divergence of vertebrates from tunicates and lancelets, but before the split between cartilaginous and bony vertebrates.\(^4\) For many single genes in invertebrates four vertebrate paralogs are known.\(^26\) A prominent example is the Fox cluster of developmental genes.\(^2\) This scenario could also explain why vertebrates produce lamin genes, encoding the lamin B1, B2, LIII and lamin A. As mentioned above, vertebrate lamin genes have retained the nine archetypal introns, which are present in basic metazoa. All gained an additional intron, which is positioned in the coil 1b region (Figs. 2 and 7). Two further changes have occurred during evolution of the vertebrate lamin: The LMNB2 gene gained an additional intron in the linker 1 region\(^1,6\) and the tail domain of lamin A was extended by insertion of a new exon into the archetypal intron IX (Fig. 7A). Alignments of lamin B1 and B2 from different vertebrates are given in the Figures S6 and S7, respectively.

The genomes of fish, amphibians and birds encode all four genes while mammals have lost the LIII gene.\(^36,37\) Transcriptional initiation at different start sites and alternative splicing of primary transcripts gives rise to an even greater number of lamin proteins.\(^36\) All vertebrate lamins are differentially expressed, but to a very different extent.\(^36,37\)

Lamin B2 is nearly universally expressed in somatic cells while lamin B1 expression is more restricted.\(^33,34,37\) B1 and B2 are closely associated with the nuclear envelope and remain associated with membranes after disassembly of the nuclear lamina during M-phase.\(^31,39,111,112\) The assumption that lamin B1 and B2 are essential for nuclear integrity, cell proliferation, and development\(^36,37,101,102\) has recently been challenged by experiments with mammalian lamin B2-KO mice.  While lamin B2-KO mice do not survive for more than eight weeks post gestation, lamin B1 seems to have more specialized functions. It interacts with a large number of nuclear protein partners.\(^19\) Filaments formed by lamin A differ from those made of B-type lamins\(^27\) and light microscopic analysis indicates that A- and B-type lamins form separate meshworks within the lamina.\(^113\) Moreover, experimental evidence reveals that lamin A contributes significantly to nuclear mechanics.\(^111,113\)

In amphibians LIII is the major lamin of oocytes and early embryos.\(^117\) In somatic cells it is restricted to a few differentiated cell types.\(^29\) EST data indicate that this might also hold true for birds. There are two variants of lamin LIII. They are generated by alternative splicing of two C-terminal CxxC motif-encoding exons. Both LIII proteins are isoprenylated, but differ greatly in their membrane association. LIIIa becomes soluble upon lamina disassembly during M-phase while LIIIm remains membrane associated. For the stable association of LIIIm with membranes six basic amino acid residues and a cysteine residue, which serves as a potential palmitoylation site act as secondary membrane targeting motifs.\(^19\) The two alternative exons, and the secondary membrane targeting signals are precisely conserved between fish, amphibians and birds, which underlines the functional importance of these motifs (Fig. S8). The fact that mammals have lost the lamin LIII gene rather than it has remained undetected has been proven by analysis of the flanking genes.\(^2\) While in Xenopus and chicken, the LIII gene is flanked by the genes SH3GL3 and ADAMTS24, these two genes are directly adjacent on the respective mammalian chromosomes (i.e., chromosome 15 in human, chromosome 7 in mouse, chromosome 1 in rat). Whether LIII is still present in the monotreme platypus (Ornithorhynchus anatinus) would be of particular interest with respect to its function in oocytes. Platypus exhibits a combination of non-mammalian and mammalian characters; for example the females lactate, yet they lay eggs. Like other egg-laying vertebrates, but unlike mammals they produce small vitellogenin-rich eggs. The egg yolk proteins are still present in platypus but have been lost from the eutherian lineage.\(^101\) Preliminary results obtained in our group do not preclude that the LIII gene still exists in the platypus genome. However, a definite answer to this question has to await further releases of the platypus genome project.

Lamin A

The structure of lamin A differs from all other lamins. Its tail domain is by about 50 to 100 amino acid residues longer than that of the B-type lamins. These extra amino acid residues are encoded by a single exon, which suggests that the LMNA gene arose by insertion of a new exon in the last intron of a B-type progenitor gene\(^39\) (Fig. 7A). An alignment of lamin A from different vertebrate species is given in Figure S9. Lamin A is synthesized as prelamin A. After isoprenylation and carboxyl-methylation it undergoes a further proteolytic cleavage by the endoprotease ZMPSTE24, which results in the loss of the hydrophilically modified C-terminus. The lack of a permanent lipidation might explain why mature lamin A becomes soluble during M phase. If for one or another reason there is improper processing of lamin A, this will cause severe diseases such as premature aging (Hutchinson-Gilford progeria syndrome) or mandibuloacral dysplasia.\(^114,115,116\)

Lamin A is expressed late in development and its appearance is normally correlated with differentiation.\(^114,116,117\) It seems to be dispensable for embryonic development but homozygote LMNA KO mice do not survive for more than eight weeks post gestation.\(^118\) Lamin A seems to have more specialized functions. It interacts with a large number of nuclear protein partners.\(^19\) Filaments formed by lamin A differ from those made of B-type lamins\(^27\) and light microscopic analysis indicates that A- and B-type lamins form separate meshworks within the lamina.\(^113\) Moreover, experimental evidence reveals that lamin A contributes significantly to nuclear mechanics.\(^111,113\)

In its implication in a multitude of cellular functions is highlighted by mutations in the LMNA gene, which cause a broad spectrum of human diseases.\(^119,120,121\) This has made lamin A the most intensely studied of all nuclear envelope proteins.

For the LMNA gene a large number of misense mutations have been described (http://www.umd.be/LMNA/). These are usually found at highly conserved positions. The Lamin A sequences of mammalian species are very similar, e.g., 97–98% of the residues of the dog, pig, and human lamin A are identical (Fig. S9). In dog and pig there is a serine residue at position 602, while there is a glycine in humans. The G602S mutation has been described to
cause type A insulin resistance syndrome. It is hard to imagine that this serine residue in an otherwise almost identical context represents the wild type in one case and in the other causes a disease. In lamin A proteins, which have a lower degree of identity with the human lamin A, such as the fish laminus, it is less surprising that changes in amino acid residues are found, which cause disease in humans. Lamin A1 of the puffer fish Takifugu contains for example four residues, which in humans cause Emery-Dreifuss muscular dystrophy or dilated cardiomyopathy. Additional examples are found in A-lamins of other fishes.

Lamin A seems to be the evolutionarily most derived member of the vertebrate lamin family. Apart from the lamin A-specific domain in the tail it bears somewhat greater overall resemblance to lamin LIII than to B1 or B2. The following evolutionary scenario might therefore be plausible, regardless of whether the four genes have arisen in the course of two whole genome duplications or by two rounds of selective duplication of the ancestral lamin gene: The first gene duplication led to the formation of two precursor genes. From one of these precursors arose the LMMV1 and LMMV2 gene after the second duplication event. LMMV2 acquired an additional intron (see above). From the other precursor arose the LHI gene and a gene that became the LMNA gene after insertion of the new exon. The insertion of the new exon into an otherwise conserved gene generated a new lamin type. Concomitant with the insertion of the new exon a ZMPSTET24 cleavage site might have been introduced. This would explain the proteolytic processing of prelamin A to the mature protein, a feature unique to lamin A. Processing of prelamin A to lamin A was confirmed experimentally for mature protein, a feature unique to lamin A. Processing of prelamin A to lamin A was confirmed experimentally for all differentiated cells albeit the ratio of the two proteins may be resolved after the completion of genome projects of the elephant shark (Callorhinchus milii), a cartilaginous fish, and the lamprey (Petromyzon marinus). All bony fish analyzed so far have a LMNA gene. Takifugu, Tetraodon (two puffer fishes), and Gasterosterus (stickleback) even have two, which arose from a further gene duplication. The first 20–30 amino acid residues encoded by the lamin A-specific exon are highly conserved in all vertebrate classes. In lamin C, the head and coil 1a of lamin C are replaced by a new N-terminal domain, which in humans cause disease in humans. Lamin A1 of the puffer fish Takifugu contains for example four residues, which in humans cause Emery-Dreifuss muscular dystrophy or dilated cardiomyopathy. Additional examples are found in A-lamins of other fishes.

Lamin C is produced as an alternative splice product of the LMNA gene. It is identical to lamin A up to amino acid residue 566 but lacks the residues encoded by exons 11 and 12.

In humans a third splice variant of the LMNA gene has been described. Lmnb2 acquired an additional intron (see above). From the other precursor arose the LHI gene and a gene that became the LMNA gene after insertion of the new exon. The insertion of the new exon into an otherwise conserved gene generated a new lamin type. Concomitant with the insertion of the new exon a ZMPSTET24 cleavage site might have been introduced. This would explain the proteolytic processing of prelamin A to the mature protein, a feature unique to lamin A. Processing of prelamin A to lamin A was confirmed experimentally for mature protein, a feature unique to lamin A. Processing of prelamin A to lamin A was confirmed experimentally for all differentiated cells albeit the ratio of the two proteins may be resolved after the completion of genome projects of the elephant shark (Callorhinchus milii), a cartilaginous fish, and the lamprey (Petromyzon marinus). All bony fish analyzed so far have a LMNA gene. Takifugu, Tetraodon (two puffer fishes), and Gasterosterus (stickleback) even have two, which arose from a further gene duplication. The first 20–30 amino acid residues encoded by the lamin A-specific exon are highly conserved in all vertebrate classes. In lamin C, the head and coil 1a of lamin C are replaced by a new N-terminal domain, which in humans cause disease in humans. Lamin A1 of the puffer fish Takifugu contains for example four residues, which in humans cause Emery-Dreifuss muscular dystrophy or dilated cardiomyopathy. Additional examples are found in A-lamins of other fishes.

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Expression of the amphibian lamin LIV is restricted to male germ cells. The protein is distributed in a characteristic punctuated pattern along the nuclear envelope of elongated sperm nuclei. The LIV protein is a differential splice product of the lamin LIII gene. The protein contains 40 extra amino acid residues in coil 2b of the rod domain. The extra residues disrupt the heptad repeat pattern of coil 2b and disrupt filament formation. Data bank searches gave no hints for the existence of lamin LIV outside the amphibian lineage. It therefore seems that LIV is restricted to amphibians.

Mammals express two germline-specific lamins, lamin B3 and lamin C2. Lamin C2 is an alternative splice product of the LMNA gene. In lamin C2, the head and coil 1a of lamin C are replaced by a short non-helical stretch of amino acid residues (Fig. 7C). The unique N-terminus serves as a myristylation site that is essential for nuclear envelope targeting of lamin C2. Lamin C2 is expressed in the male germine in spermatocytes and is enriched at telomere attachment sites of meiotic chromosomes. It is involved in the remodeling of the nuclear envelope of spermatocytes.

Lamin B3 is an alternative splice product of the LMMV2 gene. In the lamin B3 protein a new non-helical N-terminal domain replaces the head, coil 1a, and 3b of lamin B2. The size of the new N-terminal domain differs between different mammalian species. It is specifically expressed in post-meiotic male germ cells. Lamin B3 is assumed to affect filament formation and consequently alter the properties of the nuclear lamina. These lamins function in the remodeling of nuclei during meiotic chromosome movements and the morphogenesis of sperm nuclei. The generation of germline-specific lamins follows diverse evolutionary paths in different vertebrate classes.

Lamin LIV of Amphibians

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Germline-Specific Lamins of Mammals

Mammals express two germline-specific lamins, lamin B3 and lamin C2. Lamin C2 is an alternative splice product of the LMNA gene. In lamin C2, the head and coil 1a of lamin C are replaced by a short non-helical stretch of amino acid residues (Fig. 7C). The unique N-terminus serves as a myristylation site that is essential for nuclear envelope targeting of lamin C2. Lamin C2 is expressed in the male germine in spermatocytes and is enriched at telomere attachment sites of meiotic chromosomes. It is involved in the remodeling of the nuclear envelope of spermatocytes.

Lamin B3 is an alternative splice product of the LMMV2 gene. In the lamin B3 protein a new non-helical N-terminal domain replaces the head, coil 1a, and 3b of lamin B2. The size of the new N-terminal domain differs between different mammalian species. It is specifically expressed in post-meiotic male germ cells. Lamin B3 is assumed to affect filament formation and consequently alter the properties of the nuclear lamina. These lamins function in the remodeling of nuclei during meiotic chromosome movements and the morphogenesis of sperm nuclei. The generation of germline-specific lamins follows diverse evolutionary paths in different vertebrate classes.
Are There Lamins Outside the Metazoans?

Up till now there is no direct evidence of nuclear lamins present outside the metazoans. Neither in the genomes of unicellular eukaryotes (Dictyostelium discoideum), fungi (e.g., Saccharomyces cerevisiae, Laccaria bicolor) nor in those of plants (Arabidopsis thaliana, Physcomitrella patens) lamin sequences have been found. Because the sequence coverage of these genomes is large and the annotations are complete, it can be ruled out that these organisms possess lamins. However, a lamin-like organization at the nuclear envelope has been demonstrated also in non-metazoan eukaryotes. Notably, the first observations were made in unicellular eukaryotes.1-4 Since lamins are involved in key nuclear functions, that are common to all eukaryotes, proteins must exist in the other eukaryotes, which take over these functions. Those nuclear envelope proteins that have been characterized in trypanosomes and plants that might fulfill the function of lamins show no similarity to lamins, except for the fact that they contain coiled-coil domains.154

Recently, a nuclear envelope protein, NE81, has been characterized in the unicellular amoeba Dictyostelium discoideum.131-134 NE81 has several features in common with lamins. It has a tripartite protein structure with a central \( \alpha \)-helical domain, predicted to form coiled-coil. The rod domain is of similar length as that of the lamins. It carries an NLS, a C-terminal CaaX motif, and a CDK1 phosphorylation site in front of its \( \beta \)-pleated domain. NE81 is associated with the inner nuclear membrane. The central rod domain of NE81 is subdivided by non-helical linkers: A match of the helix-linker pattern between NE81 and lamins and IFs in general is at least not completely clear. However, as discussed above, some heterogeneity in the rod domains and, more importantly, interruptions of the heptad repeat pattern by deletions of a length that are not equivalent to one or more entire heptads are also found in some lamins (e.g., protostomes). More important for the classification as a lamin might be the presence or absence of the IF consensus sequences. While the sequence at the N-terminus of the NE81 rod bears some similarity with the corresponding IF-consensus, at the C-terminus no IF-consensus is present. The properties of NE81 justify, however, its denomination as a lamina-like protein; it is the first discovered in an unicellular eukaryote. The future will tell whether it will open the door for discovery of similar proteins in other unicellular eukaryotes or even in plants.

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Supplemental Material

Supplemental material may be downloaded from: http://www.landesbioscience.com/journals/nucleus/article/18927/
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