Nuclear Actin: A Lack of Export Allows Formation of Filaments

Actin has been found in nuclei of many cell types, but little is known about its form and function. A recent study has shown that a lack of specific export allows actin to accumulate in the nucleus, where it forms a network of actin filaments that may be required to stabilize the giant nucleus of the Xenopus oocyte.

Melina Schuh and Jan Ellenberg

That the nuclei of eukaryotic cells — most prominently those of amphibian oocytes — contain the cytoskeletal protein actin was reported in many papers in the 1970s and 1980s, but the significance of these findings was doubted for a long time. Only recently has work been published that dispels these doubts and starts to shed light on multiple possible functions of nuclear actin, including roles in chromatin remodeling, transcription, transport within the nucleus and traffic between nucleus and cytoplasm. But the conformation(s) of nuclear actin, and its molecular mode(s) of action, have remained unclear. A recent study by Bohnsack et al. [1] has now revealed how actin can accumulate to relatively high levels in the nuclei of amphibian oocytes and discovered a new nuclear actin conformation: a sponge-like meshwork composed of filamentous actin (F-actin) which may be required to stabilize the giant nucleus of a Xenopus oocyte.

In most cell types, the concentration of actin in the nucleus is much lower than that in the cytoplasm [2] — too low to provide enough material for an actin-based filament system. The nuclei of amphibian oocytes, in contrast, have been known for a long time to contain actin at very high levels [3,4]. What are the mechanisms controlling these differences in intranuclear actin concentrations? Monomeric actin is small enough to enter the nucleus through nuclear pore complexes by passive diffusion. In most cell types, this passive influx is counteracted by an active actin export mechanism mediated by the nuclear transport receptor exportin 6 (Exp6), which keeps nuclear actin levels very low. Conversely, actin levels rise when the Exp6 pathway is shut down [2].

Is this the origin of the high actin concentration in amphibian oocyte nuclei? To answer this question, Bohnsack et al. [1] monitored whether radiolabeled actin was exported into the cytoplasm after injection into the nucleus of the Xenopus oocyte. In contrast to other cell types, actin did not leave the nucleus and only co-injection of Exp6 induced export of actin, suggesting that this pathway is inactive in the oocyte.

Nevertheless, a functional Exp-6 orthologue is produced in somatic Xenopus cells and, surprisingly, also in the Xenopus egg [1]. An egg directly evolves from an oocyte in a process called meiotic maturation, during which the oocyte extrudes one set of homologous chromosomes in a small polar body, becoming an egg that is ‘mature’ for fertilization. Bohnsack et al. [1] found that Exp6 protein is absent from the oocyte, but its levels constantly increase during meiotic maturation, reaching similar levels to somatic Xenopus cells in the egg (Figure 1). Exp6 mRNA is maternally stored in the oocyte, but its translation seems to be prevented at this stage [1]. Only after induction of maturation is translational repression released and Exp6 levels rise, demonstrating a tight developmental regulation of this specific nuclear transport pathway.

Bohnscak et al. [1] had thus found that a lack of Exp6 activity in Xenopus oocytes accounts for their high nuclear actin levels. In their next experiments they aimed to understand whether the elevated actin levels had a biological function. To do this, they specifically depleted oocytes of nuclear actin by injection of Exp6 into the nucleus and tested how they withstood the mechanical stress during manual isolation from the oocyte into buffer. Nuclei that were depleted of actin in this way could no longer be isolated with a standard dissection technique, as they became extremely fragile outside of the oocyte. Similar results were obtained after nuclear injection of an F-actin depolymerizing drug. This demonstrates first, that the high nuclear actin levels are required to maintain the mechanical integrity of the 400 μm large Xenopus nuclei during isolation, and second, that actin must exert this stabilizing function in a polymerized, filamentous conformation. To test if filamentous actin exists in the oocyte nuclei, Bohnsack et al. [1] visualized the intranuclear actin conformation. Employing an oocyte fixation method based on quick-freezing to preserve the native actin structures as much as possible, they found that the nucleus is filled with an extensive sponge-like network composed of F-actin bundles. They suggest that this structure could have shock-absorbing and stabilizing properties that are required to maintain the mechanical integrity of the giant Xenopus oocyte nucleus.

Bohnscak et al. [1] have thus convincingly elucidated the mechanism underlying the high nuclear actin levels of Xenopus oocytes, and obtained intriguing evidence for a possible biological function. In the light of the controversial history of nuclear actin research, it is useful to compare these new findings with
what has been known previously. The existence of filamentous actin in the nucleus of frog oocytes has been observed in the past using both light and electron microscopic techniques [5–7]. Furthermore, several early studies of buffer isolated amphibian oocyte nuclei have described a ‘nuclear gel’ [8–10]. A possible explanation for the ‘nuclear gel’ is the sponge-like nuclear actin scaffold described by Bohnsack et al. [1], which might mechanically stabilize the nuclear contents resulting in a gel like consistency. Alternatively, the isolation procedure and contact with buffer might have caused the spontaneous formation of an actin gel, which may not exist in the intact cell [11]. Regardless, it is clear that the high actin levels observed in amphibian oocyte nuclei [3,4] are not an artifact, that a fraction of actin is already in a highly organized filamentous form in the intact cell, and that actin stabilizes these giant nuclei at least after isolation [1], but likely to some degree also normally in the oocyte, as depolymerization of F-actin in vivo results in nuclear deformations, as demonstrated previously by Roeder and Gard [5]. If this stabilizing function of nuclear actin is a general in vivo phenomenon, a similar scaffold should also be observable in other large nuclei.

In one study [12] consistent with this view, an expandable nuclear endoskeleton has been reported to be at work in the giant polyploid nuclei of Drosophila larval salivary glands. The nuclear protein ‘enhanced adult sensory threshold’ (EAST) seems to be a component of this nucleoskeleton. In response to heat shock, nuclear EAST levels rise, accompanied by an expansion of the nuclear volume. Interestingly, overproduction of EAST was found to cause nuclear accumulation of actin, indicating that actin might be involved in the reorganization of this nucleoskeleton in response to stress [12]. Nuclear actin might also become important when cells with large nuclei, such as oocytes, divide: in an intact starfish oocyte, for example, a contractile nuclear actin network mediates chromosome congression to the cortex, and is thus required for correct spindle formation in the first meiotic division [13]. Importantly, when this actin network is depolymerized or hyperstabilized, chromosomes are lost from the first meiotic spindle, generating an aneuploid oocyte [13]. Similarly, there are reports that in a maturing Xenopus oocyte actin depolymerization can lead to abnormal or delayed spindle formation, although the effect here seems to be less clear cut than in starfish [14,15]. We will have to wait for more studies to see how general this function is conserved in different species.

Despite these lines of evidence suggesting that filamentous nuclear actin might have important functions in special cells and physiological situations, several questions remain open. For example, what would be the advantage of the sponge-like nuclear actin scaffold found by Bohnsack et al. [1], compared to a hypothetical ‘intranuclear actin cortex’ [16], if the function was just mechanical stabilization of the oocyte nucleus? A space-filling nuclear F-actin network might in principle provide tracks through the nucleus for intranuclear transport, not possible for an ‘intranuclear cortex’. While intranuclear movements in the small nuclei of somatic cells have been shown to be largely based on diffusion, there is evidence that some traffic, especially that of large particles, is ATP and F-actin dependent [17]. Indeed, two recent studies found that intranuclear movements of chromatin loci [18] and
herpes virus capsids [19] can be inhibited by the presence of non-polymerizable actin or depolymerization of F-actin. It is unclear from these studies just how direct the requirement for actin in transport within nuclei is, but it is reasonable to suppose that directed transport would be important in a massive nucleus such as that of an amphibian oocyte, with a volume ~25 000 times larger than that of a typical somatic cell. Intriguingly, a recent ultrastructural study [20] of isolated Xenopus oocyte nuclei observed filaments, which could be decorated with anti-actin antibodies and which were sensitive to actin depolymerizing drugs, connecting nuclear pore complexes to intranuclear structures like nucleoli.

We are just beginning to understand forms and functions of nuclear actin. Bohnsack et al. [1] have unraveled why actin is allowed in nuclei of Xenopus oocytes and showed that it can form a crosslinked filamentous structure in them. It remains to be shown, however, which fibrous actin structures can be found in nuclei of different cells in vivo and what their molecular functions are — exciting questions for future research.

References

Social Learning: Ants and the Meaning of Teaching

Recent research on ants shows that running in tandem might serve the function of teaching naïve ants about the path to a target. Although these new experiments represent perhaps the most highly controlled study of teaching in animals to date, the findings prompt the question of how teaching formally differs from other forms of communication.

Ell Louise Leadbeater, Nigel E. Raine and Lars Chittka

Learning from others is so fundamental to humans that we actively speed up the social learning process — we teach. Non-human animals can also learn from members of their own species, and they might be expected to accrue considerable inclusive fitness benefits by ‘coaching’ kin to facilitate the rapid development of adaptive behaviour [1–3]. Surprisingly, however, convincing demonstrations of teaching behaviour in animals are rare. Caro and Hauser [4] laid out the following minimum criteria for information transfer between animals to be classified as teaching. The animal that conveys information must incur a cost, or at least not reap an immediate benefit from the subsequently altered behaviour of the receiver. The candidate behaviour has to be performed only when uninformed individuals are present. Hence, although juvenile songbirds learn their songs by listening to adult males, the adult is not teaching because he will sing irrespective of the youngsters’ presence. Finally, the teaching must lead the pupil to learn a skill, or acquire knowledge that it would not otherwise obtain, or at least that it would take longer to acquire. Perhaps the most convincing candidates for teaching among vertebrates involve carnivores learning to hunt (reviewed in [4,5]). Mother cheetahs that would normally capture and kill prey without delay bring live prey back to the nest when their cubs are very