

Actin's many actions start at the genes

Werner W. Franke

Over more than three decades, sporadic and circumstantial evidence has accumulated to suggest that nuclear actin has crucial functions in RNA polymerase II-based transcription. Now, using a biochemical approach, β -actin has been identified as a highly specific, constitutive component of the active transcriptional complex required for formation of the pre-initiation transcription complex.

For more than three decades it was considered cell-biological heresy to suggest that actin might exist and function in the nucleus. Actin was understood to mean filaments, cables, contraction, motility and so on — all cytoplasmic functions for actin; the nucleus was off limits; after all, in immunolocalization experiments actin was demonstrably absent from the nucleus, and in fact, it was often used as a negative control. So, not surprisingly, authors who dared to report results indicating that this famous structural protein also exists in the nucleus, mostly in a soluble form, and — even more heretical — suggest that it might be involved in classic nuclear functions such as transcription, processing of transcripts and ribonucleoprotein transport to the cytoplasm, had a difficult time. Nuclear actin was also a notorious grant proposal poison. Nevertheless, at intervals there were isolated reports on ‘the nuclear actin problem’, as it was often called, and one prominent developmental biologist classified it as a typical “axolotl”: strange and interesting, but never metamorphosing into a mature entity.

The idea that actin may be in the nucleus and may be involved in transcription emerged in 1969 as a possible explanation for the observation¹ that inhibition of transcription in amphibian oocytes by actinomycin D resulted in the formation of intranuclear bundles of filaments resembling cytoplasmic microfilaments. This observation was then followed by reports showing not only the presence of actin in isolated nuclei but also the association of

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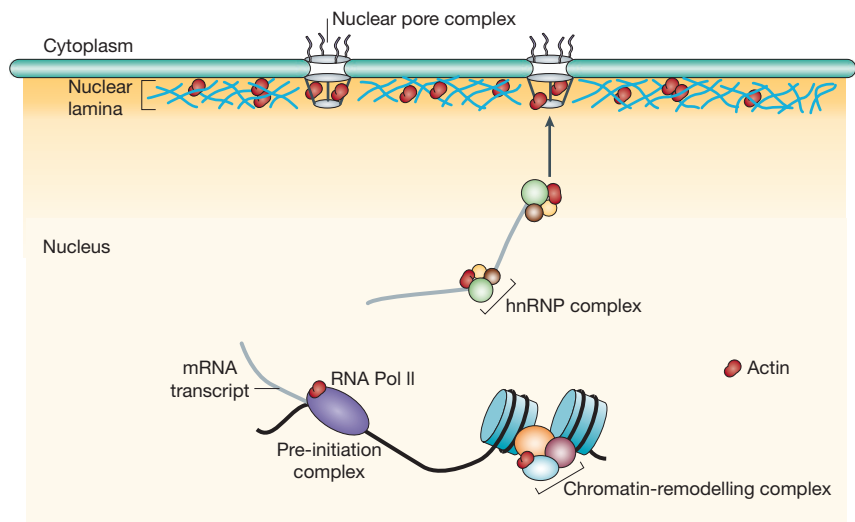


Figure 1 Nuclear functions for actin. β -actin is required for formation of the RNA Pol II pre-initiation complex, but it is unclear whether in this context actin functions as a monomer, short filament or a novel oligomeric form¹². Actin has also been suggested to have structural or regulatory roles with chromatin remodelling complexes, and to associate with mRNA transcripts through interactions with heterogenous ribonucleoprotein (hnRNP) complexes. At the nuclear pore complex, actin may participate in export of the mRNA transcripts. Finally, actin may also have a role in establishing or maintaining nuclear structure through interactions with the nuclear lamina. This figure was modified and reproduced with permission from ref. 10., © (2004) Macmillan Publishers Ltd.

actin with RNA polymerase II during purification^{2,3}, which was supported and extended by the *in vivo* and *in vitro* experiments of others^{4,5}. Only slowly did the presence of sizeable amounts of actin, actin-related-, actin-binding- and actin-regulatory proteins in the nucleus become accepted facts^{6,7}. More recently, however, cell biologists have become more open-minded — considering the possibility that actin is also involved in diverse

nuclear functions, including transcription, ribonucleoprotein packaging and transport, chromatin remodelling and the formation of karyoskeletal elements^{8–11} (Fig. 1).

Now finally, Hofmann *et al.* report on page 1094 of this issue¹² the results of a comprehensive study of a consortium of a dozen scientists from five institutions, led by Primal de Lanerolle, which leaves little doubt that at least one member of the actin family

of proteins is indeed needed as a constitutive and functionally crucial component of the RNA polymerase II-based transcriptional machinery. These authors conclude that it is exclusively β -actin, the non-muscle isoform that is involved in this process. However, their finding that all other isoforms, including γ -actin, another non-muscle actin, and the four muscle-specific actins, are nearly inactive in their transcription assay, is remarkable in view of the few amino-acid exchanges between these diverse actins. After all, γ -actin differs only in four amino acids clustered at the amino terminus, and all four exchanges are conservative in nature.

The central aspect of this report is a series of biochemical experiments, using HeLa cells to demonstrate that it is the β -actin isoform that selectively associates with RNA polymerase II *in vitro* and *in vivo*, and that only the β -actin-specific antibodies inhibit transcriptional activity in assays using purified RNA polymerase II and pure transcription factors. The authors report that the addition of recombinant β -actin can markedly stimulate transcription. The actin-containing transcription complex associates with the gene promoter in a manner that is strictly dependent on the induction of gene expression. The assembly and activity of this complex does not depend on chromatin remodelling, an activity that itself depends on actin. They also demonstrate that the formation of the initiation complex and the continuation of transcription is absolutely dependent on β -actin. In particular, Hofmann et al. emphasize a requirement for actin in the formation of the pre-initiation and the TATA-box complexes. Co-immunoprecipitation experiments identified a specific complex containing β -actin, RNA polymerase II and the TATA-box-binding protein.

The report by Hofmann *et al.*¹² is highly provocative in several ways. Certainly, the specificity claim — that a single isoform of non-muscle β -actin is involved — will be challenged by assays using alternative isoforms and mutational analyses¹³. Moreover, this claim also raises the more general question of whether and how the four muscle-type actins are excluded from the transcriptional process or possibly from the nucleus in the diverse cells of myogenic lineage. After all, nuclear actin that is recruited to the transcriptional complex represents only a minute fraction of nuclear actin¹⁴, and the mechanism by which transcriptionally involved actin molecules could be sorted from the bulk of nuclear actin is at present an enigma.

The present paper¹² has to be seen in the wider perspective, of recent reports that actin, accompanied by myosin, is an essential constituent of the RNA polymerase I complex responsible for the nucleolar transcription of rDNA¹⁵. Here too, actin is needed throughout the entire process, from initiation to the final release of the large RNA polymerase–pre-rRNP complex (I. Grummt, personal communication). And to complete the trio, a role for β -actin has concurrently been found in RNA polymerase III transcription¹⁶. Certainly, examination of possible requirements of other cytoskeletal proteins in the regulation of RNA polymerases is now generally mandatory, given that transient nucleoplasmic storage particles of RNA polymerase III contain plakophilin-2, a ‘hard-core’ cytoskeletal protein originally identified in desmosomal plaques¹⁷.

Finally, much remains to be learnt about how actin complexes with the RNA polymerase and how this complex is stored and disassembled. In this regard, the Cajal body

— the main nuclear substructure known as the assembly site and transitory stockpile of all three types of RNA polymerase complexes — is obviously of special interest¹⁴. Remarkably, these prominent nuclear bodies also harbour certain actin-binding proteins, such as profilin¹⁸ — a protein involved in the regulation of actin dynamics. Most of what we have learnt in recent years about the functions of actin in the cytoplasm has come from the study of the functions of its partners, and it does not need a prophecy to predict that this approach will also be successful in the elucidation of the nuclear functions of actin. □

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MRL proteins: Leading Ena/VASP to Ras GTPases

John A. Legg and Laura M. Machesky

The MRL protein family is emerging as a new connection between signalling, adhesion and cell motility. Two members, lamellipodin and RIAM, can regulate both actin assembly and cell adhesion, which raises many questions about how their interactions with Ena/VASP proteins, Ras-related GTPases and membranes orchestrate these key processes.

Signals from the environment are connected to cellular shape and movement through large protein assemblies at the plasma membrane that link to the actin cytoskeleton. This includes regulation of proteins such as the Ena/VASP protein family and the Arp2/3 complex that directly control actin polymerization. Both of these sets of proteins regulate cell protrusions termed lamellipodia, which are important for driving the leading edge of cells and enabling them to move and modulate their shape. In the October issue of *Developmental Cell*, Lafuente *et al.* and Krause *et al.* introduce a new family of proteins that contain membrane-association domains, signalling domains and Ena/VASP-binding sequences and which probably function as signal adaptors to modulate the actin cytoskeleton^{1,2}. The two proteins studied, Lamellipodin and RIAM (Rap1-GTP-interacting adaptor molecule, also known as RARP1, for retinoic acid response proline-rich protein 1; ref. 3) have a common domain structure, but seem to have distinct functions in signalling, motility and adhesion.

The Ena/VASP proteins (in mammals, Mena, VASP and EVL) are defined structurally by a proline-rich core domain, which is important for interaction with the small actin-binding protein profilin, flanked by amino-terminal EVH1 and carboxy-terminal EVH2 (Ena/VASP homology 1 and 2) domains. The EVH1 domain recognises the consensus (D/E)-FPPPP-X(D/E)(D/E) sequence and is important for the localization of Ena/VASP proteins to focal adhesion complexes. The EVH2 domain binds to both G- and F-actin and is thought to be important for elongation of actin filaments. To discover new potential EVH1-domain-binding proteins, Krause *et al.* screened databases for proteins that contain FPPPP motifs. Through this approach, they identified Lamellipodin, so named for its ability to colocalize with the Ena/VASP proteins in lamellipodia and

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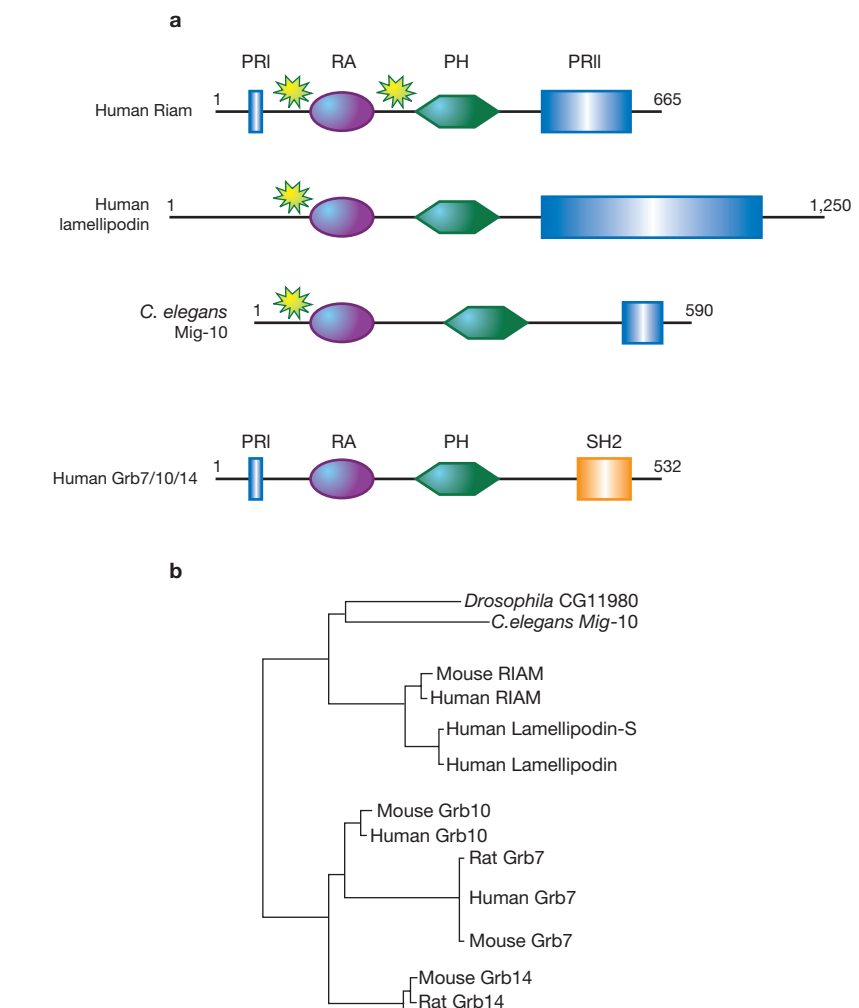


Figure 1 RIAM and Lamellipodin are part of the MRL protein family (Mig10/RIAM/lamellipodin). (a) RIAM, Lamellipodin and Mig-10 share a conserved domain structure and are related to the Grb7, Grb10 and Grb14 adaptor proteins. PR1 and PR2, proline-rich domains; RA, Ras-association domain; PH, pleckstrin-homology domain; SH2, Src-homology-2 domain; yellow star, putative coiled-coil domain. (b) Dendrogram of the MRL and Grb7 full-length proteins.

filopodia. Closer examination of the sequence revealed that Lamellipodin is a homologue of the *Caenorhabditis elegans* Mig10 protein.

In a separate screen, Lafuente *et al.* used a yeast two-hybrid screen to identify new binding proteins for the small GTPase Rap1a. Using a constitutively active Rap1E63 mutant as bait, they identified a potential interacting protein and named it RIAM. RIAM also

seemed to have the potential for cytoskeletal interactions: it contains six putative profilin-binding sites and six FPPPP motifs. They proceeded to show that RIAM could indeed bind Ena/VASP proteins, both *in vitro* and *in vivo*.

Lamellipodin, RIAM and Mig-10 share numerous structural properties and are related to the Grb7 adaptor proteins (Fig. 1). Owing to the similarity to Mig-10, Lafuente

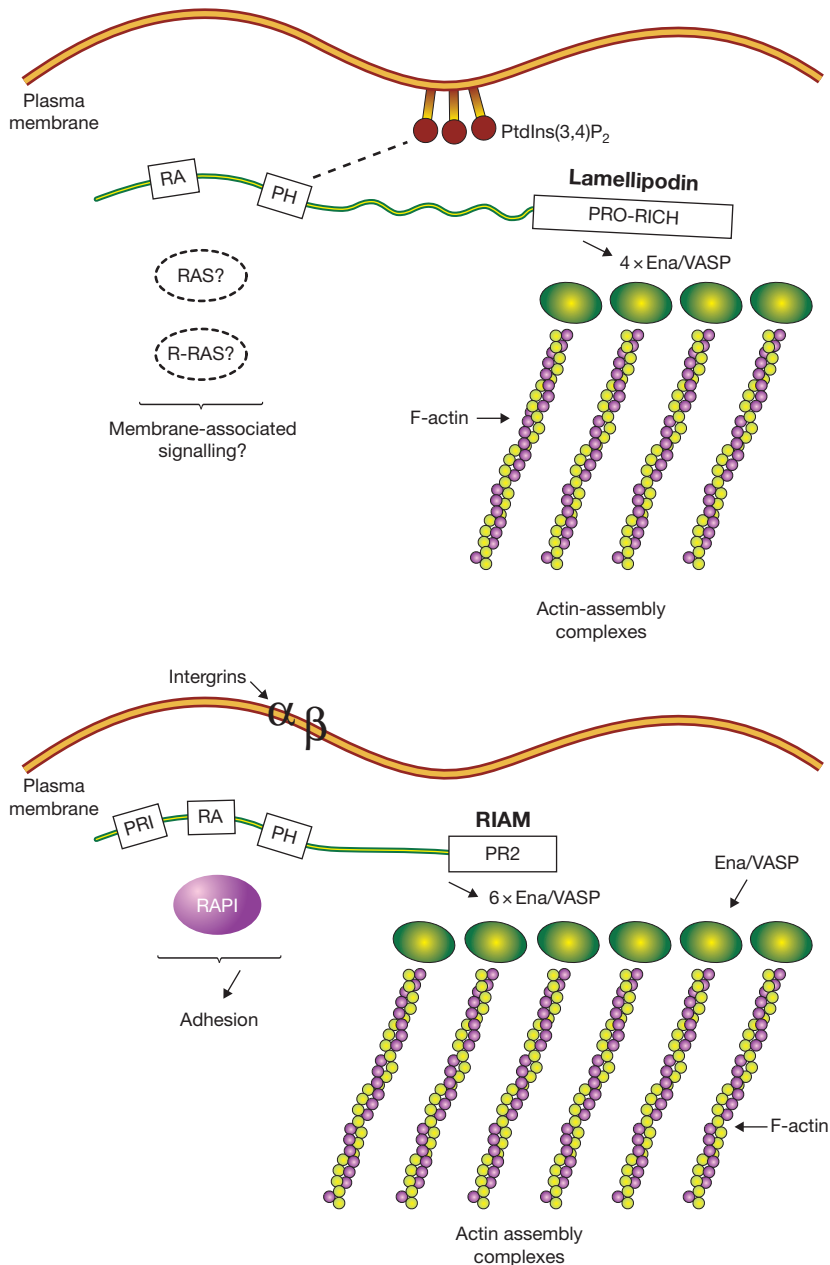


Figure 2 Comparison of the domains and putative activities of Lamellipodin and RIAM at the plasma membrane. Lamellipodin contains an RA (Ras-association) domain that may associate with Ras or R-Ras GTPases, a PH (pleckstrin homology) domain that associates preferentially with PtdIns(3,4)P₂ and up to four binding domains for Ena/VASP proteins. Lpd seems to be crucial for Ena/VASP localization at the plasma membrane and for the dynamics of lamellipodial actin assembly. By contrast, the RA domain of RIAM associates with Rap1 and the PH domain has not been characterized. Association with Rap1 mediates β-1 and β-2 integrin adhesion. The C-terminal portion of RIAM contains several proline-rich motifs that could bind up to 6 Ena/VASP molecules and possibly link actin assembly to adhesion.

et al. have proposed naming the RIAM-related adaptor molecules the 'MRL family' (Mig10/RIAM/Lpd family). The MRL family contain pleckstrin-homology (PH) and Ras-association (RA) domains along with a more

divergent proline-rich C terminus, which contains the potential profilin- and Ena/VASP-binding sites. All three proteins also contain a conserved patch of 27 residues that are predicted to form a coiled-coil region. Thus

these proteins are likely to have both structural and functional similarities, although the binding specificities of each of these domains may be unique.

In both studies, the roles of RIAM and Lamellipodin *in vivo* were further investigated using overexpression and knockdown experiments. When lamellipodia are deficient for Ena/VASP proteins, these structures protrude slower, but more persistently, leading to increased cell translocation rates⁴. Conversely, when Ena/VASP are overexpressed, lamellipodia contain longer, less branched filaments. Krause *et al.* found that when Lamellipodin is overexpressed the resulting phenotype is similar to Ena/VASP overexpression and that this phenotype can be suppressed by blocking Ena/VASP function. Knockdown of Lamellipodin with short-hairpin RNA showed severe lamellipodia and F-actin depletion, suggesting that Lamellipodin might signal to actin regulatory proteins.

Lafuente *et al.* showed that overexpressed RIAM colocalized with actin at the leading edge of the cell, consistent with the localization of Ena/VASP proteins. In Jurkat T cells, RIAM overexpression had two effects: it led to the formation of extensive lamellipodia and caused increased β1 and β2 integrin-mediated adhesion. This latter phenotype was particularly important given that the authors originally found RIAM based on its ability to bind to Rap1, which controls adhesion by β1 and β2 integrins. Rap1, the closest relative to Ras, is a small GTPase that is activated through numerous receptors, including receptor tyrosine kinases, cytokine receptors, cell adhesion molecules and heterotrimeric G-protein coupled receptors (reviewed in ref. 5). Furthermore, both Rap1 and Rap2 are implicated in actin-based processes, such as cell spreading, the extension of membrane projections and integrin-mediated cell adhesion⁶. Lafuente *et al.* proceeded to test whether the interaction of RIAM with Ena/VASP proteins might also be relevant for adhesion. It was not: although knockdown of RIAM using shRNA showed that RIAM was necessary for Rap1-induced adhesion, disrupting the interaction of the Ena/VASP proteins with RIAM had no effect on the ability of RIAM to mediate adhesion. Thus, the effect of RIAM on adhesion is likely to be through Rap1 and not Ena/VASP.

The study from Krause *et al.* suggests that Lamellipodin can also affect adhesion. In cell adhesion assays, Lamellipodin had the opposite effect to RIAM, negatively regulating adhesion. Unlike RIAM, the RA domain of Lamellipodin does not seem to interact with Rap1; although in another study, it interacted with K-Ras, N-Ras, H-Ras and R-Ras-3

through this region⁷. As RA domains have differing specificities for small GTPases (ref. 7), further studies will be required to assess which small GTPases physiologically associate with RIAM and Lamellipodin and how this affects their cellular activities.

In addition to the RA domains, RIAM and Lamellipodin contain PH domains — protein modules of 100–120 residues that bind to phosphoinositides — which could be used for interactions with lipid membrane compartments and/or with proteins (Fig. 2). Indeed, Krause *et al.* provide evidence that the PH domain in Lamellipodin binds to phosphatidylinositol(3,4)bisphosphate (PtdIns(3,4)P₂). PtdIns(3,4)P₂ levels can be regulated by extracellular signals and this lipid is preferentially distributed at the plasma membrane and at the nuclear membrane, although its role in cell motility is unclear⁸. Because the PH domain in lamellipodin localizes to the plasma membrane, Krause *et al.* propose that the binding of PtdIns(3,4)P₂ to Lamellipodin could be a mechanism by which Ena/VASP proteins localize at the leading edge. No phosphoinositide has so far been found to bind to the PH domain of RIAM, although the combined interaction of a phosphoinositide at the

PH domain and Rap1 to the RA domain could in theory be important for the localization of RIAM. PH domains have also been implicated in GTPase activation; for example, the PH domain of ELMO binds to the Rac GEF Dock180 and stabilises the nucleotide-free state of Rac, thereby aiding the GEF activity of Dock180 (ref. 9). This trimeric complex is also implicated in phagocytosis and cell migration. Thus, one possibility is that the PH domain of RIAM and Lamellipodin might also cooperate with a GEF to activate GTPase(s) associated with their RA domains.

Previous studies have shown that the C-terminal EVH2 domain of Ena/VASP is sufficient to complement the loss of Ena/VASP function in random cell motility¹⁰ and that the EVH1 domain alone fails to mediate robust targeting of full-length Ena/VASP proteins to the leading edge. EVH1-domain binding to adhesion proteins, such as vinculin, has also been implicated in recruiting Ena/VASP proteins to focal adhesion complexes. Therefore, it will require further study to determine the importance of these interactions between the EVH1 domains of Lamellipodin or RIAM and the Ena/VASP proteins, as well as how these interactions affect the location and function

of these proteins in actin-based protrusions.

The identification of RIAM/RARP1 as a protein upregulated by ATRA (all-*trans* retinoic acid) differentiation of HL-60 cells³ adds to the mystery surrounding the function of MRL proteins. This study implicated RIAM in transcriptional regulation and possibly the differentiation of myeloid cells, including T-cells and megakaryocytes/platelets. Whether the other MRL proteins are also connected to differentiation and transcriptional regulation remains to be determined and understanding how this might tie in with their actin cytoskeletal functions will be equally important. □

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