3-19-2008

As functional nuclear actin comes into view, is it globular, filamentous, or both

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Published March 17, 2008

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The idea that actin may have an important function in the nucleus has undergone a rapid transition from one greeted with skepticism to a now rapidly advancing research field. Actin has now been implicated in transcription by all three RNA polymerases, but the structural form it adopts in these processes remains unclear. Recently, a claim was made that monomeric nuclear actin plays a role in signal transduction, while a just-published study of RNA polymerase I transcription has implicated polymeric actin, consorting with an isoform of its classical partner myosin. Both studies are critically discussed here, and although there are several issues to be resolved, it now seems reasonable to start thinking about functions for both monomeric and assembled actin in the nucleus.

The notion of nuclear actin has been with us since the early 1970's. Over the subsequent two decades, its functional significance was increasingly addressed, while the key question of its form remained relatively murky (Pederson and Aebi, 2002). Attempts to demonstrate classical F-actin in the nucleus (of physiologically happy cells) yielded findings that were far from convincing. But subsequently, actin—in whatever form—was being increasingly implicated in nuclear functions (Rando et al., 2000; Pederson and Aebi, 2002), including roles in transcription by all three RNA polymerases (Pederson and Aebi, 2005; Miralles and Visa, 2006; Percipalle and Visa, 2006). This meeting of a likely function for an unknown form is where things stood for nuclear actin as recently as early 2006.

There soon appeared a key study of the mobility of actin within the nucleus of cultured mammalian cells by fluorescence recovery after photobleaching (FRAP). This study indicated the existence of both more rapidly and more slowly moving forms of actin (McDonald et al., 2006). Although it is possible that the slower moving form was the monomeric protein bound to some larger particle (always an issue in FRAP studies), McDonald et al. (2006) addressed this and demonstrated that the magnitude of this lower mobility actin population decreased after treatment of the cells with F-actin depolymerizing drugs. Notwithstanding the possibility that this effect was due to a global change in the cytoskeleton feeding back on the nucleus, the results suggested that the lower mobility fraction was likely a polymeric actin. Although this important paper may have bolstered the argument for the existence of nuclear F-actin, it was not the end of the story.

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The importance of being single: a claim for monomerism

In June 2007, a paper appeared that seemed to define a function for nuclear actin in the context of signal transduction (Vartiainen et al., 2007). A link between nuclear actin and signal reception had been previously suggested by the finding that actin binding to the SWI/SNF-like chromatin remodeling complex BAF is phosphoinositol dependent (Zhao et al., 1998; Rando et al., 2002). Vartiainen et al. (2007) came to their recent findings through a series of studies on the serum-induced activation of gene transcription in mammalian fibroblasts, culminating in a detailed analysis of a transcription factor called SRF, for serum response factor (Copeland and Treisman, 2002; Geneste et al., 2002; Posern et al., 2002, 2004; Miralles et al., 2003; Posern and Treisman, 2006). Treisman's group had identified a SRF transcriptional coactivator protein termed MAL, and knew at the outset that it was a G-actin interactive protein (Posern et al., 2002, 2004; Miralles et al., 2003). But they had not yet obtained direct evidence that an interaction with actin was required for the role of MAL in the SRF-mediated pathway. This, then, was how the stage was set when the Treisman group began the recent study (Vartiainen et al., 2007).

The first step in this recent study was the finding that MAL shuttles between the nucleus and cytoplasm and, under normal conditions, is concentrated in the cytoplasm because it is quantitatively exported from the nucleus. In a key finding, the authors found that this rapid nuclear export of MAL was significantly reduced when the serum response pathway was triggered. Vartiainen et al. (2007) next studied the interaction between recombinant MAL and purified actin. As would have been anticipated form their earlier work, MAL and rabbit skeletal muscle monomeric actin displayed in vitro binding. In addition, alanine substitutions in the RPEL domains of MAL reduced actin complex formation, and fluorescence loss in photobleaching experiments with these mutants revealed that their nuclear export rates were reduced, like the effects of the actin-binding drugs on wild-type MAL.

The Journal of Cell Biology, Vol. 180, No. 6, March 24, 2008 1061–1064
http://www.jcb.org/cgi/doi/10.1083/jcb.200709082

Published March 17, 2008

These results fueled the notion that the nuclear export of MAL is somehow related to its interaction with actin.

The next step in this study evolved from the observation that a nuclear accumulation of MAL alone did not activate the set of SRF-regulated genes in the absence of serum. One might interpret this result as evidence that MAL itself is not a coactivator of SRF-responsive gene transcription, but the investigators made the inductive leap that perhaps the MAL–actin interaction needed to be disrupted in order for MAL to exert a transcriptional coactivator role.

The authors next performed FRET in fixed cells. They used an experimental design in which the donor fluor was GFP-MAL and the acceptor fluor (Cy3) was coupled to an antibody to a myc-tagged actin, the latter protein having been cotransfected along with the GFP-MAL construct. Using this approach they detected FRET in the fixed, immunostained cells. However, the evidence for FRET in the nucleus consisted of a very small shift on the color-coded fluorescence lifetime map, and is thus not entirely convincing. The more convincing FRET results were that treatment of cells with latrunculin B, to depolymerize F-actin, led to a striking increase in both nuclear and cytoplasmic FRET and that little or no FRET signal was observed in the nucleus with an actin noninteractive mutant of MAL. Thus, although the evidence, collectively, for a nuclear actin–MAL interaction is somewhat persuasive, the FRET results are less convincing.

Though observers may differ in their opinion of its degree of cogency, the Vartiainen et al. (2007) study inescapably lifts monomeric actin into a more functional setting than in most previous work on nuclear actin. There had been no lack of evidence for monomeric actin in the nucleus (Pederson and Aebi, 2002, 2005), but these wandering single actin molecules have always been in search of a function. Studies in *Xenopus* oocytes had indicated that in these specialized cells G-actin equilibrates between the nucleus and cytoplasm simply because it is in passive exchange (Clark and Merriam, 1977) but is not actively exported from the nucleus (Bohnsack et al., 2006), a view that emerged from studies on mammalian cells as well (Wada et al., 1998). The concentration of actin in the oocyte nucleus ($\sim$4 mg/ml) is vastly above the critical concentration for polymerization and instantly gels when the nucleus is slightly perturbed (Clark and Rosenbaum, 1979). This has been addressed recently in a particularly incisive review (Gall, 2006). In addition, the relationship of nuclear actin and RNA polymerase II has been studied in an extensively characterized in vivo model system of transcription (Percipalle et al., 2003). The actin that interacted with RNase polymerase II also showed an affinity for DNase I and thus, by this well established criterion, was presumably G-actin (Percipalle et al., 2003). The Vartiainen et al. (2007) study, with some issues, nonetheless elevates the journey to understand nuclear actin to a new level of interest, not only because the context, signal transduction, is such a broadly significant corridor of contemporary cell biology, but because a regulatory role of monomeric actin in the nucleus had been considered by many to be less likely than its mere obligatory presence resulting from an equilibrium with assembled forms of actin. It will be exciting to see if parallels to the MAL–actin–SRF story will emerge in other signal transduction contexts, as well as perhaps even for "intracrine" nuclear events such as cell cycle progression checkpoints, because what Vartiainen et al. (2007) have uncovered has the distinct aroma of a general phenomenon.

Assembled nuclear actin arrives, in the nucleolus

If we accept the existence of monomeric nuclear actin in mammalian cells, we immediately come to the vexing question of concentration. No quantitatively rigorous measurement of G-actin or F-actin (or any other form of assembled actin) has been made in the nucleus of mammalian cells. However, from the FRAP study discussed earlier (McDonald et al., 2006) it seems likely that assembled forms exist. There is also electron microscopic evidence for actin-containing filaments emanating inward from nuclear pores in *Xenopus* oocytes (Kiseleva et al., 2004); however, these filaments do not appear to be classical F-actin. Additionally, it remains unclear if somatic cell nuclei contain such nuclear pore–associated actin-containing filaments (Pederson and Aebi, 2002, 2005).

While the studies implicating nuclear actin in transcription progressed in several laboratories (Pederson and Aebi, 2005; Percipalle and Visa, 2006), a leading group working on the transcription of ribosomal RNA genes entered into a collaboration that produced evidence for a role of actin and an isoform of myosin I localized to the nucleus (Philimonenko et al., 2004). Significantly, this was the first time that a study of nuclear actin function had embraced myosin. One of the groups from that seminal collaboration has now extended these findings in a paper just published (Ye et al., 2008). These investigators provide evidence that both nuclear myosin I (NMI) and a polymerized form of actin support the transcription of ribosomal RNA genes by RNA polymerase I. Their findings implicate a classical ATP hydrolysis–dependent actomyosin-like involvement that, once again, repaints the picture of nuclear actin.

In earlier studies it had been observed that antibodies against nuclear actin and NMI do not inhibit transcriptional initiation by RNA polymerase I, but, rather, block the elongation step (Philimonenko et al., 2004; Percipalle et al., 2006). Ye et al. (2008) investigated pol I transcription after preincubating nuclear extracts with peptide antibodies specific for particular regions of actin. They observed that preincubation with an antibody against the N terminus blocked transcription in a dose-dependent manner, whereas no inhibition was observed with antibodies against either the C terminus or the DNase I–binding domain of actin. Inhibition was also seen after preincubation with an antibody to NMI, and this effect was overridden by addition of excess NMI. The inhibition of transcription by antibodies against the N terminus of actin could be rescued by the addition of exogenous recombinant actin. An additional observation was that this rescue was more efficient if both actin and NMI were added, suggesting to the authors that the two proteins were acting cooperatively.

Ye et al. (2008) next sought to determine whether actin and NMI actually occupied sites along the rDNA by using chromatin immunoprecipitation (ChIP). The presence of different regions of the transcription unit was assessed by PCR, using appropriate primers, of the immunoselected DNA. Antibodies to

Published March 17, 2008

actin and NMI selected both the transcribed and, surprisingly, the nontranscribed regions of the rDNA, thus demonstrating that actin and NM1 occupy both active and silent genes. The authors go on to show that inhibition of pol I elongation by actinomycin D did not affect the ChIP-assayed presence of actin and NMI on the rDNA. This demonstrated that their association with rDNA does not require active transcription.

The author's next foray was to treat cells with drugs that influence the state of actin polymerization. Phalloidin and jasplakinolide, which favor actin assembly, did not influence rDNA transcription as measured by PCR on cellular RNA. Conversely, they found that cytochalasin D and latrunculin B, which inhibit actin assembly, inhibited rDNA transcription. To ensure that this inhibition was not the consequence of changes in cytoplasmic actin, they tested the effects of the drugs on the nuclear extract system. Here again, the two actin assembly–promoting drugs had no effect on rDNA transcription, whereas the actin assembly–inhibiting drugs did, leading the authors to conclude that polymeric actin must be at play. These experiments did not address the issue of whether a cycle of actin assembly–disassembly operates during transcription, and yet the results do point to an involvement of assembled actin. The authors' case was also bolstered by the finding that addition of profilin (which stabilizes F-actin) did not affect pol I transcription, whereas addition of cofilin (which cleaves and depolymerizes F-actin) inhibited pol I transcription.

Ye et al. (2008) then present their most powerful finding, namely the capacity of various actin mutants to rescue the antibody-induced inhibition of pol I transcription. Only actin mutants that have the capacity to stabilize F-actin were observed to restore transcription. Given the absence of rescue with a negative control, i.e., an actin mutant that does not stabilize F-actin, these results are convincing, especially because the presence of the F-actin stabilizing mutants on the rDNA was confirmed by ChIP as was the absence of the F-actin nonstabilizing mutant in these add-back experiments. The authors went on to demonstrate that only the F-actin stabilizing mutants that scored positively by ChIP also co-immunoprecipitated with pol I and TIF-1A. Ye et al. (2008) closed out their story by overexpressing actin and showing that this increased the ChIP capture of pol I on rDNA, and that overexpression of the F-actin stabilizing mutant led to even more pol I on the rDNA.

The Ye et al. (2008) study would seem to have nailed the issue of a role of both assembled actin and myosin in transcription to a degree not achieved in previous work, however suggestive the earlier attempts have been (and they have been variable in their cogency). The immediate question is whether roles for assembled actin and myosin in pol II and III transcription will similarly emerge. Related issues include how the envisioned actomyosin-like complex mechanistically impacts the polymerases, and whether an actomyosin-like complex might also play a role in creating force needed to kick the finished transcript away from the transcription termination site, before its diffusive travels to reach nuclear pores (Politz and Pederson, 2000; Politz et al., 2003). And, of course, the question of how monomeric actin concentrations are regulated at transcription sites now looms large (see below). The observation of Ye et al. (2008) that cofilin inhibited pol I transcription is relevant here, and it would have been interesting if they had run ChIP assays with cofilin antibody. We should also bear in mind that one of the first demonstrations of a role for actin in the nucleus was as a collaborating factor in chromatin remodeling (Olave et al., 2002), an idea that has been increasingly confirmed and extended (Sjolinder et al., 2005; Percipalle et al., 2006). Could monomeric actin be somehow elevated at very specific chromosomal sites so as to actually turn on a silent gene? These are all exciting new dimensions of the nuclear actin field that could have only been vaguely imagined a few years ago.

Evolutionary perspectives

We sometimes lapse into regarding G-actin as nothing more than an obligatory term in the equation for the equilibrium constant of F-actin polymerization. From the time of its emergence, monomeric actin would have been expected to have, and indeed today still has, biological roles of its own. If polymerizing actins evolved after nonpolymerizing ones, the ancestral monomeric forms would have been long selected for. New phenotypes surely would have arisen promptly upon the advent of the polymerizable form but, also, cells would have for the first time confronted the equilibrium between the two actin forms, and thus the opportunity would have existed for selection of genes that regulate the equilibrium, known in evolutionary biology as the principle of exaptive space (Gould and Lewontin, 1979). The closest actin-like proteins present in the extant eubacteria include the polymerizing MreB (Erickson, 2007; Vats and Rothfield, 2007), ParM (Garner et al., 2004), and ActM, the latter apparently having arisen by horizontal gene transfer from a eukaryote (Guljamow et al., 2007). We can't play the videotape of evolution backward, and distinguishing today's eubacterial or archaeal actin-like proteins from the true ancestral precursors of either muscle or β-actin is beyond bioinformatics. Did the first actins exist and operate exclusively as nonpolymerizing proteins, perhaps interacting with other proteins, nucleic acids, or other cellular components, these erstwhile interactions now long lost? Is it predominantly this envisioned primordial nonpolymeric landscape of actin that now operates monomerically in the nucleus, as suggested in the Vartiainen et al. (2007) study reviewed here, or has this capacity evolved on a separate branch, astride the refinements of actin polymerization that led to muscle and stress fibers? And how has the transcription machinery for ribosomal RNA come to be functionally interactive with polymerized actin and a nuclear isoform of myosin? Moreover, studies beyond the scope of this review have recently begun to implicate nuclear actin in the repositioning of chromosomal loci within the nucleus, an entirely new theater of actin function in the nucleus (Chuang et al., 2006; Wang et al., 2006; Dundr et al., 2007). These emerging vistas now stand before us, beckoning us to look further and exemplifying what President Harry Truman's wise science adviser, Vannevar Bush, once termed "the endless frontier"—the siren of science.

Conclusion

The key point to emerge from the two studies reviewed here is that roles for both monomeric and assembled actin have become plausible. Because the nucleus is highly territorialized as

Published March 17, 2008

regards function, the immediate question that arises is how actin assembly is spatially controlled within the nucleus, presumably favoring monomerism at some locations and assembly at others. It is striking to note that this is the very same issue that has concerned the entire cytoskeleton field since nonmuscle actin was first discovered. Just as we still ponder how actin assembly is regulated at various sites in the cytoplasm (at the leading edge of motile cells, at focal adhesions, and elsewhere, impacted by any of numerous actin-interactive proteins that promote, destabilize, bundle, branch, cap, or sever), the two studies reviewed here suggest that we now need to consider these same regional issues of differential actin assembly status for the nucleus itself. To borrow the title of a grand old song: "Oh Happy Day."

I thank Tom Pollard (Yale University) for constructive comments on an early draft of the manuscript, Edward Korn (National Institutes of Health) for overall encouragement, and Ingrid Grummt (German Cancer Research Center, Heidelberg) for communicating her findings as a preprint.

Supported by National Science Foundation grant MCB-0445841.

Submitted: 13 September 2007
Accepted: 25 February 2008

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