Diverse functions for different forms of nuclear actin

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Abstract

In addition to its essential roles as part of the cytoskeleton, actin has also been linked to many processes in the nucleus. Recent data has demonstrated the presence of both monomeric and polymeric actin in the nucleus, and implied distinct functional roles for these actin pools. Monomeric actin seems to be involved in regulation of gene expression through transcription factors, chromatin regulating complexes and RNA polymerases. In addition to cytoplasmic actin regulators, nuclear proteins, such as emerin, can regulate actin polymerization properties specifically in this compartment. Besides of structural roles, nuclear actin filaments may be required for organizing the nuclear contents and for the maintenance of genomic integrity.

Introduction

Actin is a globular protein that has the unique feature to polymerize into filaments. Controlled polymerization of actin filaments (F-actin) from actin monomers (G-actin), regulated by numerous actin-binding proteins (ABPs), creates the foundation for the cytoskeleton and is essential for many cellular processes such as cell adhesion, cell motility and intracellular trafficking. However, the functions of actin are not limited to the cytoplasm, since actin is present also in the cell nucleus. Although the amount of actin in the nucleus is lower than in the cytoplasm, the level of nuclear actin is constantly maintained by active nucleocytoplasmic transport. Actin is imported into the nucleus in complex with cofilin by Importin-9 [1], and is exported with profilin by Exportin-6 [2]. This active transport mechanism suggests the need to carefully balance the cellular actin distribution (Figure 1). Also many ABPs can be translocated to the nucleus where they could regulate nuclear actin polymerization [3]. For a long time it was unclear whether nuclear actin could actually polymerize, but recent studies have shed light into this issue [4]. Development of actin probes that recognize different forms of actin has revealed localization of monomeric actin to nuclear speckles and filamentous actin as submicron length structures excluded from chromatin-rich regions [5]. In addition, certain signals, such as serum-stimulation, cell adhesion and DNA damage can induce polymerization of nuclear actin to canonical, phalloidin-stainable filaments [6,7*,8**]. It has therefore become clear that like in in the cytoplasm, nuclear actin exists in both monomeric and polymeric forms. Here we review the recent progress in elucidating the functional significance of these different nuclear actin pools.

Monomeric actin in MKL1 regulation

Actin has been linked to many processes that control gene expression. Mechanistically, the best described example relates to the regulation of the essential transcription factor serum response factor (SRF) by actin. This takes place via megakaryoplastic leukemia protein 1 (MKL1, also called MAL or MRTF-A), which is a coactivator of SRF and regulated by actin monomers through several mechanisms. G-actin binding to MKL1 inhibits its nuclear import by masking the nuclear localization signal (NLS) in MKL1 [9] [10]. Nuclear export of MKL1 is actin-dependent, and actin-binding also prevents MKL1 from activating SRF in the nucleus. Consequently reducing G-actin retains MKL1 in the nucleus and activates SRF [10]. In line with this, expression of NLS-tagged actin inhibits expression of many adhesive and cytoskeletal SRF target genes, leading to decreased cell motility [11]. Stimulating cells with serum leads to formin mDia-dependent nuclear actin polymerization, followed by retention of MKL1 in the nucleus [6]. Another mechanism to regulate MKL1 via nuclear actin involves Mical-2, an atypical actin-regulatory protein with monooxygenase activity. Mical-2 localizes to the nucleus, binds to actin filaments and triggers their depolymerization through a redox modification of a conserved methionine residue in actin. Mical-2 activity leads to decreased nuclear G-actin, resulting in nuclear retention and activation of MKL1, adding another layer to SRF control [12**]. However, the mechanism by which Mical-2 activity reduces nuclear G-actin must still be resolved. Additionally, MKL1 nuclear translocation is reduced in cells deficient for nuclear envelope proteins lamin A/C or emerin. This is due to increased mobility, and thus reduced polymerization, of both nuclear and cytoplasmic actin. Expression of emerin, but not of an emerin mutant incapable of binding actin, can rescue the phenotype [13]. Emerin, together with lamin A/C, linker of nucleoskeleton and cytoskeleton (LINC) complex at the nuclear envelope as well as formins mDia1/2 are also required for cell spreading-induced nuclear actin polymerization, which activates the MKL1/SRF pathway [7*]. Interestingly, also nuclear pore protein Nup98 and *Drosophila* Lamin have been linked to nuclear actin polymerization [14], but the mechanisms are still unclear. Phosphorylation adds yet another layer to MKL1 control. Actin-binding inhibits MKL1 phosphorylation, which is required for transcriptional activation and also regulates MKL1 localization. N-terminal phosphorylation promotes nuclear export, while phosphorylation in the RPEL domain, which is the actin-binding regulatory domain of MKL1, inhibits actin-binding and thus promotes nuclear import of MKL1 [15*]. In conclusion, actin

dynamics controls MKL1-SRF transcriptional activity, with actin monomers eliciting the negative regulatory effect (Figure 2).

Monomeric actin in chromatin remodeling and transcription regulation

Monomeric actin is known to tightly associate with several chromatin remodeling complexes, including SWI/SNF, SWR1 and INO80. These remodelers share a Helicase-SANT-associated (HSA) domain, which acts as the binding site for actin, usually together with actin related proteins (Arps) [16]. Structural studies of the INO80 complex revealed features of Arp4 that differ from actin, making it incapable of polymerizing. In fact, Arp4 can depolymerize actin with the help of Arp8, likely explaining why actin stays monomeric in this complex [17]. Additionally, the barbed end of actin is not accessible in the INO80 complex, further preventing actin polymerization. Moreover, chromatin remodeling activity and chromatin binding of INO80 was reduced when the pointed end of actin was mutated, suggesting that the accessible pointed end of actin is involved in chromatin binding [18]. Recent structural studies of actin bound to Arp4 and the HSA domain showed that actin is twisted in this complex and the pointed end is reshaped in a way that further prevents both polymerization and ATP-binding [19*]. Additionally, the architecture of the whole INO80 complex revealed that actin and Arps are located in the flexible foot module and chromatin is positioned in the cradle between the foot and the head module. Folding of the foot module could bring the DNA-binding subunits of the complex close to nucleosomes and promote remodeling [20] (Figure 2). In summary, it is evident that actin is kept monomeric at least in the INO80 chromatin remodeling complex, and that actin is required for chromatin-binding through the pointed end. Whether the twisted actin conformation favors the binding and whether the same principles apply also to other actin-containing remodeling complexes remains to be studied. In addition, actin has also been linked to chromatin modifying complexes. Monomeric actin associates with, and inhibits the activity of histone deacetylases 1 and 2 [21].

Actin has also been implicated in RNA polymerase function, and suggested to be involved in transcription elongation by polymerase (Pol) I [22], initiation [23] and elongation [24] by Pol II, and to associate with Pol III [25]. In addition to the polymerases themselves, monomeric actin was reported to associate with P-TEFb, a transcription elongation factor that releases Pol II from

pausing [26] (Figure 2). This finding seems particularly interesting when taking into account that many recent studies suggest pause-release to be a key step in transcription regulation. Even though the mechanisms of actin in transcription remains unknown, several studies have shown that adequate nuclear actin levels are required for maximal transcription. Depleting the nuclear import receptor for actin, Importin-9, decreases nuclear actin levels and hinders transcription [1]. In mammary epithelial cells, growth factor withdrawal or laminin 111 addition induces depletion of nuclear actin, destabilizing Pol II and III binding to transcription sites. This leads to decreased transcription and eventually quiescence [27]. A more recent study from the Wickström lab linked nuclear actin levels to mechanical regulation of epidermal stem cell lineage commitment. Mechanical strain induces translocation of emerin from the inner to the outer nuclear membrane, resulting in recruitment of non-muscle myosin IIA and local actin polymerization. This reduces nuclear import-competent actin monomers, and thus reduces nuclear actin levels, leading to decreased transcription as well as large scale chromatin rearrangements [28**]. It therefore appears that emerin can regulate both nuclear actin polymerization (see above) and its levels, and that it can act on both sides of the nuclear envelope (Figure 1).

Due to the lack of biochemical data, the functional form of actin during transcription is still debatable. Especially the involvement of nuclear myosin I [29], and several other regulators of actin polymerization, such as cofilin [30], the actin nucleating Arp2/3 complex [31], as well as its activator N-WASP [32] in transcription has evoked models of coordinated actin filament formation during the transcription cycle [33]. However, a recent study showed that persistent nuclear actin filaments inhibit transcription through depletion of the dynamic actin pool, and that sequestering actin monomers inhibits transcription *in vitro* [34*]. This suggests a role for actin monomers in transcription by Pol II, but the molecular mechanism still remains inconclusive. Nevertheless, it is interesting that in the context of MKL1-SRF target genes, actin monomers have a negative effect on transcription, whereas the overall impact of actin on other transcription-related processes appears positive. How these opposing activities are resolved on genes is an interesting open research question.

Organizing the nucleus with nuclear actin filaments

Recent studies have shown that nuclear actin can indeed polymerize [5,6,7*], but the mechanistic implications of these polymers, apart from their indirect role in activating MKL1-SRF

transcriptional activity (see above), still require further studies. Nevertheless, a common theme so far seems to be the organization and maintenance of nuclear integrity. *Xenopus* oocytes lack Exportin-6, and therefore contain huge amounts of nuclear actin, which forms a stable F-actin network [35]. Due to their large size, the oocyte nucleus is under significant gravitational forces. The nuclear F-actin scaffold prevents the oocyte nucleus from collapsing and resists the gravitational sedimentation of ribonucleoprotein droplets such as nucleoli [36]. Actin is responsible for organizing and rearranging the contents of the cytoplasm. This function may extend to the nucleus as well, because actin has been linked to repositioning of nuclear organelles and chromosomes. For example, chromosome relocation as well as movement of gene loci to active transcription sites is actin polymerization and myosin-dependent [37-39]. A direct link between active movement of a gene locus and its transcriptional activity was provided by the Belmont-lab. They showed directed motion of the HSP70 transgene towards nuclear speckles during heat shock. Inhibiting actin polymerization prevented the movement and gene activation [40**]. Another study found that actin polymerization induces movement of latent HIV-1 away from transcription repressing PML nuclear bodies for activation of viral transcription [41]. Nuclear F-actin thus clearly has a role in chromatin repositioning, and may be used to bring genomic regions to transcription permissive locations (Figure 3), but neither the regulators nor the mechanism by which actin attaches to genes are known.

In addition to organizing nuclear contents, actin may also play a role in maintaining genomic integrity (Figure 3). Formin-2, an actin nucleator, accumulates to the nucleus and mediates, together with another type of actin nucleators, Spire-1/Spire-2, nuclear actin polymerization in response to DNA damage. Inhibition of this pathway leads to increased DNA double strand breaks, demonstrating that nuclear actin polymerization is required for proper DNA damage repair [8**]. Actin may be involved in chromatin maintenance also by regulating the centromere protein A (CENP-A) incorporation into centromeres. Formin mDia2 operates downstream of the Rac GTPase activating protein (MgRacGAP) to regulate centromere association of holiday junction recognition protein (HJURP), which is required for CENP-A loading [42*]. Direct evidence for nuclear actin in this process must still be clarified.

Conclusion

Recent advances in imaging techniques and development of probes to detect and resolve different forms of nuclear actin have given us insights into forms and functions of nuclear actin. It is more evident than ever that actin in the nucleus is just as versatile as it is in the cytoplasm. It is also clear that cytoplasmic and nuclear actin pools are in close crosstalk through constant nucleocytoplasmic exchange of actin and ABPs. Another level of communication comes from mechanical interactions, where emerin and the nuclear lamina play important roles. While new functions for nuclear actin arise, we still lack the knowledge about the molecular mechanisms involved. For instance, actin has been connected to the RNA polymerase already 30 years ago, but we still do not understand how actin operates during transcription. The presence of polymerized nuclear actin, and its links to important nuclear organizing and maintenance processes, also raises new questions regarding the mechanisms by which actin operates in the nucleus. Do the polymers operate similarly as in the cytoplasm to provide force for movement, or do they only contribute to the viscoelastic nature of the nucleoplasm? One likely answer is that actin polymers come in different flavors, with distinct functional properties. Further development of imaging methods, utilizing for example super-resolution or electron microscopy, together with biochemical understanding of what these actin polymers interact with in the nucleus, will help us understand the versatile nuclear functions of one of the most versatile proteins of the cell: actin.

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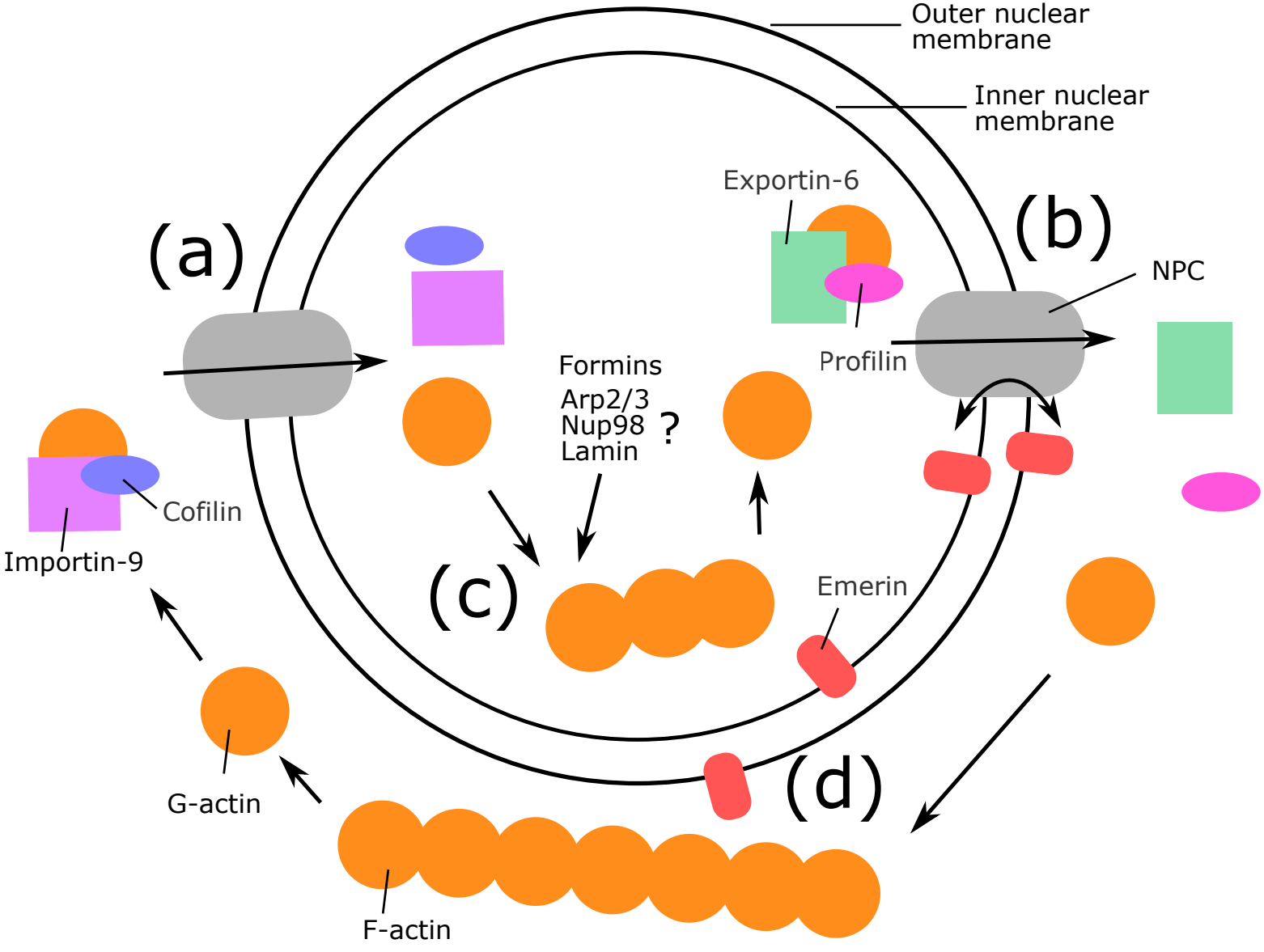
Figure legends

Figure 1. Regulation of nuclear actin pool. (a) Actin is imported into the nucleus with cofilin by importin-9 and **(b)** exported from the nucleus with profilin by exportin-6. **(c)** Actin polymerization can be controlled in the nucleus by known actin binding proteins, such as formins and Arp2/3 complex, as well as nucleus specific proteins such as emerin, Nup98 and Lamin. Question mark indicates that the proteins, excluding formins, have not been linked to nuclear polymerization of phalloidin-stainable actin filaments. **(d)** Emerin can reside at both inner and outer nuclear membrane and control local actin polymerization inside and outside the nucleus. NPC; nuclear pore complex.

Figure 2. Monomeric actin controls gene expression. (a) Actin is an integral part of the INO80 chromatin remodeling complex. It is located in a flexible foot module with Arp4 and Arp8. The barbed end of actin is attached to Arps preventing actin from polymerizing. The pointed end is accessible and possibly interacting with chromatin. **(b)** Actin monomers can bind to MKL1 keeping it inactive. Polymerization or export of monomeric actin releases MKL1 allowing it to be phosphorylated and to activate the serum response factor (SRF). **(c)** Nuclear actin is required for efficient transcription by RNA polymerases, where it possibly acts as a monomer. Actin has been suggested to interact with both the RNA polymerase II (Pol II) itself, as well as with P-TEFb which phosphorylates RNA polymerase II releasing it from pausing. P; phosphorylation

Figure 3. Functions of polymerized actin in the nucleus. (a) Nuclear actin polymerization is able to reposition an activated gene locus to transcription permissive area. **(b)** DNA damage induces Formin-2 and Spire-1/2 -dependent nuclear actin polymerization, which is required for efficient DNA damage repair. **(c)** Actin polymerization by the formin mDia2 might be involved in CENP-A loading to centromeres. In all cases, the mechanism by which the actin polymer operates, as well as how it is bound to chromatin, is not known.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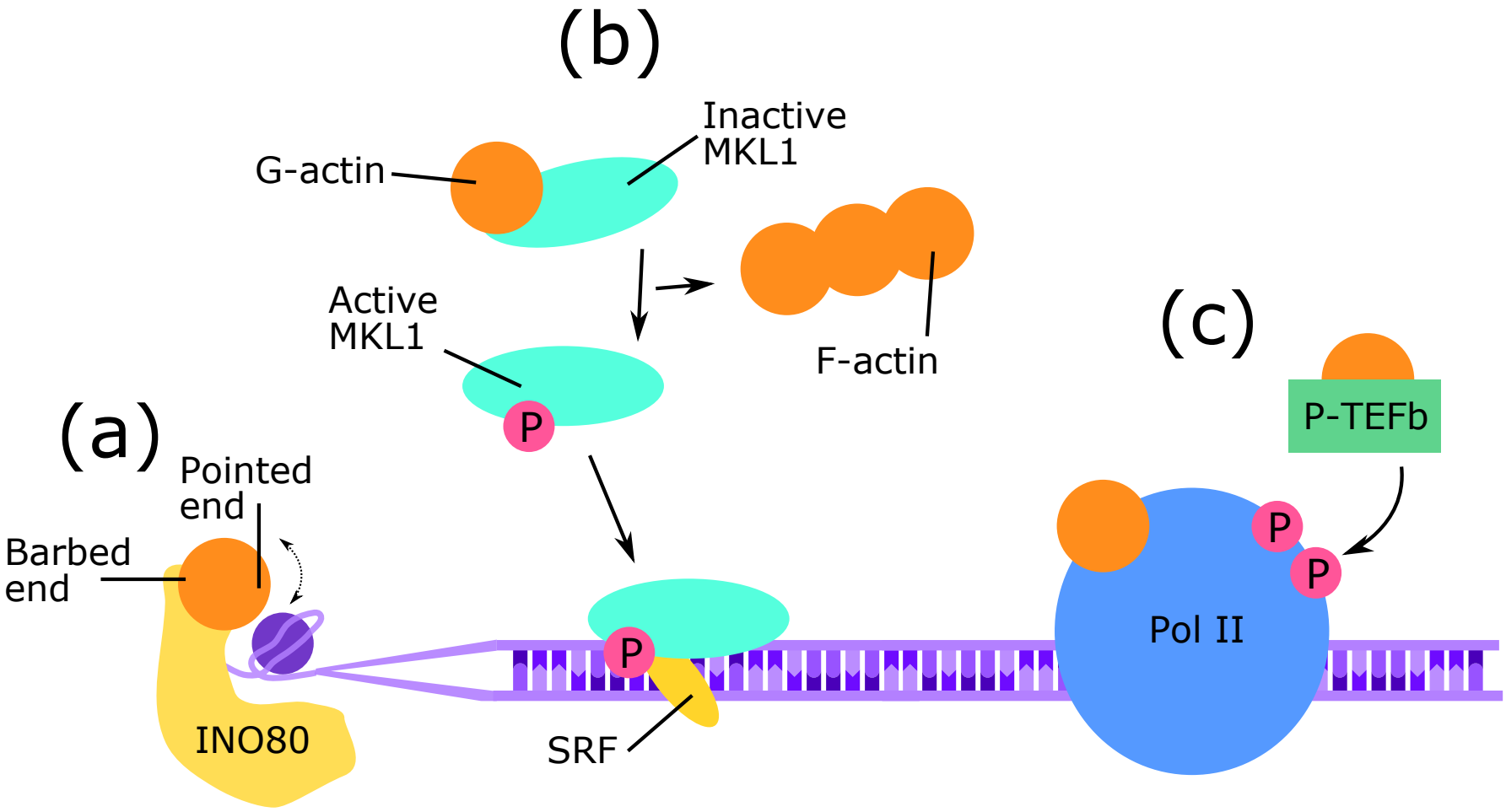
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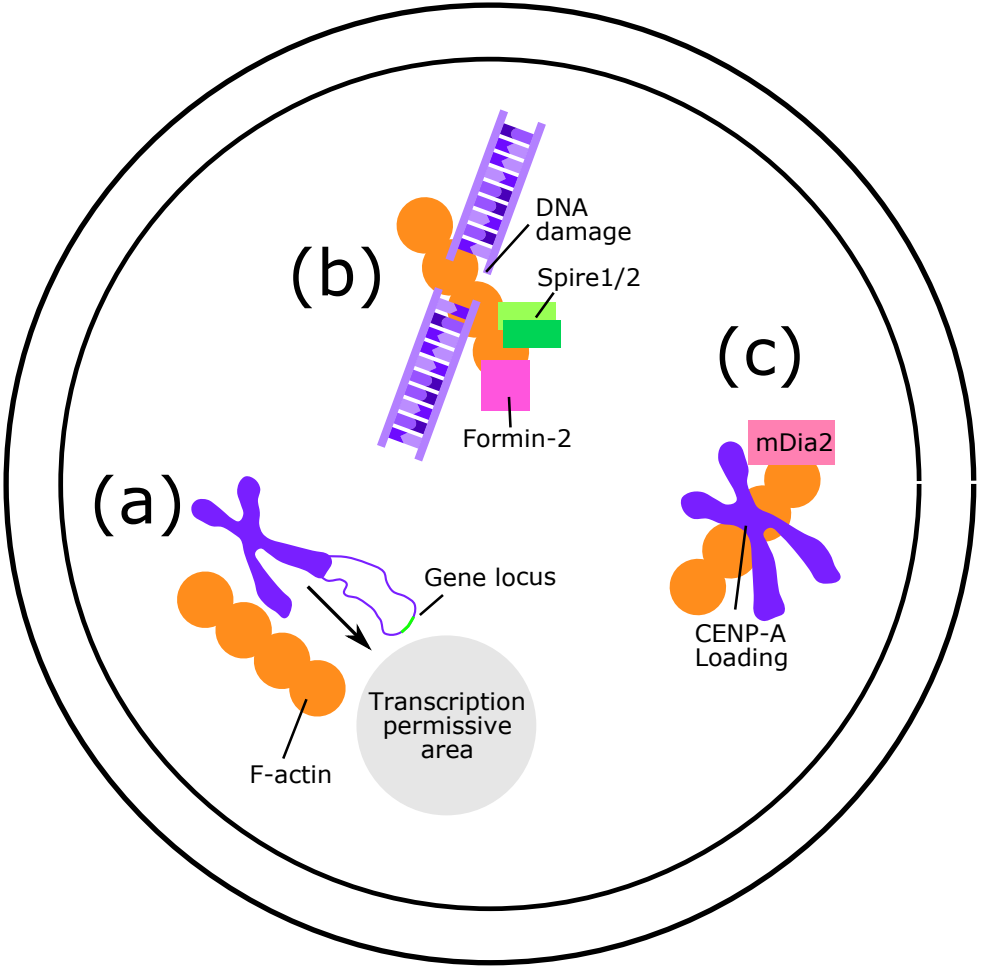
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