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SPOTLIGHT

The bioenergetics of nucleocytoplasmic transport

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How nucleocytoplasmic transport (NCT) rates change due to cellular physiology-mediated fluctuations in GTP availability remains unclear. In this issue, Scott et al. (https://doi.org/10.1083/jcb.202308152) demonstrate that cell migration, spreading, and nucleocytoskeletal coupling impact GTP levels, thereby regulating NCT, RNA export, and protein synthesis.

The nucleus regulates molecular movement between the cytoplasm and nucleoplasm via nuclear pore complexes (NPCs) embedded in the nuclear envelope (NE) (1). Importins and exportins facilitate this process, regulated by the small GTPase Ran, which maintains a gradient by binding GTP and GDP in the nucleoplasm and cytoplasm, respectively. In this issue, Scott et al. investigate how changes in GTP availability, influenced by cellular physiology, affect nucleocytoplasmic transport (NCT) rates (2).

Using biosensors for nuclear import (live-cell light-inducible nuclear localization signal [LINuS] [3]) and GTP levels (4) in human fibroblasts, they observed that reducing GTP levels by inhibiting its precursor synthesis impaired NCT rates, while inhibiting protein synthesis increased GTP levels and NCT rates. These results indicate that altering GTP-consuming processes impacts NCT, with increased GTP availability enhancing NCT rates.

Next, the authors examined the impact of substrate stiffness and cell spreading on GTP levels and NCT rates. They found that cells on softer substrates had higher GTP levels and NCT rates compared to those on stiffer substrates. Trypsinizing cells to round them and then replating to initiate spreading showed that NCT significantly decreased in spread cells compared to rounded ones. To validate these findings, a scratch-wound assay using biosensor-expressing human epithelial cells showed decreased GTP levels and NCT rates in migrating, wound-edge cells relative to less spread or motile monolayer cells. Scott et al. (2) concluded that natural changes in cellular behavior, like spreading induced by substrate rigidity or migration, significantly alter GTP levels and NCT rates.

The role of the cytoskeleton and linker of nucleoskeleton and cytoskeleton (LINC) complex–dependent nucleocytoskeletal coupling was also investigated. LINC complexes are molecular bridges that span the NE, linking the nucleus to cytoskeletal filaments (5). They consist of SUN and KASH proteins in the inner and outer nuclear membranes, respectively. LINC complex inhibition via RNAi-mediated co-depletion of SUN1 and SUN2, or overexpression of a SUN1-based dominant negative construct increased GTP levels and NCT rates. SUN2 depletion alone mirrored the effect of the double knockdown, indicating SUN2 as a key component influencing GTP availability and NCT rates. Depletion of the actin- and microtubule-interacting KASH proteins Nesprin-1 or -2, but not the intermediate filament-interacting Nesprin-3 (5), led to increased GTP levels and NCT rates. Moreover, depolymerizing actin or microtubules increased GTP levels and NCT rates, while depleting vimentin did not change either parameter.

The differential requirement for SUN2 in affecting GTP levels and NCT rates relative to SUN1 needs further investigation considering SUN1's established roles in de novo NPC assembly and mRNA nuclear export (6). Since the actin cytoskeleton consumes ~50% of the total ATP consumption (7) and GTP is synthesized from ATP (8), perhaps this is related to the fact that SUN1 and SUN2 function separately to support the coupling of nesprin-2 to microtubules and actin filaments, respectively (9). Clearly, many questions remain regarding how LINC complexes regulate NCT.

Scott et al.'s findings contradict recent studies suggesting that increased forces on the nucleus enhance NCT by dilating NPCs (10, 11). Instead, Scott et al. (2) propose that transportin-cargo binding and release are the rate-limiting steps in NCT. Surprisingly, the authors were unable to reproduce the previous findings, as LINC complex inhibition enhanced the nuclear export of the light-inducible nuclear export (LEXY) biosensor (3) in mouse embryonic fibroblasts. As an explanation for this discrepancy does not currently exist, further work is needed to reconcile these seemingly contradictory results.

To see if these findings apply to natural NCT processes, Scott et al. (2) examined dexamethasone-inducible glucocorticoid receptor nuclear import (12) and found similar results to those obtained via LINuS or LEXY. Changes in GTP levels also influenced Ran dynamics, with conditions that elevate GTP availability increasing Ran's nuclear export and cytoplasmic localization.

Scott et al. (2) further explored the impact of GTP levels on RNA export and protein synthesis. They found that inhibiting GTP precursor synthesis decreased RNA

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export, while increasing GTP levels increased cytoplasmic RNA levels. Protein synthesis rates were higher in fibroblasts on softer substrates and increased with SUN2 depletion. These findings suggest that GTP levels influence all Ran gradient-dependent NCT processes, not just specific transportins. Reduced GTP availability particularly hinders the export of rRNA and tRNA, which are crucial for ribosome function, likely restraining protein synthesis without affecting mRNA levels that use Ran-independent export (13).

The authors propose a feedback loop where the sensitivity of Ran-mediated NCT to GTP levels decreases RNA export and protein synthesis, conserving GTP during cellular energy shortages. Normally, GTP availability can limit NCT. Processes that reduce GTP- or ATP-consuming activities enhance NCT. Ran's lower affinity for GTP compared to GDP, the similar affinity of Ran's guanine nucleotide exchange factor RCC1 for GTP- or GDP-bound Ran, and the high GTP consumption needed to maintain the Ran gradient make Ran highly sensitive to GTP level fluctuations (14). Elevated NCT rates correlate with increased cytosolic Ran levels, likely due to the inability of the rate-limiting NCT factor NTF2 to reimport Ran-GDP as quickly as Ran-GTP is exported.

These results suggest that NCT rates are indirectly regulated by cellular forces primarily through their impact on free GTP levels. Previous studies suggesting mechanical forces enhance NCT by stretching NPCs are not supported by these findings (10, 11). Scott et al. (2) hypothesize that LINC complex perturbation increases GTP levels and NCT rates, potentially through direct interference with cytoskeletal dynamics or cell motility. The sensitivity of the Ran gradient to GTP levels affects RNA export and protein synthesis. Bioenergetic regulation of NCT allows cells to adapt to energy fluctuations associated with various cellular processes. This mechanism may contribute to defects in NCT observed in neurodegenerative diseases linked to altered bioenergetics (15), potentially explaining why postmitotic cells like neurons are more vulnerable to bioenergetic declines and reduced NCT.

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