Nuclear positioning is an important process during development and homeostasis. Depending on the affected tissue, mislocalized nuclei can alter cellular processes such as polarization, differentiation, or migration and lead ultimately to diseases. Many cells actively control the position of their nucleus using their cytoskeleton and motor proteins. We have recently shown that during Drosophila oogenesis, nurse cells employ cytoplasmic actin cables in association with perinuclear actin to position their nucleus. Here, we briefly summarize our work and discuss why nuclear positioning in nurse cells is specialized but the molecular mechanisms are likely to be more generally used.

Keywords: nuclear positioning, Drosophila, nurse cells, dumping, actin cables, perinuclear actin, filamin, LINC complex

Abbreviations: hts-RC, hu li tai sao ring canal isoform; LINC, linker of nucleoskeleton and cytoskeleton; Msp-300, muscle-specific protein 300; MACF1/ACF7, microtubule actin crosslinker factor 1/actin crosslinker family protein 7; TAN lines, transmembrane actin-associated nuclear lines

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Nuclear positioning by actin cables and perinuclear actin
Special and general?

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Introduction

Cells use their cytoskeleton to exert force on their nucleus and move it into position within the cytoplasm. All three cytoskeletal structures, actin microfilaments, microtubules, and intermediate filaments, can mediate nuclear positioning, either acting alone or together.1,2 Even though different cell types use similar proteins to ensure nuclear positioning, the exact molecular mechanisms are tissue specific and thus reflect adaptations to cell type-specific conditions.3,4 For example, LINC complexes are essential during nuclear positioning via microtubules during neurogenesis, or via actin in fibroblasts, but they are not essential for nuclear positioning via intermediate filaments in astrocytes.4,7 Similarly, mutations in genes that lead to human diseases associated with defects in nuclear localization have tissue-specific symptoms; periventricular heterotopia is associated with defects in nuclear migration during neurogenesis,8 while Emery Dreifuss Muscular Dystrophy is associated with mislocalized nuclei in muscles.2 We presented recently a new mechanism for nuclear positioning employing actin cables and perinuclear actin in Drosophila nurse cells.9 Here we argue that an understanding of the molecular mechanisms in the special case of nurse cells might reveal general molecular mechanisms of nuclear localization and thus help to understand molecular aspects of human diseases associated with nuclear mispositioning.

A Revised Model for Nuclear Positioning in Drosophila Nurse Cells

Nurse cells are an ideal model system to study nuclear positioning as it is an essential process during Drosophila oogenesis; loss of nuclear positioning leads to small infertile eggs.10 Each egg is generated by 15 nurse cells and one oocyte within each egg chamber (Fig. 1). The nurse cells produce most of the material that is important for the early embryonic development within the laid egg. This material is transported from nurse cells into the oocyte, the future egg, through cytoplasmic bridges called ring canals that connect nurse cells with each other and with the oocyte. At the end of oogenesis,
Nurse cells contract and dump all their cytoplasmic content through the ring canals into the oocyte to generate a full-size mature egg. During this dumping process, the localization of nurse cell nuclei is critical, as the loss of nuclear positioning leads to nuclei clogging the ring canals, blocking cytoplasmic flow into the oocyte and thereby resulting in small dumpless eggs with impaired development.

Seminal work by several groups led to a model in which segmented actin cables form a cage around the nucleus, blocking cytoplasmic flow into the oocyte and thereby resulting in small dumpless eggs with impaired development. Seminal work by several groups led to a model in which segmented actin cables form a cage around the nucleus, blocking cytoplasmic flow into the oocyte and thereby resulting in small dumpless eggs with impaired development.

We provided a revised model for nuclear positioning in nurse cells, in which filopodia-like actin cables position nuclei in association with perinuclear actin. Whereas previous evidence suggested that actin cables were built from segments of actin bundles, we found that actin cables were unsegmented and continuously synthesized from tip complexes at the membrane, like filopodia except that the actin cables extend inward rather than outward. When nurse cells shrink, we found that the actin cables wrap around the nucleus, rather than retracting. Furthermore, in the live imaging it was clear that the growth of the actin cables was pushing the nucleus away from the ring canals, rather than just being a passive barrier to nuclear movement. In addition, we never saw actin cables bypassing the nucleus once they had reached it, despite the continuous cable growth and the obvious tension on bending actin cables.

For these reasons we sought to understand how the actin cables were anchored to the nucleus. We were surprised that the most likely candidate, the LINC complex, was not involved, even though it localized to the perinuclear ends of actin cables. We identified a perinuclear actin meshwork associated with numerous actin-binding proteins that decorate the end of the actin cables close to the nucleus. While we think that the link between actin cables and the perinuclear actin is critical for nuclear positioning, we were not able to demonstrate this conclusively, because removal of candidate actin crosslinkers either had no effect on nuclear positioning, or blocked oogenesis at an earlier step. For example, the actin crosslinker filamin has an early perinuclear localization before actin cable formation and later accumulates

Figure 1. Nuclear positioning in Drosophila nurse cells. Nuclear positioning is essential during oogenesis for the development of layed eggs (above). Before dumping (until stage 10A), the round nurse cell nuclei have perinuclear actin, to which filamin localizes. At stage 10B filopodia-like actin cables in association with perinuclear actin position nuclei toward the outside, away from the ring canals. Filamin localizes to the perinuclear ends of actin cables. Dumping, a process in which nurse cells expel their content into the oocyte (orange arrow), starts at the same time. The continuous contraction of nurse cells leads to nuclei turning in order to accommodate the long actin cables in the shrinking cells (here shown at stage 12). The middle row shows egg chambers of the mentioned stages, each egg chamber with one nurse cell marked by a blue nucleus. Zooms into these marked nurse cells are shown in the row below (nc, nurse cell; oc, oocyte; A, anterior; P, posterior; D, dorsal; V, ventral).
at the perinuclear ends of actin cables. Our data suggest that filamins crosslink actin cables to perinuclear actin: the accumulation at the perinuclear ends of actin cables required the proximity of the nucleus, as actin cables that did not reach the nucleus failed to accumulate filamin at their perinuclear ends. Furthermore, both localizations of filamin, the early and the late, depend on a dynamic actin structure since Latrunculin B treatment abolished the early localization and reduced the localization to the ends of actin cables (without affecting the cables). However, the multiple functions of filamin and actin during oogenesis prevented us from testing whether the association of actin cables with perinuclear actin is essential for nuclear positioning. The knockout or knock-down of filamin affected the formation of ring canals, which are essential for dumping,\textsuperscript{16} and the treatment of egg chambers with Latrunculin B blocked nurse cell contraction, also blocking dumping. Our results suggest that multiple proteins function redundantly in linking the actin cables to the perinuclear actin and the nucleus. These redundant proteins include actin crosslinkers like filamin and α-actinin, the actin-microtubule crosslinker spectraplakin, the adducin-like hts-RC, and the LINC complex.

The identification of this new way of positioning nuclei raises the question of whether any other types of cells use similar molecular machinery to control the movement of their nuclei.

### Nuclear Positioning in Nurse Cells, a Special Case Using Conserved Molecular Machinery

There are increasing numbers of examples where actin structures utilize force from actin polymerization to push nuclei into position. One example is the role of transmembrane actin-associated nuclear (TAN) lines in repolarizing migrating fibroblasts that mediate nuclear localization by coupling the nucleus to moving dorsal actin cables.\textsuperscript{5,6,17,18} These actin cables develop from an isotropic actin mesh close to the nucleus in a myosin II-dependent manner. LINC complexes, which connect the cytoskeleton to the nucleoskeleton via a bridge of KASH proteins in the outer and SUN protein in the inner nuclear membrane,\textsuperscript{19} link the actin cables to the nuclear lamina, forming the TAN lines on the dorsal side of the nucleus. Defects in the linkage between actin cables and the nucleus impair nuclear positioning and thereby lead to compromised cell migration and wound healing. A second example is the apical actin caps that regulate nuclear movement and nuclear shape and are also formed by apical (or dorsal) actin cables.\textsuperscript{20-23} The actin cables of the apical cap differ from the actin cables of TAN lines by the fact the former link to basal focal adhesion sites whereas the latter do not.\textsuperscript{24} The formation of actin cables of the apical cap relies on the bundling of perinuclear actin filaments mediated by filamin A and refilin B.\textsuperscript{25} Apical actin caps regulate shape and movement of nuclei and these processes have been associated with an efficient epithelial-mesenchymal transition and cell migration.\textsuperscript{22,25} Similar to TAN lines, LINC complexes connect the actin cap to the nucleus and the nuclear lamina.\textsuperscript{25} Other examples of actin structures mediating nuclear positioning are the actin mesh that is essential to move the nucleus forward during the interkinetic nuclear migration in pseudostratiﬁed epithelia of zebrafish,\textsuperscript{26} actin ﬁlaments that restrict nuclear movement and anchor nuclei to the cortex during early Drosophila embryogenesis,\textsuperscript{27} and actin-LINC complexes that anchor muscle nuclei in the worm Caenorhabditis elegans.\textsuperscript{28}

As discussed above, also in nurse cells actin structures generate forces by actin polymerisation to push the nucleus into position. But whereas nuclei connect in mammalian cells via LINC complexes to dorsal actin cables in TAN lines or to apical actin caps, or to actin in worm muscle cells, nurse cells do not rely on the LINC complexes to connect their force-generating actin structure to the nucleus.\textsuperscript{29,30} We speculate that dumping nurse cells use an adapted mechanism for nuclear positioning because of their special developmental program. Before dumping, nurse cells nuclei are highly polyploid (1024C),\textsuperscript{31} relative round, and dynamic with their “wriggling” mobility depending on microtubules.\textsuperscript{32} When nurse cells build their actin cables and dumping starts, their nuclei stop moving and are multilobed, indicating a lower stiffness of the nuclear envelope. Shortly after that, or at the same time, nurse cells initiate a cell death program that involves so far unknown molecular mechanisms.\textsuperscript{33} During dumping and nuclear positioning, signs for occurring cell death appear, including DNA fragmentation and permeabilization of the nuclear envelope.\textsuperscript{34} This cell death of nurse cells is closely linked with dumping, but does not dependent on it; cell death occurs in dumpless mutants, even though later as in controls.\textsuperscript{35} Thus, nurse cells overcome the challenge of localizing their large, soft, degenerating nucleus against the cytoplasmic flow with the help of actin cables. In this scenario, the engagement of the LINC complex appears counterproductive since LINC complexes stabilize the nucleoskeleton by linking it to the (perinuclear) cytoskeleton. This would prevent the breakdown of the nuclear envelope, which is part of the developmental program of nurse cells. In fact, a delay in the breakdown of the nuclear envelope or cell death correlates with inefficient nurse cell dumping.\textsuperscript{36} Instead, our data suggest nurse cells have adapted the molecular machinery to their special “doomed” condition to engage multiple links between actin cables and the perinuclear actin in order to localize their large, soft nucleus. This molecular machinery includes nesprins (Msp-300), the actin crosslinkers filamin and α-actinin, the actin-microtubule crosslinker spectraplakin, and the adducin-like hts-RC. Our data suggest that these factors might act redundantly to ensure the localization of the soft nuclei in nurse cells: we found multiple actin crosslinking proteins localized at the perinuclear ends of actin cables and that nesprins were not essential for nuclear positioning and dumping,\textsuperscript{29,30} even though the localization of nesprins to the perinuclear ends of actin cables required the proximity of cables and nucleus.
Future experiments will be needed to determine how far conserved are in fact the functions of these proteins and the perinuclear actin during nuclear positioning in different tissues and organisms. Filamins and spectraplakins are particularly promising candidates for more general use during nuclear positioning. Filamins control nuclear shape in apical actin cap, even though by a different function; in the apical actin cap, they control the bundling of actin filaments to actin cables rather than the crosslinking of actin filaments. The spectraplakin MACF1/ACF7 impairs cell polarization and cell migration in wound healing assays, similar to mutations affecting nuclear positioning by dorsal actin cables in fibroblasts, but its role during nuclear positioning is unknown.\(^{37}\)

Finally, other cellular processes might employ the proposed molecular machinery regulating the connection of actin to the nucleus during nuclear positioning. For example, actin filament, which link the membrane with the nuclei, are important for mechanotransduction to the nucleus.\(^{38}\) The molecular nature of these connections between membranes, actin filaments, and nuclei are not well known, but cells might employ the same molecular machinery, adapted for mechanotransduction instead of nuclear positioning.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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