FRAP ANALYSIS OF NUCLEAR EXPORT RATES IDENTIFIES INTRISIC FEATURES OF NUCLEOCYTOPLASMIC TRANSPORT

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Targeting a quantitative description of carrier-mediated nuclear export in live cells, we fused a prototypical leucine-rich nuclear export signal (NES) to GFP as a model cargo and expressed the fluorescent chimera into live CHO-K1 cells. The relevant parameters of NES-mediated nucleocytoplasmic transport were recovered by Fluorescence Recovery After Photobleaching (FRAP) following an established theoretical description of kinetic exchanges between the cytoplasm and the nucleus [1-3]. By this approach we were able to calculate the affinity of the expressed NES for the export machinery and the maximum rate of nuclear export achievable at saturation of endogenous carriers. We found out that the active-export time through the Nuclear Pore Complex (NPC) is 18 ms, similar to the maximum import rate that we previously determined by both relaxation [1] and fluctuation measurements [4, 5]. Additionally, we highlighted that export is unaffected by the co-expression of saturating levels of a fluorescently-labeled nuclear import signal (NLS), thus indicating that nuclear import and export are uncoupled. The same uncoupled behavior was demonstrated between nuclear export and passive diffusion. Our results suggest gating properties of single nuclear pores.

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