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A selective block of nuclear actin export stabilizes the giant nuclei of *Xenopus* oocytes

Markus T. Bohnsack[1], Theis Stüven[1], Christa Kuhn[1], Volker C. Cordes[1] and Dirk Görlich[1,2]

Actin is a major cytoskeletal element and is normally kept cytoplasmic by exportin 6 (Exp6)-driven nuclear export. Here, we show that Exp6 recognizes actin features that are conserved from yeast to human. Surprisingly however, microinjected actin was not exported from *Xenopus laevis* oocyte nuclei, unless Exp6 was co-injected, indicating that the pathway is inactive in this cell type. Indeed, Exp6 is undetectable in oocytes, but is synthesized from meiotic maturation onwards, which explains how actin export resumes later in embryogenesis. Exp6 thus represents the first example of a strictly developmentally regulated nuclear transport pathway. We asked why *Xenopus* oocytes lack Exp6 and observed that ectopic application of Exp6 renders the giant oocyte nuclei extremely fragile. This effect correlates with the selective disappearance of a sponge-like intranuclear scaffold of F-actin. These nuclei have a normal G2-phase DNA content in a volume 100,000 times larger than nuclei of somatic cells. Apparently, their mechanical integrity cannot be maintained by chromatin and the associated nuclear matrix, but instead requires an intranuclear actin-scaffold.

The nuclear envelope divides eukaryotic cells into a nuclear and a cytoplasmic compartment. It thereby enforces a strict division of labour between the two compartments and necessitates the nucleo-cytoplasmic exchange of material[1–3]. Nuclear pore complexes allow two modes of such nucleo-cytoplasmic transport — passive diffusion and facilitated transport[4]. Passive diffusion is rapid for small molecules, but becomes increasingly restricted as the molecules approach or exceed a molecular weight (M_r) of approximately 30–40 K. Facilitated transport, in contrast, can accommodate even very large cargoes. It is typically receptor-mediated, coupled to an input of metabolic energy and therefore capable of cargo transport even against steep gradients of chemical activity (active transport).

Importin β-related factors represent the largest class of nuclear transport receptors[2,3]. In humans they include 11 factors that function primarily or exclusively in nuclear import (importins), seven exportins and two family members of as yet unknown function.

The primary function of the nuclear transport machinery is biosynthetic transport; that is, the supply of proteins to the nucleus and of nuclear products (such as tRNAs and ribosomal subunits) to the cytoplasm. However, five nuclear-export pathways are partially or entirely devoted to the task of keeping cytoplasmic proteins in the cytoplasm[5–10]. At first, this may seem a waste of cellular resources. However, nuclear pore complexes are only imperfect barriers that cannot prevent, but only delay, leakage of cytoplasmic components into the nucleus. This leakage must then be counteracted by steady nuclear export of mislocalized molecules. Also, the cytoplasmic and nuclear contents of higher eukaryotic cells mix during mitosis. Their separation on re-entry into interphase includes not only active import of nuclear components, but also exportin-mediated exclusion of cytoplasmic constituents from the nucleus.

Actin is one of these actively excluded cytoplasmic components[11]. It forms cytoplasmic microfilaments (F-actin) that have a cytoskeletal function, are involved in cellular motility, and serve as tracks for myosin-type motor proteins. Unpolymerized, monomeric actin has an M_r of 40 K. Its flux through nuclear pore complexes is thus significantly restricted, but it is still small enough to passively enter nuclei with a half time of approximately 30 min to a few hours (depending on cellular dimensions and nuclear pore complex density; data not shown). Nevertheless, actin is firmly excluded from the nuclear compartment of most cell types. Recently, we showed that this exclusion results from continuous, active nuclear export of actin mediated by Exp6 (ref. 6).

Exp6 is conserved from amoeba to vertebrates and, at least in *Drosophila melanogaster,* is encoded by an essential gene[6]. As actin represents the only directly recognized cargo of this pathway[6], it seems that high levels of nuclear actin either interfere with developmental programmes or are toxic at a cellular level.

Nevertheless, enormous amounts of nuclear actin have been reported in a highly specialized cell type — the amphibian oocyte[12]. The function of these high levels of nuclear actin was unclear so far. Here, we show that the Exp6 pathway is inactive in *Xenopus* oocytes, thus explaining why oocyte nuclei accumulate actin. Ectopic application of Exp6 excluded actin from the nuclear compartment and rendered the nuclei so fragile that they consistently broke during standard oocyte dissection procedures. Thus, the most straightforward explanation of actin function in

[1]Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), INF 282, D-69120 Heidelberg, Germany.
[2]Correspondence should be addressed to D.G. (e-mail: dg@zmbh.uni-heidelberg.de)

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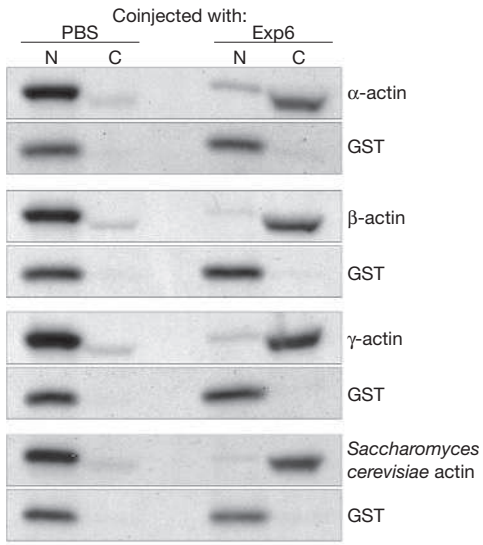

Figure 1 Actin export from nuclei of *Xenopus* oocytes requires exogenous Exp6. ^{35}S-labelled actin isoforms were generated by *in vitro* translation and injected into the nuclei of *Xenopus* oocytes. After 1 h, oocytes were dissected into cytoplasmic (C) and nuclear (N) fractions. Labelled proteins were detected by SDS–PAGE followed by autoradiography. Note that actin was not exported to the cytoplasm unless 8 μM human Exp6 was included into the injection mix (15 nl per oocyte). GST served as an injection control.

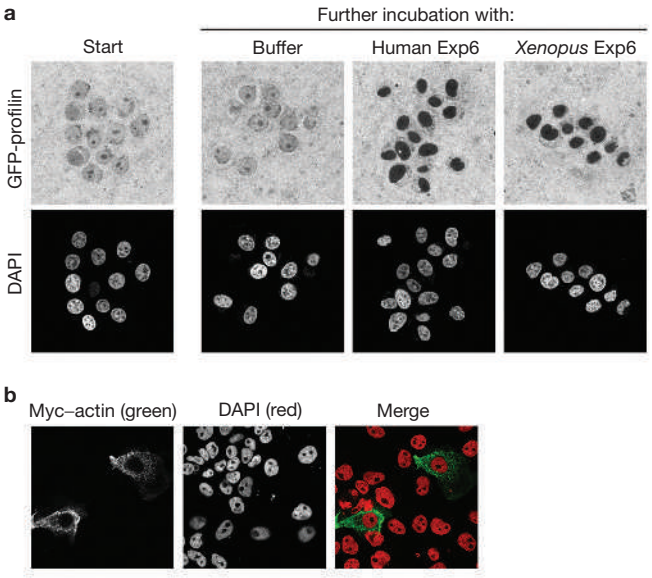

Figure 2 *Xenopus* Exp6 is active in nuclear actin export. (**a**) HeLa cell nuclei were incubated in a *Xenopus* egg extract that was depleted of endogenous nuclear transport receptors. For 1 h, 3 μM GFP–profilin was allowed to equilibrate between nucleus and cytoplasm (Start). The reaction was split and supplemented with buffer or 0.5 μM Exp6. After 15 min, the distribution of GFP–profilin was assessed by confocal laser scanning microscopy (CLSM). *Xenopus* and human Exp6 were equally efficient in excluding the GFP–profilin–actin complex from the nuclei. (**b**) Myc-tagged actin was expressed by transient transfection in the *Xenopus laevis* KE A6 cell line (ATCC CCL 102), and detected by anti Myc-staining and CLSM. Note that actin is excluded from the DAPI-stained nuclei.

oocyte nuclei holds true: it fulfils a cytoskeletal function and stabilizes these vastly inflated nuclei. There has also been a long-standing debate about the state of actin inside the nuclei of amphibian oocytes[13,14]. Our data indicate that this actin form is not as exotic as previously thought. Instead, it stains with phalloidin and therefore represents mostly F-actin. We also show that it forms a branched, three-dimensionally interconnected scaffold that spans the entire nucleus, an arrangement that is ideal for the elastic absorption of compression forces.

RESULTS

Human Exp6 exports not only β-actin, but also α- and γ-actin, and even actin from yeast

β-actin has previously been identified as an Exp6-cargo and it has been demonstrated that loss of Exp6 function in *Drosophila* cells results in nuclear actin accumulation[6], which in turn causes embryonic lethality. As a next step, we wanted to test if Exp6 exports only β-actin or also other actin isoforms. We therefore microinjected *in vitro*-translated, radiolabelled actin into nuclei of *Xenopus* oocytes and monitored the arrival of the injected protein in the cytoplasm. Surprisingly, none of the injected actin isoforms, including β-actin, showed any signs of export (Fig. 1). Instead, they remained in the nucleus, even after several hours of incubation. This may indicate that *in-vitro*-translated actin is not export-competent or that actin-export activity is very low in this particular cell type.

To distinguish between these possibilities, we co-injected 8 μM human Exp6 (resulting in a cellular concentration of ≈0.2 μM) and now observed rapid export of not only of β-actin, but also of α- and γ-actin and even of actin from the yeast *Saccharomyces cerevisiae* that lacks an Exp6 orthologue (Fig. 1). These data demonstrate that Exp6 recognizes all actin isoforms and suggests that Exp6 adapted during evolution to pre-existing actin features. The experiment also indicates that *Xenopus* oocytes possess no significant Exp6 activity.

The *Xenopus Xpo6* gene is functional, even though oocyte nuclei lack active actin-export

Exp6 is apparently inactive in *Xenopus* oocytes, but is essential for viability of *Drosophila*. This may reflect a general difference between the two species and mean that *Xenopus* lost this pathway during evolution. Alternatively, it may be a cell-type specific phenomenon and imply that the pathway is selectively inactivated in oocytes.

To clarify this issue, we asked whether *Xenopus* possesses a functional *Xpo6* gene and isolated the full-length cDNA of a *Xenopus Exp6*-orthologue with 79% amino-acid sequence identity to the human exportin. Recombinant *Xenopus* Exp6 was then prepared and tested for export activity. HeLa cell nuclei were incubated in a *Xenopus* egg extract that lacked nuclear transport receptors[6], and the export marker, green fluorescent protein (GFP)–profilin, was allowed to equilibrate between nucleus and cytoplasm. Addition of either human or *Xenopus* Exp6 resulted in rapid nuclear exclusion of GFP–profilin (Fig. 2a), which is known to be co-exported with actin[6]. Taken together, these data indicate that *Xenopus* produces an mRNA for an Exp6 orthologue that is fully functional in the nuclear export of profilin–actin complexes.

Exp6 expression and the nucleo-cytoplasmic distribution of actin in the *Xenopus laevis* kidney epithelium (KE) A6 cell line was then investigated. Immunoblotting with *Xenopus* Exp6 antibodies indicated that these cells contain Exp6 in a cellular concentration of approximately 0.1 μM (data not shown; see below). Consistent with this, a clear exclusion of Myc-tagged β-actin from nuclei of this cell line was observed (Fig. 2b), indicating that the Exp6 pathway can be operational in *Xenopus* cells.

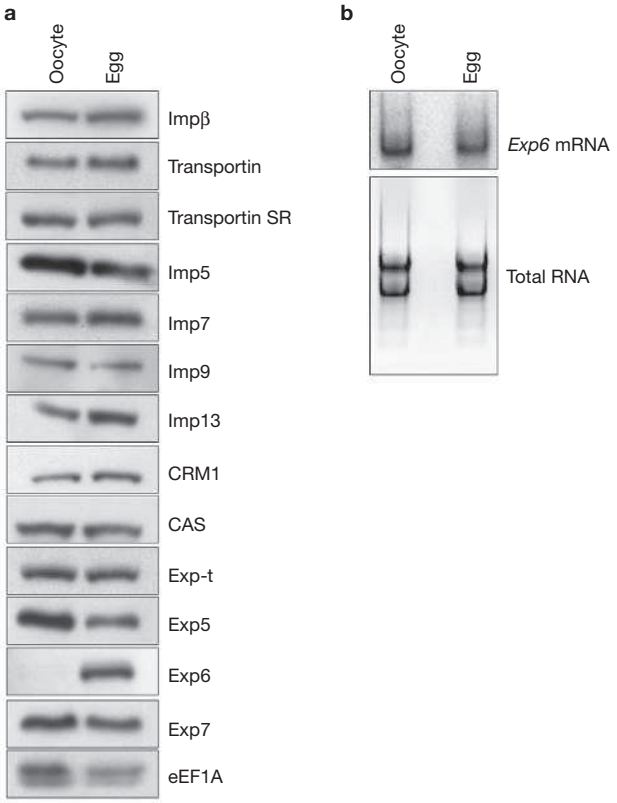

Figure 3 Xenopus oocytes stockpile *Exp6* mRNA, but selectively lack the Exp6 protein. (**a**) Total extracts from *Xenopus* stage-VI oocytes and eggs were analysed by immunoblotting with antibodies against the indicated transport receptors and eEF1A as a loading control. (**b**) Total RNA from oocytes and eggs was analysed by denaturing agarose gel electrophoresis, followed by ethidium bromide staining (total RNA) or Northern blotting with an Exp6-specific probe.

Exp6 is undetectable in oocytes, but markedly induced during and after meiotic maturation

The expression pattern of Exp6 during development was examined. Exp6 levels in oocytes were extremely low (less than 1 nM; Figs 3a, 4), explaining the negligible nuclear-export activity for actin within this cell type. In eggs, however, strong signals corresponding to a cellular Exp6 concentration of approximately 30 nM were observed (Fig. 3a). Strikingly, none of the other nuclear-transport receptors examined showed similar concentration differences between oocytes and eggs (Fig. 3a). The expression pattern of Exp6 therefore seems unique amongst nuclear transport receptors. This points to a strong selective pressure to prevent actin export in the growing oocyte and to only activate this pathway later in development.

Xenopus oocytes grow to their enormous size and accumulate large stockpiles of mRNAs, ribosomes, histones and nuclear pore complex components while arrested in their first meiotic prophase[15]. Progesterone, or related steroid hormones, then trigger maturation into a fertilizable egg. This process not only comprises the two meiotic divisions until metaphase II (when the egg can be fertilized), but also the selective synthesis of certain maternal gene products[16]. These include mostly signalling molecules (such as MAPKKK), components of the meiotic machinery (such as cyclins B1 and B4), or the chromokinesin, Xkid. This meiotic induction occurs at the translational level: that is, the corresponding maternal mRNAs are stored in the oocytes and relieved from translational repression when meiotic maturation is induced. *Exp6* mRNA is also abundantly present in stage-VI oocytes and levels do not increase further during the transition from oocyte to egg (Fig. 3b). Thus, meiotic Exp6 synthesis is also induced by selective translation of a stockpiled mRNA.

Fig. 4a illustrates how Exp6 levels change during meiotic maturation. Exp6 can first be detected when breakdown of the germinal vesicle occurs, but Exp6 levels become significant only after the first meiotic division. They continue to increase long after fertilization, until a peak is reached around stage 12 of embryonic development (Fig. 4b). The average cellular Exp6 concentration (approximately 0.1 μM) at this stage is then close to the level required for maximal *in vitro* export of the profilin–actin complex (Figs 1, 2a; data not shown).

Ectopic Exp6 makes *Xenopus* oocyte nuclei extremely fragile

We wondered why nuclear-actin export is blocked during oogenesis. The crucial clue came from the microinjection experiments that we performed in *Xenopus* oocytes. To monitor export, each oocyte must be dissected into a cytoplasmic and a nuclear fraction: The animal pole of the oocyte is punctured and the nucleus is squeezed through the resulting hole, out of the oocyte. Oocyte nuclei are remarkably elastic and so only very few of them break during dissection (<0.2%; see Table 1 and Supplementary Information, Movie 1). After Exp6 injection, however, this situation is markedly changed. When dissection was performed 30 min after injection, 57% of the nuclei broke at the squeezing step and the liquid nuclear contents were ejected into the dissection buffer (see Supplementary Information, Movie 2). By 60 min post-injection it was impossible to recover any intact nuclei by the standard dissection procedure. This effect was highly specific and not observed after injecting buffer or nuclear transport receptors other than Exp6. It should be noted that our export experiment (Fig. 1) only succeeded after modifying the dissection technique such that the hole punched into the oocyte was larger than normal. Even then, only 23% of Exp6-injected nuclei survived the procedure.

Exogenous Exp6 depletes the large nuclear-actin pool of *Xenopus* oocytes

How could Exp6 destabilize oocyte nuclei? It was known that high concentrations of nuclear actin occur in the amphibian oocyte[12]. Therefore, it appeared possible that these nuclei were stabilized by nuclear-actin structures, and that these stabilizing elements dissolved on Exp6-injection. Indeed, nuclear injection of the actin-depolymerizing drug, cytochalasin B, also made the nuclei very fragile (data not shown), whereas co-injection of phalloidin[17] together with Exp6 prevented nuclear fragility (Table 1).

When the nucleo-cytoplasmic actin distribution was analysed by immunoblotting, the loads of the nuclear and cytoplasmic fractions were normalized to the volumes of these compartments. Concentrations could thus be directly compared. Remarkably, in control oocytes, the nuclear actin concentration was found to exceed even that of the cytoplasm (Fig. 5a; see also ref. 12). This nuclear pool disappeared 30–60 minutes after Exp6 injection (Fig. 5b).

Analysis of oocyte nuclei by SDS–PAGE and Coomassie-staining identified a prominent actin band that disappeared after Exp6 injection (Fig. 5c). Other changes in protein composition were not evident, which in turn suggested that loss of nuclear actin is the principal, if not the only, direct consequence of the treatment and the immediate cause of the resulting nuclear fragility.

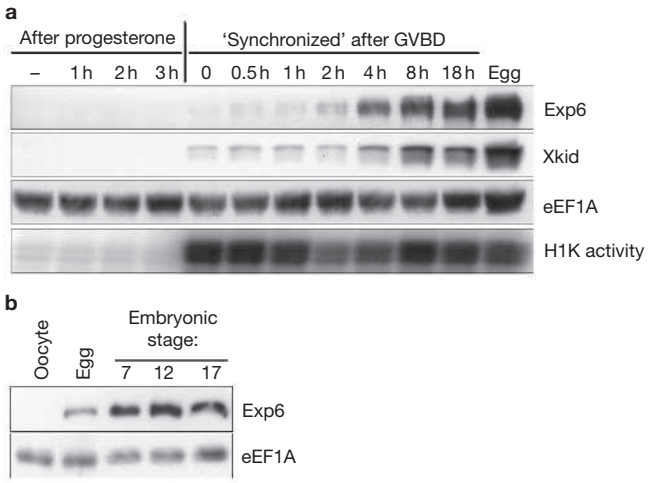

Figure 4 Synthesis of the Exp6 protein is induced during meiotic maturation. (**a**) *In vitro* maturation of stage-VI oocytes was triggered with progesterone. Changes in cellular Exp6 concentration during meiotic maturation were examined by immunoblotting. GVBD, germinal vesicle breakdown. The anti-Xkid-signal and MPF (histone H1-kinase)-activity served as maturation markers; the anti eEF1A-signal served as a loading control. (**b**) A comparison of Exp6 levels in oocytes, unfertilized eggs and embryos at the indicated stages[15]. Stages 7, 12 and 17 were reached 8, 12 and 30 h post fertilization, respectively.

Quantitative immunoblots indicate a nuclear actin concentration of 50 μM (2 mg ml^{-1}) in untreated stage-VI oocytes (data not shown). This compares to approximately 2.5 μM of nuclear Exp6 after injection. Complete export was thus achieved with catalytic amounts of Exp6, with each Exp6 molecule participating, on average, in approximately 20 rounds of export.

Actin forms an extensive network within oocyte nuclei

The fact that actin stabilizes oocyte nuclei raises the question as to which actin structures provide the mechanical support. Therefore, actin was visualized on oocyte sections (see Methods). Both the anti-actin antibody and fluorochrome-labelled phalloidin showed strong staining not only of cortical actin, but also of the oocyte nucleus (Fig. 6a). As phalloidin is known to specifically bind F-actin[17], this experiment suggested that the nuclear actin of amphibian oocytes is predominantly in the filamentous form. Fortunately, an ideal control to determine specificity was possible: this nuclear actin-staining completely disappeared after depleting the nuclear actin-pool by injection of Exp6.

Individual actin-filaments could hardly stabilize an oocyte nucleus, simply because they are too short to span this compartment. Also, they are known to be very flexible and must therefore be bundled to bear compression forces[11]. Indeed, higher magnification revealed nuclear F-actin bundles (Fig. 6b) of a similar diameter as cytoplasmic stress fibres, which are probably comprised of several hundred protofilaments. In contrast to stress fibres however, the nuclear F-actin bundles are branched into an interwoven three-dimensional meshwork, which in turn seems ideal for the elastic absorption of compression forces.

DISCUSSION

The field of 'nuclear actin' has a history of healthy scepticism that focused largely on three issues. The foremost one comprises two seemingly contradictory sets of observations: namely that the nuclei of amphibian

Table 1 Ectopic Exp6 renders nuclei of *Xenopus* oocytes extremely fragile.

Pre-treatment of oocytes	Nuclei burst during dissection	
Not injected with Exp6	<0.2%	(n>2000)
30 min after Exp6 injection	57%	(n=87)
60 min after Exp6 injection	>99%	(n=104)
60 min after co-injection of Exp6 and phalloidin	0%	(n=29)

Where indicated, oocytes were injected with a 15 nl injection mix containing 8 μM Exp6 or 8 μM Exp6 + 60 μM phalloidin. Oocytes were subsequently dissected using standard procedures[18]. The dissections of untreated oocytes, and of Exp6-injected oocytes, that burst during the procedure are documented in Supplementary Information, Movies 1 and 2.

oocytes contain enormous amounts of actin, as demonstrated by manually dissecting these giant cells into clean nuclear and cytoplasmic fractions (Fig. 5; ref. 12); and that somatic nuclei are negative in phalloidin stains and hence devoid of F-actin[11]. This discrepancy can be explained in two ways: first, one could assume that high levels of nuclear-actin concentrations are common to all cell types. One then arrives at the conclusion that nuclear actin exists in exotic conformations[13,14] that are not detectable by phalloidin[17]. Alternatively, these observations may simply reflect real differences between cell types. Our data support this second explanation and suggest that the nuclei of somatic cells do not stain with phalloidin because they express Exp6 and therefore contain very little actin (see below). The lack of Exp6 activity in *Xenopus* oocytes, however, allows nuclear accumulation of actin in this highly specialized cell type.

A second issue is the puzzling contrast between the proposed general cellular functions for nuclear actin (such as a proposed essential role in transcription in oocytes and somatic cells[19–21]) and again the fact that typical somatic cells exclude actin from their nuclei (Fig. 2B and ref. 11). Though we cannot discuss these potential non-skeletal functions of nuclear actin in detail here, it needs to be stated that even in Exp6-expressing cells, the nuclear actin concentration will not be zero. Instead, it is estimated that the exportin system can deplete the nuclear pool only down to 1% of the cytoplasmic G-actin levels[22] (that is, to a low micromolar concentration). This would still leave room for any non-skeletal function of nuclear actin[13,14,23] and for signalling the cytoplasmic actin state to the nuclear compartment[24].

Finally, there was no convincing and generally accepted explanation as to why the highly specialized nuclei of *Xenopus* oocytes accumulate such large amounts of actin. It now seems that the most straightforward of all possible explanations is correct, namely that actin fulfils a cytoskeletal function inside these nuclei.

Fig. 6 clearly shows that actin of oocyte nuclei stains brightly with phalloidin. It therefore does not represent some exotic form, but instead is comprised of mostly F-actin. This staining experiment is conceptually simple and so the reader may wonder why it was not included into the discussion of nuclear actin function before. One reason lies in the technical difficulties in the cytochemical analysis of a cell as large as a stage-VI oocyte (diameter of approximately 1.3 mm). For example, it takes hours to fix such cells with formaldehyde and it is then very hard to exclude the possibility of major rearrangements occurring during the treatment. Even with this reservation in mind, it should be noted that phalloidin-positive nuclei in formaldehyde-fixed *Xenopus* oocytes have previously been reported[25,26]. It now appears unjustified that the nuclear actin field ignored these studies to date.

The alternative method of cryofixation is very rapid, but poses the problem that the frozen sections also need to be fixed. This has normally

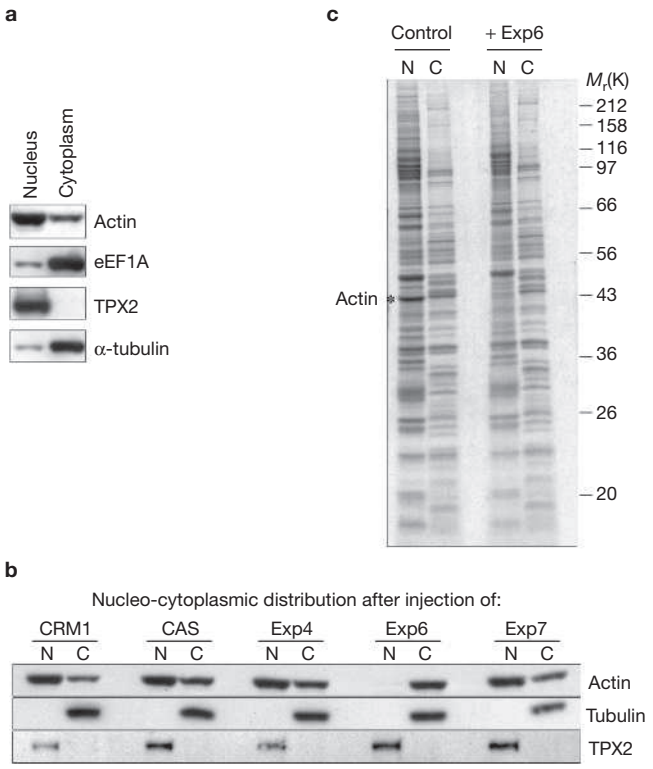

Figure 5 Exp6 depletes the large actin pool from nuclei of *Xenopus* ooctyes. (**a**) Stage-VI *Xenopus* oocytes were manually dissected, and resulting nuclear and cytoplasmic fractions analysed by immunoblotting with indicated antibodies. α-tubulin and eEF1A served as cytoplasmic markers and TPX2 as a nuclear marker. For a direct comparison of concentrations, loads were normalized to volumes of the respective compartments (nucleus ≈50 nl, yolk-free cytoplasm ≈500 nl). (**b**) Nuclei of *Xenopus* oocytes were injected with the indicated exportins (15 nl at 8 μM). After 60 min, the nucleo-cytoplasmic distribution of actin, tubulin and TPX2 was analysed by immunoblotting. (**c**) Total protein patterns of nuclei and cytoplasm from control oocytes and Exp6-injected oocytes were analysed by SDS–PAGE followed by Coomassie staining.

been done by drying the oocyte section on a glass slide, followed by fixation with formaldehyde or acetone[27,28]. We found that the intermediate steps of thawing and drying cause major rearrangements in nuclear-actin structure, which coincide with a loss of phalloidin stain (data not shown). Our protocol, which includes ultrafast freezing of the oocyte, freeze-substitution against acetone, and post-fixation with formaldehyde, avoids these problems. It revealed that nuclear actin stains with phalloidin as efficiently as cortical actin, suggesting that it is mostly comprised of F-actin.

Similarly to cytoplasmic stress fibres, the nuclear actin structures seem to be organized in bundles (Fig. 6b). However, there are clear differences to cytoplasmic actin structures. The most striking one is that these nuclear-actin bundles are not linear, but branched into a three-dimensional meshwork. It is probable that a nucleus-specific factor crosslinks F-actin to this characteristic form. Such a factor may also explain another apparent paradox: actin seems to enter oocyte nuclei passively (data not shown); the free nuclear G-actin concentration should therefore not exceed the cytoplasmic one. Yet, total actin levels in the nucleus are clearly higher than in the bulk cytoplasm (Fig. 6a). We therefore suggest that bundling of F-actin favours actin polymerization and thereby traps actin inside nuclei. It is still unknown which factors crosslink these actin filaments, but this question certainly deserves thorough investigations. Also, it needs to be seen how the atypical actin filaments[29], which appear to support the lamina and to contain actin as well as the 4.1 protein, relate to the structures we observed.

High nuclear actin levels represent clearly an exceptional situation and a cell-type-specific phenomenon. The fact, that *Drosophila Exp6* is encoded by an essential gene, indicates that too high levels of nuclear actin either interfere with developmental programmes or can be toxic on a cellular level. Likewise, we observed that injection of antisense morpholino-oligonucleotides, which prevent *Exp6* expression, into two-cell *Xenopus* embryos has a lethal effect; the embryos do not survive beyond stage 20 (data not shown). A high nuclear actin concentration therefore seems to occur only when the Exp6 pathway is inactivated by a developmental programme or is impaired otherwise. Nuclear actin has been observed after dimethyl sulphoxide treatment of cultured cells[30–32], as a consequence of senescence[33] or certain types of stress[34]. We would now suggest that these conditions primarily affect the Exp6 export pathway; nuclear actin accumulation would be a consequence of that.

Why do amphibian oocytes, but not somatic cells, require an intranuclear actin-skeleton? The most obvious difference between these cell types is size. Mature oocyte nuclei are in diameter (450 μm) approximately 50 times and in volume even approximately 100,000 times larger than somatic nuclei[15]. Larger objects experience greater mechanical forces than smaller ones and therefore need stronger mechanical support.

Oocyte nuclei harbour a similar DNA content as a normal G2 cell; why then do they need to be so exceedingly large? A mature oocyte stockpiles material for more than the next 12 cell generations. This material not only comprises cytoplasmic components, but also enormous amounts of nuclear proteins, such as 5×10^{12} chaperone-bound histone molecules[15], which suffice to pack 3.1×10^{13} base pairs of DNA, that is, the DNA-content of approximately 15,000 G1 cells[35], into chromatin. These nuclear proteins contain nuclear import signals and are therefore pumped into the nuclear compartment[36,37], where they occupy volume for themselves and for the water that keeps them in solution. It therefore seems that nuclear protein import inflates oocyte nuclei to their enormous size. This import is so massive that even this large nucleoplasm reaches a protein concentration of approximately 100 mg ml^{-1} (ref. 38).

This consideration brings us to a second and probably even more crucial difference between oocytes and 'normal' cells. As a consequence of the greatly increased volume, the DNA concentration in the oocyte nucleus is 100,000 fold lower than in somatic nuclei. The content of oocyte nuclei therefore appears to be a liquid protein solution. This can even be directly observed, when nuclei of Exp6-treated oocytes break during dissection and eject their liquid contents into the buffer (see Supplementary Information, Movies 1 and 2). In contrast, somatic nuclei are essentially filled with the solid compound of chromatin and nuclear matrix, which is much harder to deform than a liquid. This is a second clear reason why an intranuclear actin skeleton is dispensable in somatic cells, but required to maintain the mechanical integrity of the vastly inflated and seemingly liquid-filled oocyte nuclei.

Oocyte nuclei seem to be stabilized by two scaffold-types of different geometry: by the three-dimensional-interconnected intranuclear actin network and the hollow-sphere-shaped lamina, which directly supports the nuclear envelope. For certain somatic nuclei, the lamina has been shown to provide such a substantial mechanical support that it has been referred to as a 'molecular shock absorber' (see ref. 39 and

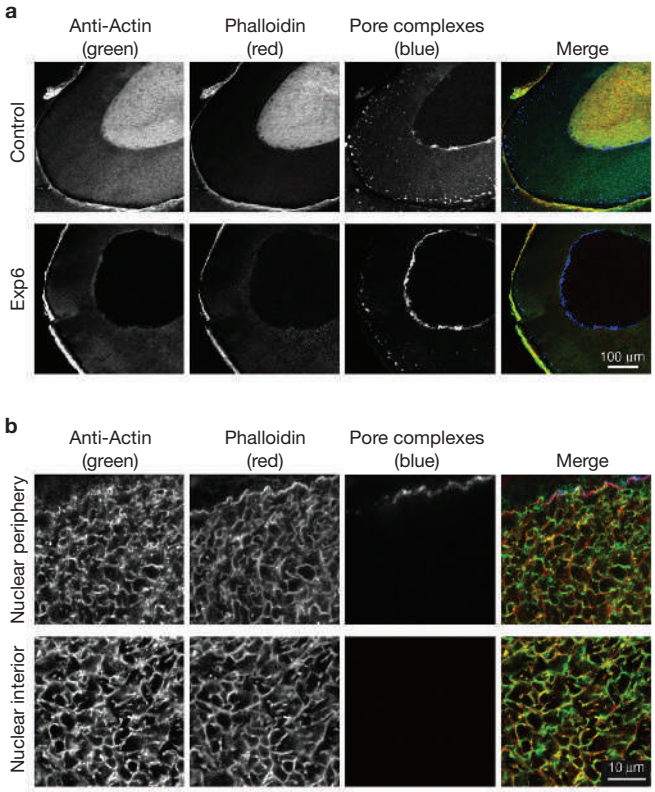

Figure 6 A sponge-like actin meshwork within nuclei of *Xenopus* oocytes. Sections of *Xenopus* oocytes (see Methods) were stained with antibodies against actin (C-terminal epitope) and TRITC–phalloidin to detect filamentous actin. mAb414 recognizes several nuclear pore complex proteins and was used to delineate the nuclear boundary (however, it also stains cytoplasmic annulate lamellae). Fluorescence images were recorded by CLSM. (**a**) A comparison of actin staining in control oocytes and 60 min after Exp6 injection. The intranuclear actin structures disappeared after Exp6 injection. The faint actin signal at the nuclear envelope of Exp6-injected oocytes probably represents export intermediates with Exp6-bound G-actin. (**b**) Higher magnification reveals a branched actin meshwork that spans the entire nucleus. This meshwork has a similar appearance in the nuclear interior and near the nuclear periphery.

references therein). This poses the question of how far the two scaffold-types contribute to mechanical stability.

Let us consider a somatic nucleus and an oocyte nucleus with a 50-fold larger diameter. Let us further assume that both types of nuclei are filled with liquid and stabilized only by the hollow-sphere-shaped lamina. To achieve the same pressure resistance, oocyte nuclei would need a lamina, whose local strength and thus thickness is 50-fold greater than in the somatic cell[40]. However, the lamina of oocytes comprises just a single layer of orthogonally crossing lamin filaments[41] and is hence not thicker than in somatic cells. This already implies that only a small fraction of the mechanical support, which oocyte nuclei need, can actually originate from the lamina. The fact that Exp6 makes oocyte nuclei so extremely fragile supports this conclusion and suggests that the intranuclear actin meshwork represents the major stabilizing element.

Up to now, numerous cases of regulated nuclear transport have been described[42], examples being the movement of transcription factors Pho4p or NFκB, which can switch between a cytoplasmic and a nuclear localization, depending on the cellular requirements. In all these cases, relocalization is achieved by regulating the access of the cargo (for example, by cargo phosphorylation) to otherwise constitutively active nuclear import or export pathways. The alternative strategy of switching an entire nuclear transport pathway on or off was, to our knowledge, not observed in interphase cells so far. The reason for that appears obvious: switching an entire pathway will change the localization of all cargoes that 'rely' on this transport receptor. In the case of a broad-spectrum receptor, such as CRM1, the effects would be pleiotropic and finely tuned physiological responses impossible.

Exp6 is now the first example, where an exportin is the immediate subject of regulation. The fact that Exp6 carries seemingly only a single transport substrate, namely actin[6], explains why, in this case, nature could make an exception to the above-mentioned rule. Normal actin function might be impaired by additional transport-regulating modifications. The option of regulating the nucleo-cytoplasmic actin-distribution independently of other parameters might therefore have been the 'reason' why Exp6 was 'invented' in evolution.

METHODS

Recombinant protein expression and purification. Human Exp6 was expressed and purified as described[6]. *Xenopus laevis* (x)Exp6 was expressed with an N-terminal His-zz-TEV-tag. Purification was performed on Ni-NTA–Sepharose, followed by cleavage with TEV protease and removal of protease and tag.

RNA work. Total RNA from *Xenopus laevis* oocytes or eggs was prepared by the guanidine isothiocyanate/phenol method. Oocyte RNA was reverse-transcribed with an oligo-dT primer and used as template for PCR-amplification of the coding region of xExp6.

For Northern blot analysis, total RNA was separated on a 0.8% denaturing agarose gel and transferred onto a nitrocellulose membrane. *xExp6* mRNA was detected with a radiolabelled DNA probe comprising nucleotides 2,251–3,408 of the open reading frame (ORF).

Microinjection of *Xenopus* oocytes and nuclear fragility assays. *In vitro* transcription–translation of actin isoforms and GST, sample preparation, oocyte injection and subsequent analysis were performed as described[10]. The stability of oocyte nuclei was studied during manual dissection. Oocytes from the same frog were analysed in parallel under all conditions shown and the experiment was repeated with material from 5 different frogs.

***In vitro* maturation of *Xenopus* oocytes.** Collagenase-treated stage-VI oocytes were incubated in 5 µg ml^{-1} progesterone, until the appearance of the maturation spot indicated germinal vesicle breakdown (after 6–10 h). From thereon, oocytes were further incubated for times indicated. Analysis was performed by immunoblotting and histone H1 kinase assays.

Fertilization of eggs and cultivation of embryos. Eggs were first mixed with macerated testes in Ringer's solution, then incubated in 0.1x Barth solution for 20 min and subsequently treated with 2% cysteine to remove the jelly coat. After several washes, embryos were finally incubated in 0.1x Barth supplemented with penicillin/streptomycin until extract preparation and analysis by immunoblotting.

Antibodies. Anti-actin (#A2066) and anti-tubulin (#T9026) were from Sigma (Taufkirchen, Germany); Antibodies against Xkid and TPX2 were kind gifts from A. Nebreda and O. Gruss, respectively. Antibodies against Impβ, transportin, CRM1, CAS/Exp2, Exp-t, Imp5, Imp7, Imp9, Imp13 (ref. 43 and references therein), eEF1A[5], xExp5 and xExp7 (ref. 10) have been described previously. Anti-xExp6 was raised in rabbits against residues 668–1135. All polyclonal antibodies were used after affinity purification.

Actin-staining in *Xenopus* oocytes. The key problem was to visualize the actin-distribution inside the nucleus without generating artefacts during sample preparation. This might easily occur when the oocytes are manually dissected in the standard (actin-polymerizing) buffer[18].

Therefore, whole oocytes were quick-frozen in −150 °C cold isopentane (initial 'cryofixation') and were subjected to freeze substitution for 48 h with anhydrous acetone at −30 °C. Water and acetone are not freely mixable at this temperature,

but separate into a solid aqueous phase and a liquid acetone-phase (with ≤10% water), in which proteins are insoluble. Importantly, the water does not melt by freezing-point depression in the acetone, but instead dissolves leaving dehydrated and fixed proteins behind. Such fixation therefore avoids an aqueous liquid phase in which larger re-arrangements of actin could possibly occur. The oocytes where then covalently fixed with 2% formaldehyde in 90% acetone + 1 mM Pipes at pH 6.8 for 2 h at room temperature. This suppresses autofluorescence of yolk platelets and prevents loss of soluble proteins in subsequent steps. After stepwise re-hydration, the specimens were equilibrated in PBS containing 25% sucrose, 8% sorbitol and 2% glycerol (w/v), quick-frozen as above and cut into 30-µm-thick cryosections. The sections were then directly transferred into PBS (without intermediate drying) and stained with indicated primary antibodies (rabbit anti-actin and mouse mAB414), fluorescent secondary antibodies (Alexa488 anti-rabbit and Alexa647 anti-mouse) and with TRITC-labelled phalloidin. After extensive washing, the specimens were post-fixed with 4% formaldehyde, mounted in Vectashield, and examined by CLSM.

DDBJ/EMBL/Genbank accession number. The nucleotide sequences of *Xenopus* Exp6 is listed under accession number AJ865375.

Note: Supplementary Information is available on the Nature Cell Biology website.

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COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.

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