Review

NMCP/LINC proteins: putative lamin analogues in plants?

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Abbreviations: BAF, barrier to autointegration factor; CSK, cytoskeleton; feSEM, field emission scanning electron microscopy; GFP, green fluorescent protein; HP1, heterochromatin protein 1; IF, intermediate filament; INM, inner nuclear membrane; LBR, lamin B receptor; LEM domain, LAP2/Emerin/MAN domain; LINC complex, linker of the NSK to CSK complex; LINC proteins, little nuclei proteins; MW, molecular weight; NE, nuclear envelope; NIF, nuclear intermediate filament protein; NLS, nuclear localization signal; NMCPs, nuclear matrix constituent proteins; NPC, nuclear pore complex; NSK, nucleoskeleton; NUA, nuclear pore anchor protein; TEM, transmission electron microscopy.

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Abstract

Lamins are the main components of the metazoan lamina, and while the organization of the nuclear lamina of metazoans and plants is similar, there are apparently no genes encoding lamins or most lamin-binding proteins in plants. Thus, the plant lamina is not lamin-based and the proteins that form this structure are still to be characterized. Members of the plant NMCP/LINC protein family share the typical tripartite structure of lamins, although the two exhibit no sequence similarity. However, given the many similarities between NMCP/LINC proteins and lamins (structural organization, position of conserved regions, sub-nuclear distribution, solubility and pattern of expression), these proteins are good candidates to carry out the functions of lamins in plants. Moreover, functional analysis of NMCP/LINC mutants has revealed their involvement in maintaining nuclear size and shape, another activity fulfilled by lamins. This review summarizes the current understanding of NMCP/LINC proteins and discusses future studies that will be required to demonstrate definitively that these proteins are analogues of plant lamins.

TEXT

The presence of a fibrous lamina underlying the nuclear envelope that binds to the nuclear pore complex (NPC) was first revealed by TEM in invertebrates in the 1960s (protozoa, gregarines and annelids)[1-4], and later in vertebrates.[5] Yet, it was not until the 1970s that this structure was isolated from rat liver nuclei[6] and its main polypeptides identified.[7] These polypeptides, now known as lamins, have since been characterized extensively and shown to be restricted to metazoans.[8] However, two lamin-like proteins were recently identified in unicellular eukaryotes: the *Dictyostelium* NE81 protein is considered to be an evolutionary precursor of lamins;[9, 10] while *Trypanosoma* NUP1 is an unrelated long coiled-coil protein with lamin-like functions, participating in the

regulation of nuclear shape and structure, chromatin organization and the distribution of NPCs.[11] Lamins not only provide mechanical support to the nucleus and the nuclear envelope (NE), and promote the association between the nucleoskeleton (NSK) and cytoskeleton (CSK), but they are also involved in a multitude of nuclear functions such as chromatin organization, gene regulation, signalling and DNA repair.[12-14]

The plant nuclear lamina was described by TEM in isolated NSKs in the early 1990s,[15-20] and subsequently, a number of the similarities between the plant and vertebrate lamina were defined by field emission scanning electron microscopy (feSEM) of whole nuclei.[21] Fully sequenced plant genomes lack genes encoding lamins[22, 23] or lamin-binding proteins, with the exception of the SUN-domain proteins.[22, 24] Given the crucial role of lamins in nuclear and cellular functions, the fact that plants possess a non-lamin-based lamina has raised certain interest and considerable research effort has been dedicated to the characterization of the proteins that compose the plant lamina.[12-14] In this review, we summarize the current understanding of nuclear matrix constituent proteins (NMCPs) / little nuclei proteins (LINCs), the main candidates to fulfil lamin-like functions in plants, and we compare and contrast these proteins with the well-characterized lamins, the main components of the metazoan lamina. In addition, we discuss the main functions attributed to NMCPs and the evidence that must be accumulated in future studies to definitively consider these proteins as analogues of plant lamins.

Organization and composition of the metazoan lamina

The nuclear lamina is a complex protein meshwork attached to the inner nuclear membrane (INM) and the nucleoplasmic ring of NPCs.[25, 26] The metazoan lamina consists of a polymeric layer of lamins that belong to the intermediate filament protein

superfamily, numerous transmembrane lamin-binding proteins that anchor the lamina to the INM, and chromatin-associated factors.[14, 27] The nuclear lamina not only provides support for the NE, NPCs and chromatin anchoring sites but also, it is involved in linking the NSK to the CSK, and in regulating signalling and gene activity.[25] The ultra-structural organization of the lamina has been well characterized in amphibian oocytes and it consists of 10 nm lamin filaments arranged in a regular orthogonal pattern. By contrast, more irregular filamentous networks have been observed in somatic cells.[25, 26]

Lamins, the building blocks of the lamina

Lamins are the main components of the nuclear lamina.[7] Sequence comparisons and those of exon/intron patterns indicate that lamins are the founding members of the IF protein family.[8] Lamins can be found in all metazoans but not in single cell eukaryotes or plants, although a distant lamin homologue has been described in the unicellular amoebae *Dictyostelium,* which under certain conditions can form a cell body.[9, 10] Based on their structure, expression pattern, mitotic behaviour and biochemical characteristics, lamins have been classified into two types (A and B).[13, 14] Most invertebrates have a single lamin gene encoding a type B lamin,[8, 28] while vertebrates possess four lamin genes: *LMNB1*, *LMNB2*, *LIII* (sometimes called *XLMNB3)* and *LMNA* that encodes lamins A and C. The *LIII* gene has been lost in mammals[8] and moreover, mammals possess an additional type A lamin, lamin C, which is produced by alternative splicing of lamin A transcripts. The tail domain of lamin A contains a unique 90 amino acid segment not found in type B lamins, probably due to the insertion of a new exon in the last intron of a type B progenitor gene. Lamin A interacts with numerous nuclear proteins, and it is involved in multiple nuclear and cellular functions, as witnessed by the broad spectrum of human diseases caused by *LMNA* gene mutations.[29]

Lamins have the conserved tripartite structure typical of IF proteins, consisting of a coiled coil central rod domain that contains four coils (1A, 1B, 2A and 2B), each separated from one another by three short linkers (L1, L12 and L2). The rod domain is flanked by a short N-terminal domain that contains a conserved phosphorylation site for cdk1,[8] which is involved in head-to-tail polymerization, and a longer C-terminal tail domain containing a second conserved cdk1 phosphorylation site required for mitotic lamin depolymerisation.[13] In addition, there is a nuclear localization signal (NLS) located between the C-end of the rod domain and the highly conserved IgG fold, as well as a C-terminal CAAX box (Fig.2).[12, 30]

The expression of lamin genes is developmentally regulated and while lamin B2 is constitutively expressed in somatic cells, the expression of lamin B1 is more restricted. Lamin LIII is the predominant lamin in oocytes and embryos, yet its expression in somatic cells is restricted to a few differentiated tissues. Lamin A is expressed late in development, normally correlating with differentiation.[29] Except for lamin C, all lamins are expressed as prelamins, and they undergo highly regulated and extensive post-translational modification of the C-terminal CAAX box via cysteine farnesylation, which is followed by proteolytic cleavage of the AAX by a prenyl protease and carboxymethylation of the C-terminal cysteine. B-type lamins remain permanently farnesylated and carboxymethylated, whereas prelamin A undergoes the removal of 15 amino acids from the C-terminus to produce the mature lamin A that lacks these farnesyl and carboxymethyl modifications.[12, 27]

Lamins also undergo other post-translational modifications, such as phosphorylation and sumoylation. The polymerization and mitotic disassembly of lamins is regulated by

extensive phosphorylation by cdk1, PKC, PKA, S6-kinaseII and Akt. Lamins have 12 conserved phosphorylation sites that are involved in mitotic lamin polymerization and that are located in the head and tail domains. Other conserved phosphorylation sites are probably involved in the regulation of conserved functions, while unique phosphorylation sites likely mediate the differential regulation of lamins in distinct tissues.[27]

The assembly of lamins into nuclear lamina is a complex multistep process. *In vitro* reconstitution and structural analysis revealed that the building blocks of lamin polymers are formed by parallel dimerization of the rod domains that then assemble longitudinally to form higher order head-to-tail lamin polar oligomers. Two head-to-tail oligomers then interact laterally to form tetrameric protofilaments, which further assemble to form 10 nm filaments, as successfully assembled *in vitro* using *Ce*-lamin (most other lamins form paracrystalline arrays *in vitro*).[13, 31] The rod segments play an important role in lamin homodimerization, and in the formation of lateral and longitudinal contacts.[32, 33] Moreover, mutational analyses suggest that the 2B coil plays a dual role in dimerization and in the interdimer interactions necessary for filament formation.[33]

Current information about the organization of lamins *in vivo* is largely based on studies of the amphibian oocyte lamina, which express a single type of lamin (LIII). By contrast, lamin organization in somatic cells remains poorly understood, probably due to the complex interactions of lamins with chromatin and other proteins or nuclear structures. *In vivo* lamins form an intricate orthogonal meshwork of filaments within the lamina,[25, 26, 34] and they also reside in the nucleoplasm in a less organised state but with much greater mobility than that observed in the lamina.[35] Although type A and B lamins form separate filament networks at the nuclear envelope and in the interior of the nucleus,

these individual networks interact to varying degrees.[26, 34, 36, 37] During the open mitosis of metazoans, type A and B lamins display different assembly and disassembly properties. When the NE is disassembled during late prophase lamins are depolymerised by mitotic kinases and while type A lamins are dispersed throughout the cytoplasm, type B lamins remain associated to the nuclear membranes that disperse throughout the endoplasmic reticulum, probably due to their permanent farnesylated state. Type A and B type lamins also undergo spatially and temporally distinct forms of assembly into the nuclear lamina at the end of mitosis. Type B lamins accumulate around decondensing chromosomes to form relatively stable complexes at telophase, while type A lamins are transported into the nucleus at a later stage, after the formation of an intact NE.[12]

Lamins are involved in many nuclear functions, such as: the regulation of nuclear shape and architecture; the association of the NSK to the CSK; epigenetic modifications; chromatin organization and positioning; DNA replication, repair and transcription; and cell proliferation and differentiation. They also perform several structural functions including the regulation of the size, shape and mechanical properties of the nucleus, and they are important for NE stabilization and the incorporation and spacing of NPCs.[12-14, 30, 35, 36, 38] In addition, lamins participate in the physical connection between the nucleus and the CSK through their interaction with the LINC complex, which is formed by SUN- and KASH-domain proteins and is essential for nuclear positioning and migration, centrosome attachment to the nucleus, meiotic chromosome pairing and mechanotransduction.[39, 40] Lamins also act as modulators of transcription through their influence on chromatin structure and organization as a result of direct interactions with either DNA, histones and/or other chromatin-associated proteins, such as LBR, HP1 and BAF, or their direct or indirect interaction with transcription factors that affect cell proliferation,

differentiation and apoptosis.[13, 41, 42] The direct or indirect interaction of chromatin with lamins also has a strong effect on the epigenetic modification of histones.[43, 44] The absence of lamins affects the organization of chromosome territories and domains, and a role for lamins in the localization and function of centromeres and telomeres has also been demonstrated.[45, 46] Lamins localize to replication foci and interact with PCNA, a component of the replication machinery,[47] while mutations in lamins can produce genomic instability by compromising DNA repair.[48] Together, all the above observations point to lamins as key determinants of nuclear architecture and function.

It is clear from the above that the interaction of lamins with distinct proteins, including structural and regulatory proteins, defines their activity.[27, 49] Lamin protein partners, mainly those of lamin A, have been studied extensively.[14, 27, 49] The proteins that interact with lamin A are involved in different nuclear activities, and they include components of the NSK and NPCs, such as lamins B1 and B2, actin (lamins have two actin binding sites in their tail domain),[50] nesprin 1α and nesprin 2, lamin companion 1 (LCO1), SUN1 and SUN2, the nucleoporins Nup153 and Nup88, and LEM domain proteins such as LAP2α, MAN1, LEM2 and emerin. Other partners include chromatin associated proteins such as BAF, PCNA, HP1 and histones, as well as transcription factors like Rb or other proteins involved in transcription and signalling.[27] The partners of type B lamins are less well known, although lamin B1 is known to interact with the lamin B receptor (LBR), which contains eight transmembrane domains, as well as with emerin, MAN1, actin, LCO1 and Nup153, PCNA and histones.[14, 27]

Some of the best characterized lamin-binding proteins are those that share the LEM domain, a motif of about 45 residues that folds as two α-helices and binds BAF, a

mobile lamin-binding protein that interacts with histones.[49] Most LEM proteins are integral proteins of the INM and have one or two transmembrane domains, while some have additional domains that bind DNA or chromatin-binding proteins. All LEM domain proteins bind type A and/or B lamins through a direct interaction with the IgG fold in their tail domains.[14] Emerin binds many other proteins in addition to lamins, including: structural proteins (*e.g.*, nesprin1α, nesprin2β, actin, nuclear myosin c and nuclear αII-spectrin); other INM proteins (*e.g.*, MAN1, LUMA); proteins involved in signalling, transcription and mRNA splicing; and BAF.[51] Emerin forms several multiprotein complexes, some of which contain mainly architectural components and others containing chromatin and gene regulators.[49, 51] LAP2α and LCO1 bind lamin A and form three-dimensional scaffolds in the nuclear interior.[35] Lamins, LEM proteins, BAF, and probably other INM proteins, form a complex multiprotein network involved in anchoring chromatin to the NE.[49] These functions and interactions of LEM proteins, lamins and BAF are strongly conserved in metazoans, emphasizing their fundamental roles in the nucleus.

Other well characterized lamin partners are the SUN-domain proteins of the INM, proteins that form trimers which interact with three KASH domain proteins of the ONM in the lumen of the NE, forming LINC complexes.[39] SUN proteins are conserved in eukaryotes, including yeast and plants,[52-54] and they are characterized by their C-terminal SUN domain, a 120 residue motif involved in binding the KASH domain, and a nearby coiled-coil domain that mediates trimerization.[40, 55] SUN domain proteins bind to lamins through a direct interaction with their nucleoplasmic N-terminal domain.[56]

The plant lamina

The presence of a peripheral layer similar to the metazoan lamina was identified by TEM in the NSK and nucleus of both dicot and monocot plants in the early 1990s.[15-19, 57] A more recent feSEM study of the nucleus revealed the presence of a plant lamina attached to the INM and linked to the nucleoplasmic ring of NPCs, with a highly organized filamentous structure similar to that of the metazoan nuclear lamina.[21, 58] Plants lack orthologues of lamins[22, 23], as well as most lamin-binding proteins except for the SUN proteins,[52, 53] although Nup136 is a functional analogue of metazoan lamin binding protein, Nup153.[59] The similar organization of the lamina and the fulfilment of the main activities of lamins in the plant nucleus suggest that plants express proteins that functionally replace lamins, and that probably share their structural and functional properties rather than sequence similarity, as described for NUP1 in *Trypanosoma*.[11] Since the first description of a plant lamina a few insoluble proteins have been identified in this structure, mainly by immunological methods.[20, 60] Some of these proteins are immunologically related to vertebrate lamins, and are of similar sizes, with comparable pI values and nuclear distributions.[16, 17, 19, 61] These include the NIF group of proteins, which not only exhibit the aforementioned similarities with lamins but they also form 6-12 nm filaments *in vitro*.[16, 61] Unfortunately the sequence of these proteins remains unavailable to compare them to lamins.

As indicated above, the best candidates to fulfil the functions of lamins in plants are NMCPs, which have a predicted secondary structure similar to that of lamins and thus, should be able to dimerize and form filaments. NMCP1 was first described in 1993 in carrot as a residual protein of the nuclear matrix with a pI value similar to that of lamins but a much higher molecular mass.[18] This protein was later shown to have a predicted

tripartite structure with a central coiled-coil domain similar to that of lamins,[57] and to assemble and disassemble in mitosis like lamins.[62] Four homologues of carrot NMCP1 were subsequently identified in a genome-wide search for coiled-coil proteins in *Arabidopsis thaliana*.[23] These four *A. thaliana* genes were named *LINC* (little nuclei) 1 to 4 after the phenotype of their corresponding mutants.[63] This term is somewhat misleading as it is already used to describe the linker of the NSK to CSK complex of the NE.[40] Accordingly, it was proposed to change this term to *CRWN* (crowded nucleus), after another phenotype of the mutants,[24] although this could add further confusion to the field and in our opinion, the original term NMCP (nuclear matrix constituent proteins) should be used to refer to these proteins. More recently, searches of plant genomes have identified genes encoding other NMCP homologues in many different species, confirming that NMCPs are well conserved in plants.[64, 65] Mutational analysis in *A. thaliana* has revealed that NMCP proteins participate in some nuclear functions mediated by lamins in metazoans, such as the regulation of nuclear size and shape.[63, 66] Although NMCPs do not share strong sequence similarity with lamins, their predicted structure and sub-nuclear distribution suggest that they may participate in the formation of the plant lamina. This observation, along with their demonstrated role in regulating nuclear shape and size, make these proteins good candidates to be lamin analogues in plants.

The NMCP protein family

The NMCP protein family has been characterized using bioinformatic and biochemical approaches, as well as with molecular biology tools. Members of this family share a high degree of sequence similarity and they have been identified in all land plants (Embryophytes) analyzed, including a moss (*Physcomitrella patens*) and vascular plants (Tracheophyte), although they appear to be absent from single cell plants (*Volvox carteri* and the

unicellular algue *Chlamydomonas reinhardtii*). However, these proteins are not conserved in metazoans, yeast or bacteria.[65] Based on sequence similarities, structural analogies and phylogenetic relationships, and in agreement with a prior study that analysed a small number of sequences, an exhaustive analysis of 97 sequences from 37 plant genomes (Supplementary table) recently classified NMCP proteins into two clusters: NMCP1 and NMCP2 (Fig.1).[64,65] All plants carry one *NMCP2* gene and while monocots have one *NMCP1* gene, dicots carry several *NMCP1* genes: one *NMCP1* gene and an additional gene encoding other NMCP1-related proteins named *NMCP3*. *A. thaliana* carries four genes, *LINC1-4*.[23, 63] *LINC1* is an orthologue of *NMCP1*, whereas *LINC2* and *LINC3* are classified as *NMCP3*-type genes and LINC4 is a NMCP2 protein (Fig.1).[64, 65] Like *A. thaliana*, some other dicots (*Dacus carota*, *Capsella rubella* and *Brassica rapa*) express two NMCP3-type proteins, while *Solanum tuberosum* and *Solanum lycopersicum* contain two NMCP1-type but no NMCP3-type proteins (Fig.1).

In vascular plants, NMCPs evolved from two genes: the *NMCP1* and *NMCP2* progenitors. The two *P. patens NMCPs* evolved from a common *NMCP* progenitor gene and are included in the *NMCP2* cluster, suggesting that the archetypal NMCP progenitor was an NMCP2 protein (Fig. 1).[65]

All *LINC* genes are expressed in whole *A. thaliana* plants.[66] The expression profile of *LINC1-4* genes show that they are co-expressed with genes encoding proteins involved in the cell cycle, DNA processing and transcription.[63] Microarray data from root tissues shows that *A. thaliana LINC1*, *LINC4* and *LINC3* are expressed most strongly, whereas *LINC2* is generally expressed more weakly. The expression of NMCP/LINC proteins is developmentally regulated. *LINC1* is strongly expressed in meristems, and less so

between the elongation and the differentiated root zones, correlating with the pattern of expression of LINC1-GFP from its native promoter.[67-70] The expression of *LINC2* and *LINC3* also decreases from the meristem to the differentiated zone, with a particularly steep decrease in *LINC2* expression between the meristematic and the elongation zones. The expression of *LINC4* decreases in the elongation zone but increases slightly again in the differentiated zone.[68-70] NMCP1 western blots and the distribution of LINC1-GFP protein support these results and demonstrate that NMCP1/LINC1 is abundant in both proliferating and quiescent meristems, although it accumulates in much smaller amounts in the cells of the mature zone.[65, 67] The expression profile of NMCP1/LINC1 resembles that of lamin B1, which is abundant in proliferating and quiescent meristematic cells but is weakly expressed in differentiated cells.[71-73]

NMCP proteins have a tripartite structure with a central coiled-coil rod domain, and non-coiled head and tail domains (Fig. 2).[57, 63-65] Most NMCPs contain two coiled coils, separated by a linker of about 20 residues, which form a central rod domain that is predicted to dimerize. Short linkers have also been predicted to reside inside the coiled-coil segments. The length of the rod domain is conserved in NMCPs, as are the positions of the linkers in the NMCP1 and NMCP2 proteins.[65] In addition, both termini of the NMCP coiled-coil domain are conserved in all NMCPs, suggesting that the structure of the rod domain is well conserved across the NMCP family and that it plays an important role in oligomerization. The NMCPs in *Physcomitrella patens* have a longer sequence than other NMCP proteins and they contain a long insert in the rod domain. This insertion results in a unique distribution of coiled-coils and altered linker positions in Ppa proteins (Ciska et al., unpublished).

The general organization of coiled coils in lamins and NMCPs is similar, although the rod domain of NMCPs is twice as long as that of lamins (Fig. 2). NMCPs exhibit a high degree of sequence similarity in the rod domain, which contains five highly conserved regions at each end and within the second coil, just before the second linker. Another conserved region includes the linker separating the two coils and it is conserved in all NMCPs except for those in *P. patens*.[65] Lamins exhibit a similar distribution of conserved motifs and those located at either end of the coiled-coil domain are prime candidates to mediate the head-to-tail associations.[32] The analogous structures, and the location of conserved motifs in the rod domain of NMCPs and lamins, suggest a similar mechanism of oligomerization and protofilament formation. This hypothesis is supported by the presence of consensus sequences recognized by cyclin dependent kinase (cdk1) and protein kinase (PKC)[74] at either side of the rod domain.

NMCPs also contain several highly conserved motifs in the less conserved tail domain, including a NLS and NMCP-specific regions.[65] A conserved region close to the NLS in NMCP1 proteins (R**YNLRR**), along with the NLS and the N-terminal region of the protein, is required for proper localization of the protein to the periphery of the nucleus in carrot (Masuda et al unpublished). This region also contains a 5 amino acid stretch that is identical to a specific region of lamin A (E**YNLRSR**T)[8] and that probably serves as an actin-binding site.[50] Point mutations in this sequence in lamins cause severe laminopathies, suggesting an important role for this actin-binding site in lamin A.[75] Like lamins, most NMCPs contain a predicted NLS in the tail domain, although the position and sequence is only conserved in NMCP1-type proteins. Few sequences lack a predicted NLS, but two such sequences (AgNMCP2 and DcNMCP2) localize to the nucleus, to which they are probably directed via an alternative pathway.[64]

NMCPs lack the C-terminal CAAX box typical of lamins, although the C-terminus of most members contains a highly conserved region that does not appear to be involved in NE association (Masuda et al unpublished). The C-terminal conserved region is present in NMCP1 clusters and in monocot NMCP2, although it is absent in dicot NMCP2,[64, 65] which coincides with the appearance of NMCP3 proteins and suggests that this new protein class fulfils some of the functions of NMCP2. This region is preceded by a stretch of acidic amino acids that is also found at the end of the tail domain of vertebrate lamins.[76]

While the predicted molecular weights of NMCPs from dicots and monocots are similar (130-140 kDa for NMCP1 and 110-120 kDa for NMCP2), the mobility of the endogenous proteins varies across species,[64, 65] probably due to post-translational modifications. In some monocots the MW of the endogenous proteins is lower than predicted, suggesting the involvement of alternative splicing or proteolytic cleavage. It is also possible that the differences in NMCP size are also present at the transcript level. NMCP genes may encode multiple transcripts and while an NMCP1 protein in *Sorghum bicolor* (Sbi04g030240.1) is predicted to contain 1,156 amino acids, for example, a protein product of the alternative transcript is predicted to lack 134 C-terminal amino acids (Sbi04g030240.3).

Like lamins, NMCP proteins are predominantly distributed in the nuclear lamina, although they are also found in the nucleoplasm, as demonstrated by immunofluorescence confocal microscopy, immuno-TEM and the expression of YFP/GFP-LINC proteins. These observations suggest that the proteins are not only involved in nuclear functions associated with the lamina but also, in those mediated by internal lamins such as transcription,

cell cycle progression, differentiation and chromatin organization.[35] A predominant distribution at the nuclear periphery has been reported for carrot and celery NMCP1 and NMCP2,[57, 64] and *A. thaliana* LINC1 and LINC4.[63, 66] *A. thaliana* LINC2 and LINC3 are exclusively nucleoplasmic,[63, 66] while onion NMCP display both an internal and peripheral localization.[65] The mechanisms that direct the proteins to these nuclear compartments are not yet understood. A very recent study demonstrated that the localization of carrot NMCP1 to the nuclear periphery involves a N-terminal 141 amino acid stretch, the conserved motif R/Q/HYNLRR/H and the NLS (Masuda, unpublished data). Nonetheless, the interactions with other proteins necessary for proper localization remain unknown. While immuno-TEM has demonstrated the presence of onion NMCP1 in the NE in close proximity to NPCs,[65] feSEM experiments will be necessary to unequivocally confirm that this protein is a component of the filaments that form the plant lamina.[21]

As demonstrated by immunofluorescence, the interphase distribution of NMCP1 in onion varies in different root cell populations, as does its expression. AcNMCP1 is regularly distributed along the nuclear envelope in meristematic cells, while its distribution in this structure in differentiated cells is discontinuous, with areas depleted of protein. A similar distribution has been reported for *Ce*-lamin in aging cells of *C. elegans*.[77] In quiescent meristematic nuclei the protein accumulates in a punctuate pattern in the nucleoplasm that may reflect sites of stored protein ready for early activation during root germination, as described for similar quiescent structures containing packed nuclear ribonucleoproteins (RNPs) and actin.[78, 79] The detection of NMCP proteins in NSK fractions[18, 65, 66] confirms that these proteins are insoluble components of the peripheral lamina and that like lamins, they are present in a minor fraction in the internal NSK.

NMCPs associate to the NE during interphase but they have a distinct spatial and temporal distribution when this structure disassembles during mitosis.[62, 64, 66] All NMCPs disassemble in prometaphase after NE breakdown but they subsequently behave distinctly. NMCP1/LINC1 proteins accumulate on segregating chromosomes during anaphase, although carrot and celery NMCP1 first associate with the mitotic spindle, and then they are finally incorporated into the NE during telophase. NMCP2, and LINC4, LINC2 and LINC3 disperse throughout the cytoplasm, and they are then incorporated into the surface of the chromosome and NE envelope via different pathways and at different times during telophase. Accordingly, NMCP2 associates to cytoplasmic nuclear membrane-derived vesicles while LINC4 binds to punctuate structures in the cytoplasm before relocating to the chromosome surface during telophase.[62, 64, 66] The pathway responsible for the assembly of the NMCP1 and LINC1 proteins during mitosis differs to that of lamins, which do not associate to segregating chromosomes,[12] suggesting that NMCPs and lamins are subject to distinct assembly processes in the NE.

The functions of NMCPs remain largely unknown. Although quadruple *LINC* mutants are unviable, indicating that NMCP/LINC proteins participate in essential processes, single, double and even some triple mutants are viable.[24] The phenotypic effects of LINC mutations are not as severe as those caused by lamin mutations and they mainly involve plant dwarfism, as well as reduced cellular and nuclear size.[63, 66, 67] Furthermore, single mutants do not produce abnormal phenotypes at the whole plant level, indicating a degree of complementation between different LINC proteins.[63, 66]

Disrupting the cytoskeleton has no effect on plant nuclear morphology, indicating that it is maintained by intranuclear factors and not by the CSK.[66] While the underlying molecular

mechanisms are unknown, several studies have implicated NMCP/LINC proteins in the regulation of nuclear shape and size,[63, 66, 80] a role also played by lamins. LINC mutations result in a decrease in nuclear size and alterations in the shape of differentiated nuclei,[63, 66, 67] while nuclear size is increased by LINC4 overexpression.[66] Analyses of mutants have revealed that although all LINCs are involved in the regulation of nuclear shape and size to different extents, LINC1 and LINC4 play predominant non-redundant roles.[63, 66, 67] LINC1 is mainly expressed in meristematic tissues but it is required to achieve a differentiated nuclear shape and it has been proposed to participate in a key differentiation step after nuclear formation.[63, 67] The increase in nuclear size that occurs during seed germination is also dependent on LINC1 and LINC2 proteins.[80] Other nuclear proteins reported to affect nuclear shape in plants include the SUN domain proteins,[55, 81] the KASH domain WIP proteins[55] and Nup136, a functional homolog of animal Nup153.[59] Nup136 mutants produce a similar phenotype to that of *linc1linc2* mutants, suggesting that plant SUNs, WIPs and Nup136 interact with NMCP/LINC proteins and act in concert to regulate nuclear morphology, similar to the way in which animal lamins interact with SUN proteins and Nup153 to regulate nuclear shape.[12]

The role of NMCP proteins in chromatin organization remains unclear. NMCP proteins do not affect DNA content as all *linc* mutants have normal ploidy levels,[63, 66] although double *linc1linc2* mutations affect the organization of heterochromatin, as witnessed by a significant decrease in the number of chromocentres, probably due to coalescence.[63] Nevertheless, similar changes in the relative heterochromatin fraction and the distribution of heterochromatic regions (a centromeric 180 bp repeat, pericentromeric subtelomeric 45s rDNA repeats and pericentromeric sequences) are observed during germination in *linc1/linc2* mutants and in the wild types,[80, 82] indicating that LINC1 and LINC2

proteins do not participate in the control of heterochromatin compaction. Functional LINC3 and LINC4 proteins may complement the loss of some NMCP functions, and thus, the involvement of LINC proteins in these functions cannot be completely ruled out. Further analysis of the nuclear organization in mutants, including those carrying mutations in other *LINC/NMCP* genes, is required to verify the role of NMCP proteins in chromatin organization.

A key function of lamins in animals is to regulate nuclear positioning and movement, processes that are mediated by the interaction of type A lamins with SUN and KASH proteins to form the LINC complex, and which requires the Samp1 protein to stabilize the binding of SUNs to lamins, as well as cytoplasmic actin.[83] In plants, nuclear movement in response to blue light is mediated by phototropin2 and it involves thick actin filaments that associate to the nucleus.[84] However, the nuclear components involved in this interaction remain unknown. Analyses of single and double *linc1/4* and *linc2/3* mutants have ruled out a role of NMCP proteins in blue light-induced nuclear movement and positioning, although protein complementation cannot be completely discounted.[66] These results, along with the lack of nesprin conservation reported (the main KASH domain proteins in vertebrates),[22] and the revelation that the SUN and KASH protein WIP are not required for nuclear movement during root and leaf hair elongation,[55, 81] strongly suggest that the organization of the bridges between the NSK and CSK, as well as the mechanisms regulating nuclear movement in plants, differ from those of animals.

Analysis of the binding partners of NMCPs will contribute to our understanding of their functions, as well as to the composition and organization of the protein networks that form the NE and NSK in plants. As discussed above, the functional analysis of mutants

suggests that NMCPs interact with some proteins whose metazoan analogues are lamin-binding proteins,[27] such as SUN[55, 81] and Nup136.[59] As yet, no NMCP partners have been unequivocally identified, although preliminary results suggest that NMCP and SUN proteins may interact.[24] The identification of NMCP-binding proteins in the INM, and NPCs such as SUN proteins, NUA and actin, would explain the association of NMCPs to these structures and is an interesting issue that deserves further investigation.

Future directions and perspectives

Although NMCPs have no sequence similarity with lamins, the two proteins share many other features including their structural organization, sub-nuclear distribution, expression and function (see Table I), making NMCPs the best candidates to perform the functions of lamins in plants. Further research is required to unequivocally demonstrate that NMCPs are plant lamin analogues, rather than structural components of the plant lamina, and to unravel their functions in the plant NE and nucleus. Key issues to be addressed in future studies include: 1) the localization of NMCPs in the filaments of the lamina by immuno-feSEM; 2) the polymerization of NMCPs *in vitro*; and the characterization of 3) NMCP binding partners and 4) the functions of NMCPs in the nucleus and cell, as may be determined using multiple mutants.

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Figure Legends

Figure 1

Evolutionary relationships of NMCP proteins.

Sequences classified as NMCP1 (NMCP1 and NMCP3) are marked in red and those classified as NMCP2 in green. The two proteins of *Physcomitrella patens* are in blue. Dicotyledon species are represented by cian rhombi, monocotyledons by yellow triangles and mosses by blue circles.

Figure 2

Comparison of the structure of NMCP proteins and lamins.

Both have a tripartite structure with a central coiled coil domain (orange boxes) flanked by cdk1 phosphorylation sites and a tail domain with an NLS (green boxes) and a conserved C-terminus (blue box in the case of the NMCPs and CaaX in the case of lamins). NMCP proteins lack the IgG fold typical of lamins (blue oval).

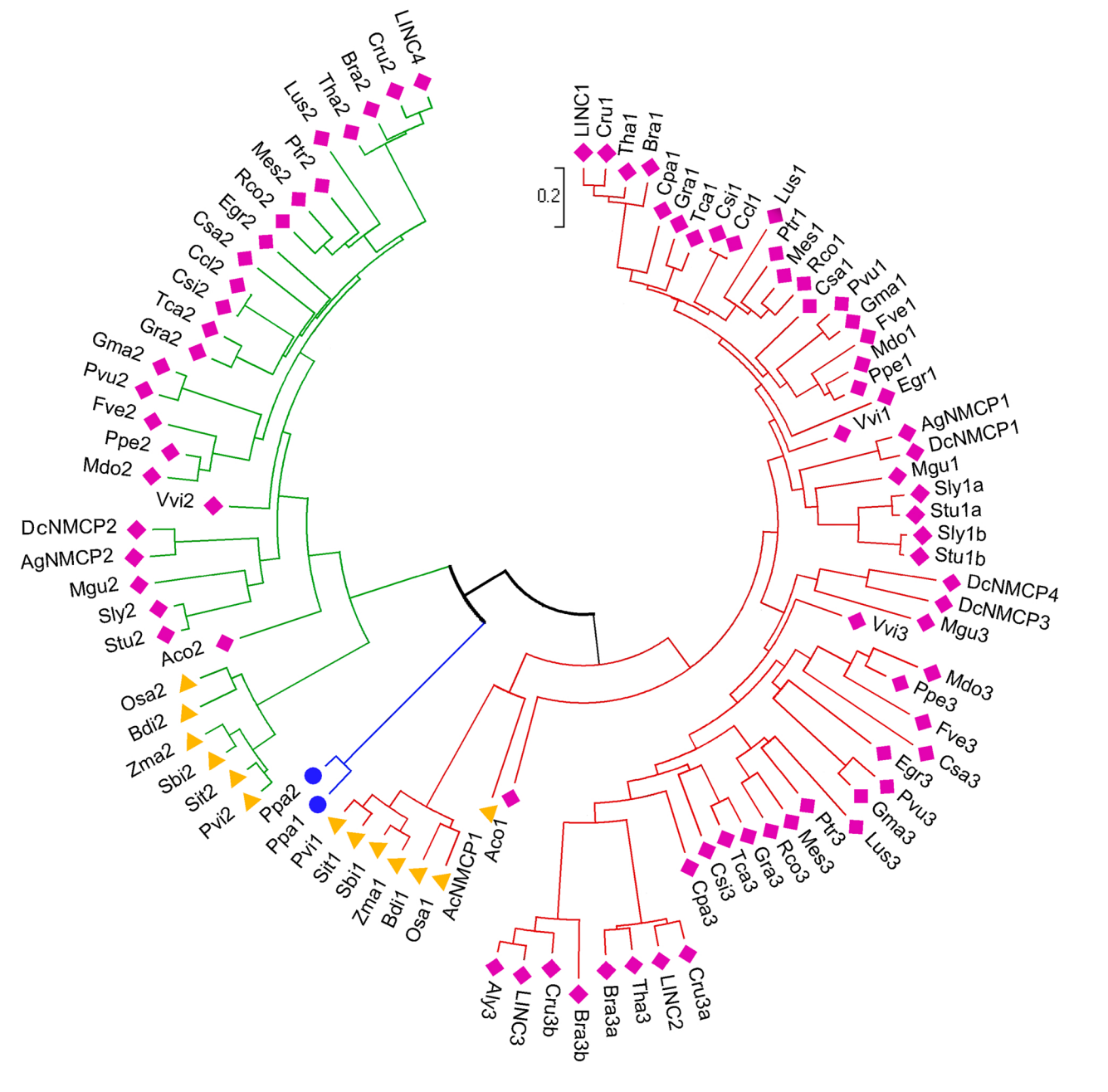
Table 1

Main features of NMCP proteins and lamins.

Similar characteristics are denoted in blue and different ones in red.

Supplementary Table.

Sequence accession data.


LINC4
Cru2
Bra2
Tha2
Lus2
Ptr2
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Rco2
Egr2
Csa2
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Tca2
Gra2
Gma2
Pvu2
Fve2
Ppe2
Mdo2
Vvi2
DcNMCP2
AgNMCP2
Mgu2
Sly2
Stu2
Aco2
Osa2
Bdi2
Zma2
Sbi2
Sit2
Pvi2
Ppa2
Ppa1
Pvi1
Sit1
Sbi1
Zma1
Bdi1
Osa1
AcNMCP1
Aco1
Aly3
LINC3
Cru3b
Bra3b
Bra3a
Tha3
LINC2
Cru3a
Cpa3
Csi3
Tca3
Gra3
Rco3
Mes3
Ptr3
Lus3
Gma3
Pvu3
Egr3
Csa3
Fve3
Ppe3
Mdo3
Mgu3
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DcNMCP4
Vvi3
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Stu1a
Sly1a
Mgu1
DcNMCP1
AgNMCP1
Vvi1
Egr1
Ppe1
Mdo1
Fve1
Gma1
Pvu1
Csa1
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Bra1
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Cru1
LINC1
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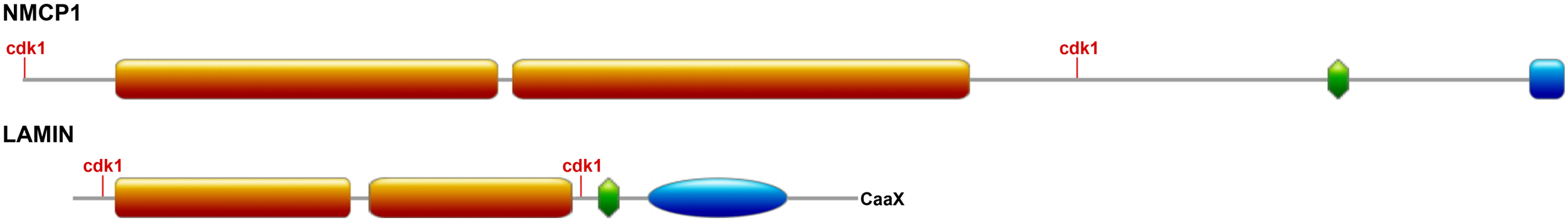
NMCP1
cdk1
cdk1
LAMIN
cdk1
cdk1
CaaX

	NMCPs	lamins
Types	**NMCP1** (monocots one; dicots two-three genes) **NMCP2** (one gene)	**B-type** (invertebrates one; vertebrates two-three genes) **A-type** (one gene in vertebrates and in few invertebrates)
Structure	Central coiled-coil rod domain, head and tail Rod domain flanked by cdk1 sites NLS in the tail domain	Central coiled-coil rod domain, head and tail Rod domain flanked by cdk1 sites NLS in the tail domain
Conserved regions	Extremes of rod domain, linkers and C-terminus	Extremes of rod domain, linkers and C-terminus
Polimerization state	Predicted to dimerize	Dimerize and form filaments
MW	Variable (70-200 kDa)	65-74 kDa
pI	Acidic	Acidic (B-type lamins); neutral (A-type)
Solubility	NSK component	NSK component
Subcellular localization	Lamina, nucleoplasm	Lamina, nucleoplasm
Functions	Nuclear shape and size Chromatin organization (?) (decreased number of chromocenters but the distribution of heterochromatic regions not changed) Not involved in light induced nuclear movement	Nuclear shape and architecture Chromatin organization and positioning Connecting NSK and CSK DNA replication, repair and transcription Cell proliferation and differentiation
Genes	Min. two in most plants	Min. three in vertebrates, one in most invertebrates
Where?	Multicellular land plants	metazoan

NAME	SPECIES	GENE ID	SOURCE (GENOME PROJECT)
Aly3	*Arabidopsis lyrata*	gene 476006	JGI release v1.0
Lus1	*Linum usitatissimum*	gene Lus10003075.g	BGI v1.0 on assembly v1.0
Lus2	*Linum usitatissimum*	gene Lus10019257.g	
Lus3	*Linum usitatissimum*	gene Lus10034383.g	
Pvu1	*Phaseolus vulgaris*	gene Phvulv091023539m.g	JGI annotation v1.0 on assembly v1.0 using published ESTs, and JGI RNAseq
Pvu2	*Phaseolus vulgaris*	gene Phvulv091014376m.g	
Pvu3	*Phaseolus vulgaris*	gene Phvulv091022727m.g	
Mdo2	*Malus domestica*	gene MDP0000322171	GDR prediction v1.0 on Malus x domestica assembly v1.0
Mdo3	*Malus domestica*	gene MDP0000208604	
Mdo1	*Malus domestica*	gene MDP0000312257	
Cru3a	*Capsella rubella*	gene Carubv10011605m.g	JGI annotation v1.0 on assembly v1
Cru3b	*Capsella rubella*	gene Carubv10019698m	
Cru1	*Capsella rubella*	gene Carubv10019693m.g	
Cru2	*Capsella rubella*	gene Carubv10025809m.g	
Bra1	*Brassica rapa*	gene Bra034012	Annotation v1.2 on assembly v1.1 from brassicadb.org
Bra3b	*Brassica rapa*	gene Bra038353	
Bra3a	*Brassica rapa*	gene Bra019819	
Bra2	*Brassica rapa*	gene Bra037827	
Tha3	*Thellungiella halophila*	gene Thhalv10006601m.g	JGI annotation v1.0 on assembly v1
Tha2	*Thellungiella halophila*	gene Thhalv10003578m.g	
Tha1	*Thellungiella halophila*	gene Thhalv10018034m.g	
Mes1	*Manihot esculenta*	gene cassava4.1_000510m.g	Assembly version 4, JGI annotation v4.1
Mes3	*Manihot esculenta*	gene cassava4.1_000491m.g	
Mes2	*Manihot esculenta*	gene cassava4.1_000625m.g	
Rco1	*Ricinus communis*	gene 29673.t000017	TIGR release 0.1
Rco3	*Ricinus communis*	gene 29738.t000030	
Rco2	*Ricinus communis*	gene 29825.t000006	
Ptr1	*Populus trichocarpa*	gene Potri.017G111400	JGI assembly release v3.0, annotation v3.0
Ptr3	*Populus trichocarpa*	gene Potri.008G114800	
Ptr2	*Populus trichocarpa*	gene Potri.012G034300	
Gma1	*Glycine max*	gene Glyma18g51560	JGI Glyma1.1 annotation of the chromosome-based Glyma1 assembly
Gma3	*Glycine max*	gene Glyma02g11335	
Gma2	*Glycine max*	gene Glyma05g23100	
Csa1	*Cucumis sativus*	gene Cucsa.238180	Roche 454-XLR assembly and JGI v1.0 annotation
Csa3	*Cucumis sativus*	gene Cucsa.280830	
Csa2	*Cucumis sativus*	gene Cucsa.103490	
Ppe1	*Prunus persica*	gene ppa000399m.g	JGI release v1.0
Ppe3	*Prunus persica*	gene ppa000415m.g	
Ppe2	*Prunus persica*	gene ppa016288m.g	
Cpa1	*Carica papaya*	gene evm.TU.supercontig_129.57	ASGPB release of 2007
Cpa3	*Carica papaya*	gene evm.TU.supercontig_1.235	
Csi1	*Citrus sinensis*	gene orange1.1g048767m.g	JGI v1.1 annotation on v1 assembly
Csi3	*Citrus sinensis*	gene orange1.1g000847m.g	
Csi2	*Citrus sinensis*	gene orange1.1g001119m.g	
Ccl1	*Citrus clementina*	gene Ciclev10013467m.g	JGI v1 assembly and v1.0 annotation
Ccl2	*Citrus clementina*	gene Ciclev10024751m.g	
Egr1	*Eucalyptus grandis*	gene Eucgr.J01462	JGI assembly v1.0, annotation v1.1
Egr3	*Eucalyptus grandis*	gene Eucgr.G02361	
Egr2	*Eucalyptus grandis*	gene Eucgr.J00661	
Vvi1	*Vitis vinifera*	gene GSVIVG01031076001	March 2010 12X assembly and annotation from Genoscope
Vvi3	*Vitis vinifera*	gene GSVIVG01011972001	
Vvi2	*Vitis vinifera*	gene GSVIVG01007428001	
Mgu1	*Mimulus guttatus*	gene mgv1a000432m.g	JGI 7x assembly release v1.0 of strain IM62, annotation v1.1
Mgu3	*Mimulus guttatus*	gene mgv1a000453m.g	
Mgu2	*Mimulus guttatus*	gene mgv1a000959m.g	
Aco1	*Aquilegia coerulea*	geneAcoGoldSmith_v1.000268m.g	JGI 8X assembly v1.0, annotation v1.2a
Aco2	*Aquilegia coerulea*	geneAcoGoldSmith_v1.019728m.g	
Sbi1	*Sorghum bicolor*	gene Sb04g030240	Sbi1.4 models from MIPS/PASA on v1.0 assembly
Sbi2	*Sorghum bicolor*	gene Sb03g035670	
Zma1	*Zea mays*	gene GRMZM2G015875	5b.60 annotation (filtered set) of the maize "B73" genome v2 produced by the Maize Genome Project
Zma2	*Zea mays*	gene GRMZM2G320013	
Sit1	*Setaria italica*	gene Si016142m.g	JGI 8.3X chromosome-scale assembly release 2.0, annotation version 2.1
Sit2	*Setaria italica*	gene Si000171m.g	
Osa1	*Oryza sativa*	gene LOC_Os02g48010	MSU Release 7.0 of the Rice Genome Annotation
Osa2	*Oryza sativa*	gene LOC_Os01g56140	
Bdi1	*Brachypodium distachyon*	gene	JGI 8x assembly release v1.0 of strain Bd21 with JGI/MIPS PASA annotation v1.2
Bdi2	*Brachypodium distachyon*	gene Bradi2g50990	
Ppa1	*Physcomitrella patens*	gene Pp1s76_81V6	JGI assembly release v1.1 and COSMOSS annotation v1.6
Ppa2	*Physcomitrella patens*	gene Pp1s200_64V6	
Fve1	*Fragaria vesca*	gene 10337-v1.0-hybrid	v1.1 assembly and v1.0 annotation from Shulaev et. al., hosted at GDR
Fve3	*Fragaria vesca*	gene 03889-v1.0-hybrid	
Fve2	*Fragaria vesca*	gene 05584-v1.0-hybrid	
Gra1	*Gossypium raimondii*	gene Gorai.001G200600	JGI annotation v2.1 on assembly v2.0
Gra3	*Gossypium raimondii*	gene Gorai.008G187500	
Gra2	*Gossypium raimondii*	gene Gorai.007G227800	
Tca1	*Theobroma cacao*	gene Thecc1EG019537	D. Gilbert public gene set 8 Mar 2012 on assembly v1.1
Tca3	*Theobroma cacao*	gene Thecc1EG011885	
Tca2	*Theobroma cacao*	gene Thecc1EG000864	
Stu1a	*Solanum tuberosum*	gene PGSC0003DMG402010047	DM1-3 516R44 (CIP801092) Genome Annotation v3.4 mapped to pseudomolecule sequence
Stu1b		gene PGSC0003DMG400003363	
Stu2		gene PGSC0003DMG400021400	
Sly1a	*Solanum lycopersicum*	gene Solyc02g089800.2	SGNTomato Genome Project ITAG2.3
Sly1b		gene Solyc03g045050.2	
Sly2		gene Solyc02g091960.2	
Pvi1	*Panicum virgatum*	gene Pavirv00005279m.g	JGI v0.0 annotation on assembly v0
Pvi2		gene Pavirv00069263m.g	
AtNMCP1/LINC1	*Arabidopsis thaliana*	At1g67230	TAIR release 10 acquired from TAIR
AtNMCP2/LINC2	*Arabidopsis thaliana*	At1g13220	
AtNMCP3/LINC3	*Arabidopsis thaliana*	At1g68790	
AtNMCP4/LINC4	*Arabidopsis thaliana*	At5g65770	
NAME	SPECIES	ACCESSION NUMBER	
DcNMCP1	*Daucus carota*	D64087.1	GenBank/EMBL/DDBJ
DcNMCP2	*Daucus carota*	AB514509.1	GenBank/EMBL/DDBJ
AgNMCP1	*Apium graveolens*	AB514506.1	GenBank/EMBL/DDBJ
AgNMCP2	*Apium graveolens*	AB514507.1	GenBank/EMBL/DDBJ
LINC1	*Arabidopsis thaliana*	NM_105392.4	GenBank/EMBL/DDBJ
LINC2	*Arabidopsis thaliana*	NM_101194.2	GenBank/EMBL/DDBJ
LINC3	*Arabidopsis thaliana*	NM_105552.2	GenBank/EMBL/DDBJ
LINC4	*Arabidopsis thaliana*	NM_125974.5	GenBank/EMBL/DDBJ
OsNMCP1	*Oryza sativa*	AB110204.1	GenBank/EMBL/DDBJ
OsNMCP2	*Oryza sativa*	AB110205.1	GenBank/EMBL/DDBJ