# Nuclear actin: to polymerize or not to polymerize

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The form and function of actin in the nucleus have been enigmatic for over 30 years. Recently actin has been assigned numerous functional roles in the nucleus, but its form remains a mystery. The intricate relationship between actin form and function in the cytoplasm implies that understanding the structural properties of nuclear actin is elementary to fully understanding its function. In this issue, McDonald et al. (p. 541) use fluorescence recovery after photobleaching (FRAP) to tackle the question of whether nuclear actin exists as monomers or polymers.

The presence of actin in the nucleus was first reported in this journal in 1969 (Lane, 1969). This report was followed by other studies that connected nuclear actin to transcription. However, this early work was met with skepticism and the very presence of actin in the nucleus was questioned for many years. The main point of criticism was the failure to detect actin filaments in the nucleus by fluorescence staining or by electron microscopy. In addition, cytoplasmic actin is virtually always found in association with myosin, yet attempts to detect myosin in the nucleus were unsuccessful. Nevertheless, in this issue McDonald et al. (p. 541) use FRAP to demonstrate the presence of both monomeric and polymeric actin species in the nucleus, although the nature of the polymers is yet to be defined.

Actin exists in equilibrium between monomers (globular- or G-actin) and polymers (filamentous- or F-actin). Under physiological salt conditions, G-actin readily polymerizes into filaments (F-actin). The tendency of G-actin to form filaments depends on the critical concentration (Cc) of actin. Actin tends to form filaments because the concentration of actin is well above the Cc in the cytoplasm. The sine-qua-non for the presence of actin filaments is staining by phalloidin, which specifically binds to actin filaments with a minimum filament size of 7 monomers (Visegrady et al., 2005). Although the concentration of actin in the nucleus is above the Cc, phalloidin does not stain the nucleus unless cells are treated with drugs like DMSO (Sanger et al., 1980). Thus, actin polymers, if they exist in the nucleus, are less than 7 monomers in length.

Actin filaments are important in defining cell shape, cell motility, cytokinesis, and intracellular transport in nonmuscle cells, and necessary for contraction and force generation in muscle cells. Importantly, virtually all known functions of actin in the cytoplasm involve actin filaments, or at least the process of polymerization into filaments, and an interaction with myosin. This is most obvious in striated muscle where thick (myosin II) and thin (actin) filaments slide past each other, resulting in contraction. Thus, the combined absence of phalloidin staining and myosin in the nucleus dealt deadly blows to the idea of a role for actin in the nucleus.

All of this has changed recently. Many studies have established the presence of actin in the nucleus and have shown that its functions are as diverse as in the cytoplasm (for review see Bettinger et al., 2004; Pederson and Aebi, 2005). Nuclear actin is essential for transcription by RNA polymerases, it is a component of chromatin remodeling complexes, it is important for nuclear transport of proteins and RNA, and it appears to have a role in nuclear envelope assembly (de Lanerolle et al., 2005). Despite the absence of obvious classical filaments in the nucleus, there is indirect evidence for the presence of some sort of F-actin. For instance, the actin-dependent nuclear export of RNAs and proteins in *Xenopus* oocytes can be inhibited by using the drug latrunculin B, which binds to actin monomers and prevents actin polymerization (Hofmann et al., 2001). Similarly, latrunculin B inhibits actin-dependent intranuclear movement of Herpes simplex virus-1 capsid (Forest et al., 2005) and nuclear envelope assembly (Krauss et al., 2003). In addition, actin dynamics in the cytoplasm are highly regulated by a vast array of binding proteins. The identification of proteins such as cofilin, profiling, and gelsolin in the nucleus (Gettemans et al., 2005), further suggests well-controlled regulation of the state of nuclear actin.

McDonald et al. (2006) provide the first convincing evidence in support of polymeric forms of actin in the nucleus. Starting with the assumption that the nuclear function of actin involves its controlled polymerization and depolymerization, they used FRAP to analyze the dynamic properties of GFP-actin in the nucleus of living cells. They found rapidly moving and slowly moving populations of both cytoplasmic and nuclear actin after FRAP. Based on studies with latrunculin B and an actin mutant that cannot polymerize, they concluded that the slowly moving population represented a polymeric form of actin. However, the authors point out that the polymers in the nucleus are inherently different from the actin stress fibers found in the cytoplasm and that the nuclear polymers are highly dynamic. By doing so, they provide a mechanism for reconciling conflicts between the observed cell biology and that predicted based on actin biochemistry.

At the same time, McDonald et al. (2006) present us with dilemmas. Their demonstration of a highly dynamic, atypical

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polymeric form of nuclear actin suggests that actin polymers in the nucleus and cytoplasm are fundamentally different. This notion is supported by immunofluorescence studies using specific antibodies that recognize structures different from classical filaments. Gonsior et al. (1999) used the monoclonal 2G2 antibody to actin to demonstrate the presence of actin aggregates in the nucleus. Based on X-ray diffraction studies, 2G2 recognizes an epitope that is buried in the F-actin structure and is unlikely to recognize classical actin filaments. Schoenenberger et al. (2005) used another mAb, 1C7, to study actin in the nucleus. This antibody was raised against the lower dimer form of actin, the first dimer that is transiently formed at the onset of polymerization. This antibody does not recognize actin filaments, but reacts with unpolymerized actin in the cytoplasm. It also recognizes nuclear actin, interestingly in a pattern different from the 2G2 antibody. Together, they suggest important differences in the actin polymers in the two cellular compartments.

The various forms of actin could well reflect different functions. Conceivably, actin in chromatin remodeling complexes exists in a monomeric form that has a structural role in complex assembly. Indeed, it was estimated that there is one actin molecule per SWI/SNF-like BAF chromatin remodeling complex, which suggests a structural role (Zhao et al., 1998). Actin could have a similar role in transcription. Actin binds to RNA polymerase I, II, and III (for review see de Lanerolle et al., 2005) and is necessary for the formation of preinitiation complexes (PICs) (Hofmann et al. 2004). Because depleting actin from a nuclear extract prevents the binding of the large subunit of RNA polymerase II to the PIC, these data also suggest a structural role for actin in PIC formation. On the other hand, studies on the role of actin in intranuclear transport or export of RNA and proteins rather point toward the requirement of a polymeric form in these processes.

Still unclear in this regard is the interaction between the nuclear forms of actin and myosin. As mentioned above, the absence of myosin in the nucleus undermined a role for nuclear actin. This issue has recently been resolved by the demonstration of an isoform of myosin I in the nucleus (Pestic-Dragovich et al., 2000). However, nuclear myosin I, unlike myosin II, is an unconventional myosin that does not form filaments. This presents another dilemma because the classical picture of force generation, as seen in muscle contraction, involves sliding filaments. But nuclear myosin I, like other myosin I molecules, has a very short tail and is unable to self-associate into filaments. Therefore, the classical model of actin filaments sliding past myosin filaments does not apply to this myosin. Nevertheless, nuclear actin and myosin I must be anchored to generate force (de Lanerolle et al., 2005) and additional experiments are required to determine how their special distribution and interactions support energy transduction in the nucleus.

Ultimately, the true significance of the work by McDonald et al. (2006) has to do with the future, not the past. They have helped to resolve a controversial issue by demonstrating polymeric forms of actin in the nucleus and by establishing that they are different from those in the cytoplasm. An important implication of their work is the need to imaginatively apply what we know about cytoplasmic actin to the study of nuclear actin. McDonald et al. (2006) have also shown that studying actin in the nucleus of living cells has its own set of intricacies. Foremost among these is the need for nuclear-specific probes of actin. For instance, manipulating actin dynamics in the cytoplasm can affect transcription by regulating the translocation of transcription factors to the nucleus (Miralles et al., 2003). Therefore, it is necessary to develop methods that selectively affect nuclear actin to obtain a detailed understanding of how monomeric, oligomeric, and polymeric forms of actin mediate nuclear functions.

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