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SPOTLIGHT

Nesprin-2G tension fine-tunes Wnt/β-catenin signaling

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How LINC complexes mediate nuclear mechanotransduction remains unclear. In this issue, Déjardin, Carollo, et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201908036) show that the LINC complex protein nesprin-2G is a mechanosensor of epithelial–mesenchymal transitions (EMTs), recruiting α-catenin to the nucleus to attenuate Wnt/β-catenin signaling.

Nuclear mechanotransduction is the ability of cells to convert physical forces into biochemical activities and changes in gene expression within the nucleus (1). Nuclear envelope (NE)–spanning molecular bridges known as LINC (linker of nucleoskeleton and cytoskeleton) complexes mediate this process. LINC complexes are composed of SUN and KASH proteins found in the inner and outer nuclear membranes, respectively. In this issue, Déjardin, Carollo, et al. (2) shed light on how LINC complexes promote nuclear mechanotransduction.

Déjardin et al. approached this problem by inserting a genetically encoded, calibrated FRET (Förster resonance energy transfer)-based tension biosensor module (TSMod; 3) into the previously described functional nesprin-2G construct, mini-nesprin-2G (4). Nesprin-2G is an ∼800-kD protein comprised of a pair of tandem, actin-binding calponin homology (CH) domains at its N terminus followed by 56 spectrin-like repeats (SRs), a transmembrane domain, and a KASH peptide that engages SUN proteins within the perinuclear space (5). The residues found between SRs 2 and 55 of nesprin-2G are deleted in mini-nesprin-2G. Since TSMod consists of a pair of donor and acceptor fluorescent proteins separated by a polypeptidic linear-elastic spring, FRET is inversely proportional to force. This method has been used to quantitatively image mechanical tension across a number of proteins in living cells, including those found at the cell cortex as well as in cell–cell and –matrix adhesions (6).

The authors' cytoskeletal binding mini-nesprin-2G construct (CB) localized properly to the NE of MDCK epithelial cells and NIH 3T3 fibroblasts. As a tension-insensitive control, the authors generated a variant of CB impaired in its ability to interact with actin due to the presence of a previously described I128,131A mutation within its CH domains (CH mutant; 4). As expected, the CH mutant exhibited a FRET index that was significantly higher than CB. Thus, the authors concluded that CB was under actin-dependent mechanical tension. Through a series of pharmacological experiments, the authors showed that CB is held under ∼8 pN of tension generated by the actomyosin and microtubule cytoskeletons, which is balanced by cell–cell adhesion.

It should be noted that Arsenovic et al. were first to insert the TSMod into mini-nesprin-2G, allowing them to demonstrate that nesprin-2G is subject to actomyosin-dependent tension and sensitive to cell elongation (7). They inserted TSMod in between SR2 and SR55 of mini-nesprin-2G (7), whereas Déjardin et al. inserted it between SR56 and the transmembrane domain (2). Interestingly, the latter group found that CB was more sensitive to changes in cell density than the one generated by Arsenovic et al. Conversely, the original construct revealed tension differences across apical versus equatorial planes of the nucleus (7). These data show that inserting TSMod at different sites within the same protein can have consequences for FRET-based measurements. These effects might arise because different regions of mini-nesprin-2G may be subject to different amounts of tension. Given such differences, it will ultimately be important to interrogate tension changes on the endogenous full-length nesprin-2G using current gene-editing techniques.

Having established the utility of their tension sensor constructs for performing FRET-based molecular tension microscopy on nesprin-2G, the authors sought to determine if nesprin-2G tension was sensitive to extracellular mechanical cues. They showed data indicating that cytoskeleton-generated tension on CB drops in squeezed nuclear regions during the migration of MDCK cells through an array of microfabricated obstacles containing constrictions that were smaller than the diameter of their nuclei. Déjardin et al. also found that substrate stretching and reduced cell density increased CB tension, suggesting that nesprin-2G acts as a mechanical sensor of cell packing.

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Next, the authors sought to test the hypothesis that LINC complexes participate in epithelial–mesenchymal transition (EMT)–dependent activation of β-catenin/Wnt signaling, based on evidence that cells lining the wound front are more sensitive to Wnt signals than cells packed behind the front (8). When they measured the FRET index of CB-expressing sheets of MDCK cells subjected to scratch wounding, they found a gradient consistent with nesprin-2G being under higher molecular tension at the wound front relative to the back of the monolayer. Since the FRET index correlated with internuclear distance and not cell velocity, Déjardin et al. concluded that the increased tension was due to reduced cell packing and not increased cell migration velocity. All together, these results strongly suggest that the wound front generates increased cytoskeleton-dependent tension on nesprin-2G due to decreased cell packing.

Déjardin et al. then asked if the molecular tension across nesprin-2G behaved similarly in a more complete model of EMT, where exposure of MDCK cell colonies to hepatocyte growth factor (HGF) results in decreased cell packing before cell scattering (9). Unlike during partial EMT, the FRET index of CB significantly increased over time regardless of HGF. This unanticipated result indicated that cytoskeletal tension experienced by nesprin-2G in MDCK cell colonies is not at a steady state and that it slowly relaxes in a time- and cell density–dependent manner. Consequently, the authors concluded that nesprin-2G is a mechanotransducer that discriminates between partial and complete EMT programs.

Finally, Déjardin et al. wanted to identify molecule(s) that might be able to differentially interact with tense or relaxed nesprin-2G at the NE. Previous work found that SRs 53 and 54 of nesprin-2G directly interact with the actin-binding region of α-catenin, recruiting it to the NE most prominently in cells lacking robust cell–cell adhesion (10). Evidence that cells found at edges of wounded epithelial sheets register stronger β-catenin nuclear accumulation and signaling than the cells further back in the monolayer (8, 9), along with evidence that α-catenin can antagonize β-catenin signaling (11, 12), raised the possibility that tension-dependent changes in nesprin-2G might alter α-catenin recruitment to the NE and therefore its capacity to limit β-catenin signaling during EMT. Indeed, α-catenin was most prominent at the NE under conditions where CB was relaxed. These data suggest that cell packing states and their effects on the conformation of nesprin-2G might serve to limit Wnt/β-catenin signaling within a narrow biophysical range (Fig. 1).

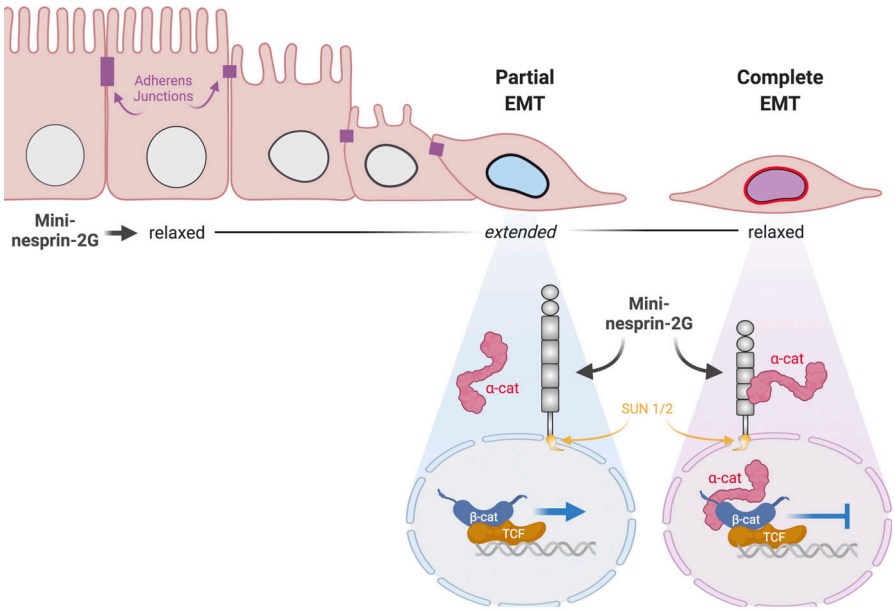

Figure 1. **A Biorender.com-generated illustration showing that mini-nesprin-2G at the NE undergoes cytoskeleton-dependent extension under partial, but not complete, EMT.** This impairs its ability to recruit α-catenin (α-cat) and thus limit β-catenin (β-cat) signaling through end point mediators like the transcription factor T cell factor (TCF).

In summary, this work advances mechanistic understanding of how LINC complexes mediate nuclear mechanotransduction. Déjardin et al. propose that nesprin-2G is a mechanotransducer that fine-tunes Wnt/β-catenin signaling during EMT (Fig. 1). Given the size of nesprin-2G, we speculate that there are many more tension-dependent interacting partners awaiting discovery. Finally, it will be interesting to investigate how the NE-independent regulation of β-catenin signaling by N-terminal nesprin-2 variants (13) influences nesprin-2G–dependent mechanotransduction as epithelial cells acquire mesenchymal, fibroblast-like properties.

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