Enzymatically Driven Transport: A Kinetic Theory for Nuclear Export

Sanghyun Kim and M. Elbaum*
Department of Materials and Interfaces, Weizmann Institute of Science, Rehovot, Israel

ABSTRACT Nuclear import and export are often considered inverse processes whereby transport receptors ferry protein cargo through the nuclear pore. In contrast to import, where the reversible binding of receptor to nuclear RanGTP leads to a balanced bidirectional exchange, termination of export by physiologically irreversible hydrolysis of the Ran-bound GTP leads to unidirectional transport. We present a concise mathematical model that predicts protein distributions and kinetic rates for receptor-mediated nuclear export, which further exhibit an unexpected pseudolinear relation one to the other. Predictions of the model are verified with permeabilized and live cell measurements.

INTRODUCTION

Macromolecular exchange between the cell nucleus and cytoplasm takes place via the nuclear pores (1,2). These large protein assemblies traverse both lipid bilayers that form the nuclear envelope, and they provide a mechanism of selectivity for passage of specific macromolecules. In particular, passage of a given molecular substrate, or cargo, can occur both autonomously and in a facilitated manner, mediated by soluble protein receptors. Autonomous (i.e., passive) transport allows free passage of small solutes, extending to proteins up to a molecular weight of tens of kDa (3). Cargo charge and hydrophobicity are also likely determinants of the nuclear-pore permeability (4,5). Such passive transport is simply described by classical Fick theory, which predicts a steady state with equal concentrations in the cytoplasm and nucleoplasm (6). Receptor-mediated transport, on the other hand, can lead to a selective accumulation on one side or the other of the nuclear envelope, i.e., nuclear import or export. According to the signal hypothesis, nuclear-localization-signal (NLS) and nuclear-export-signal (NES) peptides label protein cargo for accumulation in the nucleus or cytoplasm. The associated receptors are known as importins and exportins, respectively, or collectively as karyopherins.

The small GTPase Ran plays a crucial role in determining net transport directionality. Activities of the Ran guanosine exchange factor (RanGEF) and the GTPase-activating protein (RanGAP) create a step at the nuclear envelope, with Ran predominantly in the GTP state inside, and in the GDP state in the cytoplasm (7). Importins bind RanGTP competitively to their NLS cargo, whereas exportins bind the two substrates, NES-cargo and RanGTP, cooperatively. Accordingly, import cargo should be released from the importin receptor upon arrival in the nucleus by replacement with RanGTP, whereas export cargo is released from the exportin receptor on arrival in the cytoplasm by hydrolysis of the Ran-associated GTP. Thus, import and export are often considered to be complementary directional transport processes. An essential distinction, however, is that exchange of cargo versus RanGTP substrate on importin receptors is a reversible biochemical association, whereas GTP hydrolysis is enzymatically driven and is nearly irreversible under physiological conditions in which the GTP/GDP ratio is held far from equilibrium by cellular metabolism.

Based on titration assays and fluorescence photobleaching recovery, we have shown that steady-state nuclear accumulation, i.e., import, represents a balanced, bidirectional flux across the nuclear envelope (8). This was explained in a chemical partitioning model based on reversible molecular interactions (9) and generalized in a kinetic model that incorporates both passive and receptor-mediated transport processes (6). Nuclear export is more challenging to assay, since it is difficult to distinguish bona fide export from simple nuclear exclusion. A convenient experimental approach is to use small substrates such as green fluorescent protein (GFP)-NES with a significant autonomous flux (10). Quantitative interpretation then requires a mathematical model (10,11). Even qualitative deductions may depend on that model in subtle ways.

Here, we present a general kinetic model of nuclear export together with supporting experiments. The approach follows in spirit our earlier analysis of import (6), i.e., the solution of a minimal set of coupled equations using the classical matrix method. Again the aim is to find an analytically solvable model with only essential parameters. In comparison to import, we find a very different behavior and insight for export. Steady-state distribution and FRAP measurements were used to verify salient predictions of the model. Briefly, to the extent that the hydrolysis of Ran-bound GTP is irreversible, export will be a truly unidirectional transport process.

MATERIALS AND METHODS

Cloning

Cloning of the enhanced-GFP (EGFP)-signal constructs was performed by polymerase chain reaction with EGFP plasmid (pKW430) (13) and primers
including sequences encoding localization signal peptides (NES or NLS), followed by insertion to the mammalian cell expression vector, pCDNA3 (Invitrogen, Carlsbad, CA). EGFP-x sequences in these constructs were transferred to PET28 vector by restriction and ligation to produce the same construction with His-tag for protein expression in Escherichia coli.

**Cell culture**

HeLa cells were cultured in DMEM with 10% fetal bovine serum and supplemented with L-glutamine and antibiotics (penicillin/streptomycin) at 37°C in 5% CO₂.

**Concentration calibration and fluorescence correlation spectroscopy**

We verified the linear response of the measured fluorescence intensity versus protein concentration by fluorescence correlation spectroscopy (FCS) in vitro, performed on the PicoQuant FCS upgrade kit (PicoQuant, Berlin, Germany) for Olympus Fluoview 300 (BX50WL, Olympus Europe, Hamburg, Germany). Calibration results appear in section 1-1 of the Supporting Material. A water-immersion objective lens (60×/1.2W; Olympus) was used to focus the laser beam, and Atto488 dye (D = 560 μm/10 s) was used to determine the size of the detection volume. A wide range of concentrations of purified EGFP were prepared and diluted in Xenopus egg extract. Concentration was measured by the zero-time plateau in FCS and compared to the same imaging conditions. The linearity of the confocal fluorescence intensity with concentration is shown in section 3-1 of the Supporting Material. Calibration at high concentrations was continued by dilution. The laser power for FCS was ~65 μW of the Ar laser for EGFP. The cross-correlation function was calculated with SymphoTime software (PicoQuant) after the 50/50 beam splitter on the detection path and 496 nm long-pass emission filters.

**Transfection and live-cell imaging**

For live-cell imaging, the cells were plated 1 day before transfection on an 18-mm-diameter glass coverslip to obtain ~50% confluence. The transfected cells were mounted to a temperature-maintained chamber for the confocal microscope. The growth medium for live imaging contains supplementary HEPES to maintain pH, without Phenol red. Experiments were performed between 24 and 48 h post-transfection. New fluorescent protein expression made a negligible contribution during data collection (see section 1-2 in the Supporting Material).

**Import assay in permeabilized cells**

HeLa cells were plated 1 day before observation on an 18 mm square glass coverslip with ~10% confluence. Cells were washed with cold transport buffer (110 mM KOAc, 20 mM HEPES, pH 7.3, 2 mM Mg(OAc)₂, 1 mM EGTA, and 2 mM diithothreitol) and permeabilized for 5 min in transport buffer containing 10 μL/mL digitonin (Calbiochem, Billerica, MA). Cells were then rinsed with transport buffer five times and mounted with a spacer of 100-μm-thick double-sided tape to a 24 mm coverslip overlaid with the reaction mixture. After both coverslips were stuck together, the gap between the two coverslips was sealed with paraffin wax. The standard reaction mixture contained a total of 16 μL volume/coverslip, with 10 μL interphase Xenopus egg extract, 2 μL energy regeneration mixture (1 mM GTP, 1 mM ATP, 10 mM phosphocreatine, and 50 μg/mL creatine phosphokinase), 0.5 μL Hoechst for chromatin staining, 1 μL TRITC-dextran 150 kDa for negative control of nuclear transport, and the substrate. Xenopus egg extract was prepared as described in Kopito and Elbaum (8). Samples were monitored on the microscope at 30°C.

**RESULTS AND DISCUSSION**

**Steady-state nuclear accumulation ratio**

Images were analyzed using ImageJ (13) for region-of-interest (ROI) determination of the nucleus and cytoplasm, and for measuring the average intensity in each compartment (section 1-3 in the Supporting Material). The signal from the growth medium was used to estimate the background noise intensity.

**Fluorescence recovery after photobleaching**

FRAP measurements were performed on the confocal microscope using XYT time-lapse scanning. Photobleaching of GFP was achieved by repetitive scans of a small area (512 × 512 pixels) in the middle of the nucleus or cytoplasm for a few seconds with full laser power (630 μW), and the recovery was monitored within a few seconds after bleaching. Because the diffusion of GFP fused to the nuclear transport signal in each compartment is very fast, <0.2 s (see section 1-4 in the Supporting Material), the entire area of the bleached compartment became dimmer homogeneously during the photobleaching step, and the measured recovery is due only to transport across the nuclear envelope.

The fluorescence intensity in each compartment recovered as a function of time with a single-exponential form sharing the same time constant, τ. In each experiment, the normalized nuclear and cytoplasmic intensities were fit as an exponential function in Global fitting mode in OriginLab with time constant τ: \[ I(t) = A_0 e^{-t/\tau} + B_0 \] and \[ I_N(t) = A_1 e^{-t/\tau} + B_1. \] In the case of cells with low expression levels there was a detectable bleaching effect during the recovery, so a linear term was added to the fitting function to account for the bleaching as \[ I(t) = A_N e^{-t/\tau} + B_N + C_{IN}. \]

**Kinetic model for nuclear export**

We consider the two major kinetic processes for nuclear export, translocation of cargoes or receptor complexes across the pore on the one hand, and receptor-binding kinetics on the other. Cargos and cargo-receptor complexes are tailored separately, with the possibility of exchange between them according to simple mass-action kinetics. The model incorporates the following specific assumptions.

1. Translocation of cargos \([C^+_N]\) or export-receptor-RanGTP-cargo complexes \([ER^+_C]\) across the pore depends on the difference in concentrations times a permeability (Fick’s law). The permeabilities relevant to the two modes are \(p\) and \(a\), respectively, referring to the common nomenclature of passive and active transport. Nuclear and cytoplasmic localizations are denoted by superscripts \(N\) and \(C\).

2. Export-cargo binding to the receptor in the absence of RanGTP, \([R]\), is negligible compared to binding in the presence of RanGTP, i.e., \([EC^+_C] \approx 0\) (14). Cargo-containing components are thus \([C^+_N]\) in the nucleus and \([ER^+_C]\) in the nucleus only.

3. The cooperative assembly of transport complex (15) is considered in two steps for simplicity: \([ER^+_C] \Rightarrow [ER^+_C] + [C^+_N]\) and \([ER^+_C] \Rightarrow [ER^+_C] + [C^+_N]\).

4. GTP hydrolysis on Ran is fast and complete in the cytoplasm, so that \([ER^+_C] \cong 0\).
These components and their interactions are sketched in Fig. 1. At steady-state two more conditions hold.

5. The total exportin receptor concentration in the nucleus, \( E^N \), is constant.

6. The exportin receptor equilibrates with available nuclear RanGTP, \([R]^N\) with affinity \( K_{ER} \).

Finally, protein concentrations in the nucleus and cytoplasm are considered to be well mixed. Spatial gradients are not considered, because the time for diffusion on length scales relevant to simple somatic cells are much faster than the observed transport kinetics. Fast intracompartment diffusion has been noted previously (16) and is consistent with the data presented here (see section 1-4 in the Supporting Material).

For each cargo \( i \), we write three kinetic equations reflecting its change in compartment or binding state to the receptor:

\[
\frac{d[C_{ei}]^N}{dt} = \frac{p_i}{v_N} \left( [C_{ei}]^N - [C_{ei}]^C \right) + \left( -k_{i}[C_{ei}]^N[E_{ei}]^N + k'_{ei}[ERC_{ei}]^N \right)
\]

\[
\frac{d[C_{ei}]^C}{dt} = \frac{p_i}{v_C} \left( [C_{ei}]^N - [C_{ei}]^C \right) + \frac{a_i}{v_C}[ERC_{ei}]^N
\]

\[
\frac{d[ERC_{ei}]^N}{dt} = \frac{a_i}{v_N} (-[ERC_{ei}]^N) + \left( k_{i}[C_{ei}]^N[E_{ei}]^N - k'_{ei}[ERC_{ei}]^N \right)
\]

and in steady-state,

\[
[E]^N_{SS} + [ER]^N_{SS} + \sum_i [ERC_{ei}]^N_{SS} = E^N_{SS}
\]

\[
k_{ER}[E]^N_{SS}[R]^N_{SS} = k'_{ER}[E]^N_{SS}.
\]

\( E^N_{SS} \) refers to the total concentration of export receptor in the nucleus, which equals the sum of the free receptors, receptors in complex with RanGTP, and receptors in trimeric complex with RanGTP and export cargo. Note that the total number of cargo is conserved in the system as Eq. 1 satisfies

\[
\frac{d[V_N]}{dt} + \frac{d[V_C]}{dt} + \frac{d[ERC_{ei}]^N}{dt} = 0.
\]

As a result, for each cargo, only two of the equations in Eq. 1 are truly independent.

**NES cargo exchange kinetics at steady state**

At steady state, we have a set of first-order differential equations with constant coefficients, \( a_i/v_N, p_i/v_N, a_i/v_C, k_{ei}[E]^N_{SS}, k'_{ei}, k_{ere}, k_{ere}' \), so the set can be rewritten in matrix form and solved by diagonalization of the coefficient matrix.

\[
\frac{d}{dt} \begin{pmatrix} [C_{ei}]^N \\ [C_{ei}]^C \\ [ERC_{ei}]^N \end{pmatrix} = \begin{pmatrix} -\frac{p_i}{v_N} - k_{ere} & \frac{p_i}{v_N} & k'_{er} \\ \frac{p_i}{v_C} & \frac{p_i}{v_C} & a_i \\ k_{ere} & 0 & -\frac{a_i}{v_N} - k'_{er} \end{pmatrix} \begin{pmatrix} [C_{ei}]^N \\ [C_{ei}]^C \\ [ERC_{ei}]^N \end{pmatrix}
\]

Given the simple first-order linear form, we expect solutions as sums of simple exponentials.
Roots of the characteristic equation define eigenvalues whose negative inverse provide the characteristic time constants:

\[
\begin{align*}
\left( -\frac{p_i}{v_N} - k_e \right) [ER]_{SS}^N + \lambda & \left( -\frac{p_i}{V_C} - \lambda \right) \left( -k_e a_i/v_N - \lambda \right) \\
& - \frac{p_i}{V_N} \left\{ \frac{p_i}{V_C} - k_e a_i/v_N - \lambda \right\} \\
& + k_e \left( \frac{p_i}{V_C} + \lambda \right) [ER]_{SS}^N = 0 \\
\end{align*}
\]

One of the eigenvalues is zero. The remaining equation is quadratic, and of the two roots, one is much smaller than the other. Therefore measured kinetics should follow closely a single-exponential behavior according to the longer time constant.

FRAP is an ideal technique to measure this exchange time scale, since starting in the steady state means that all the relevant coefficients are indeed constant. Thus, we find the kinetic solution for each compartment as follows.

\[
f(t) = A + B_1 e^{-t/\tau_1} + B_2 e^{-t/\tau_2} = A + B_2 e^{-t/\tau_2}
\]

with time constants

\[
\tau_2^{-1} = \frac{1}{2} \left( \frac{a_i}{v_N} + \frac{p_i}{v_N} + \frac{a_i}{v_C} + k_e [ER]_{SS}^N + k_e \right)
\]

\[
\pm \frac{1}{2} \sqrt{\frac{a_i}{v_N} + \frac{p_i}{v_N} + \frac{a_i}{v_C} + k_e [ER]_{SS}^N + k_e} \cdot \left( -4 \frac{a_i}{v_N} \left( \frac{p_i}{v_N} + \frac{a_i}{v_C} + k_e [ER]_{SS}^N + a_i \right) \right)
\]

**Steady-state distributions**

Steady-state distributions are found by setting the time derivatives in Eq. 1 to zero. The third component of Eq. 1 gives \([ERC]_{SS}^N = [k_e,i/(a_i/v_N) + k'_e,i)] [C]_{SS}^N | [ER]_{SS}^N\), and together with Eq. 3, the total receptor concentration, Eq. 2, is written as

\[
[ER]_{SS}^N \left( 1 + \frac{1}{[R]_{SS}^N/K_{ER}} + \sum_{j \neq i} \left( \frac{a_j}{v_N} + k'_e,i \right) [C]_{SS}^N \right) \left( \frac{k_e,i}{a_j/v_N + k'_e,i} \right) [C]_{SS}^N = E_{SS}^N
\]

This includes both the cargo of interest, indexed \(i\), and all other cargoes, indexed \(j\). In considering FRAP kinetics, \(C_{e,i}\) would likely be the fluorescent species, whereas \(C_{e,j}\) would represent endogenous, nonfluorescent export cargos. For that reason, we refer to the latter set as "dark matter". Its collective effect may be gathered into a single term: \(D = \sum_{j \neq i} (k_e,i)/(a_i/v_N + k'_e,i)) [C]_{SS}^N\). Note that the effect of dark matter competition for receptors is equivalent to reducing the available RanGTP, because it appears only in the combination \(1/([R]_{SS}^N/K_{ER})\). Therefore, the transport of distinct export substrates is independent at steady state, and in the following we drop the index \(i\) for simplicity.

The final steady-state solutions for the nuclear cargo and transport complex, \([C]_{SS}\) and \([ERC]_{SS}\), are written as:

\[
[C]_{SS}^N = \frac{1}{2} \left( -\frac{a_i}{v_N k_e} + K_e \right) \left( \frac{1}{[R]_{SS}^N/K_{ER}} + 1 + D \right) - \frac{a_i E_{SS}^N}{p} + [C]_{SS}^N
\]

\[
+ \frac{1}{2} \sqrt{\frac{a_i}{v_N k_e} + K_e \left( \frac{1}{[R]_{SS}^N/K_{ER}} + 1 + D \right) - \frac{a_i E_{SS}^N}{p} + [C]_{SS}^N}
\]

\[
[ERC]_{SS}^N = \frac{1}{2} \frac{p}{a} \left( \frac{a_i}{v_N k_e} + K_e \right) \left( \frac{1}{[R]_{SS}^N/K_{ER}} + 1 + D \right) - \frac{a_i E_{SS}^N}{p} + [C]_{SS}^N
\]

\[
- \frac{1}{2} \frac{p}{a} \sqrt{\frac{a_i}{v_N k_e} + K_e \left( \frac{1}{[R]_{SS}^N/K_{ER}} + 1 + D \right) - \frac{a_i E_{SS}^N}{p} + [C]_{SS}^N}
\]

\[
+ \frac{1}{2} \frac{p}{a} \left( \frac{a_i}{v_N k_e} + K_e \right) \left( \frac{1}{[R]_{SS}^N/K_{ER}} + 1 + D \right) - \frac{a_i E_{SS}^N}{p} + [C]_{SS}^N
\]
Asymptotic behaviors for the analytical solutions

The explicit solutions appear in analytical form and can be evaluated exactly, without simulation. At the same time, they are obviously complicated combinations of the parameters; at face value, they offer disappointingly little insight. We can gain a qualitative understanding by considering relevant limits. The following statements are justified in section 2 of the Supporting Material and summarized in Table 1.

First, in the absence of passive diffusion (\( p = 0 \)), at steady state, \([E_{RC}]^N = 0\) and \([C_e]^N = 0\), i.e., export is complete, because there is no return path. Including a nonzero passive permeability, i.e., setting \( p \neq 0 \), the ratio of NES cargo in the nucleus to that in the cytoplasm will be greater than zero but \( \leq 1 \).

Under conditions of RanGTP starvation, i.e., with \([R]^N_{SS}/K_{ER} < 1\) signifying either low concentration or weak affinity, or \( D > 1 \) implying low availability due to sequestration by competing export substrates in complex with receptors, the term \((1/[R]^N_{SS}/K_{ER} + 1 + D)\) > 1. In such cases, we cannot see nuclear exclusion of the NES cargo: \([C_e]^N_{SS} \approx [C_e]^N_{SS}\) and \([E_{RC}]^N_{SS} \approx 0\). The total nuclear fluorescence, the sum of the above terms, is just equal to the cytoplasmic fluorescence, so experimentally we would observe a ratio \( N/C \approx 1 \).

As \([R]^N_{SS}/K_{ER}\) becomes comparable to 1 and increases, we may see an effective nuclear export. However, there will also be a passive inward flux. Saturation of the directional export will set in as a function of cytoplasmic concentration, \([C_e]^C_{SS}\), with a transition at \([C_e]^C_{SS} \approx [a/p]E^N_{SS}\). In the low-cargo limit, where \([C_e]^C_{SS} < [a/p]E^N_{SS}\), we find \([C_e]^N_{SS} \approx 0\) and \([E_{RC}]^N_{SS} \approx [p/a][C_e]^C_{SS}\); there is little free nuclear cargo, as most of it is bound in complex with exportin and RanGTP. The measured nuclear fluorescence is then the sum of \([E_{RC}]^N_{SS} + [C_e]^N_{SS} \approx [p/a][C_e]^C_{SS}\). Therefore, from the observed fluorescence ratio \( N/C \approx p/a\), we obtain a direct measure of the ratio of passive to active permeability.

On the other hand, for high cargo concentration the total export concentration will be limiting. Thus, for \([C_e]^C_{SS}>[a/p]E^N_{SS}\), we find \([C_e]^N_{SS} \approx [C_e]^C_{SS} - [a/p]E^N_{SS}\) and \([E_{RC}]^N_{SS} \approx E^N_{SS}\). This yields \([E_{RC}]^N_{SS} + [C_e]^N_{SS} \approx [C_e]^C_{SS} - ([a/p] - 1)E^N_{SS}\). The term involving \( E^N_{SS}\) becomes less significant as \([C_e]^C_{SS}\) increases, so asymptotically \( N/C \rightarrow 1\). We call this situation "export failure".

The kinetic time constant (Eq. 4) also shows a simple asymptotic behavior in the same limits (see section 3 in the Supporting Material). At low cargo concentration, \( \tau = (\tau_0/\nu) [p/(p + 1)], \) is the time constant for passive flux, whereas at high NES cargo concentration, we find \( \tau \approx \tau_0\). In both cases, the permeabilities are more significant than the receptor-binding kinetics, and at high concentrations, export failure in the steady state correlates with passive transport kinetics.

Predictive plots of the theory

Predictions of the model appear in Fig. 2, where we plot solutions to Eqs. 4–7 using parameters relevant for GFP substrates (see figure caption). The total nuclear exportin concentration was taken to be 100 nM, comparable to CRM1 expression measured in Saccharomyces cerevisiae (17,18), inhibitory leptomycin B concentrations in Xenopus oocytes (15), and quantitative leptomycin B binding assays (19). The binding affinity, \( K_E = k_E/k_{E_0}\), was set at 20 nM according to its value for cooperative binding (20). The level of free RanGTP (which appears only as \([R]^N_{SS}/K_{ER}\) was adjusted to replicate the transition of export performance. Families of curves are shown in Fig. 2 scanning both exportin and RanGTP concentrations. The lefthand panels show steady-state nuclear concentrations and nuclear/cytoplasmic ratios versus cytoplasmic concentration. A small \( N/C\) ratio reflects effective export. As seen, starvation for RanGTP (or, equivalently, overabundance of competing NES-cargo; see above) results in loss of nuclear depletion.

<table>
<thead>
<tr>
<th>TABLE 1 Summary of predictions in various asymptotic regimes</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{1}{[R]^N_{SS}/K_{ER}} + 1 + D )</td>
</tr>
<tr>
<td>Limited RanGTP</td>
</tr>
<tr>
<td>( [C_e]^N_{SS} )</td>
</tr>
<tr>
<td>( [C_e]^N_{SS} )</td>
</tr>
<tr>
<td>( [E_{RC}]^N_{SS} )</td>
</tr>
<tr>
<td>( N/C = \frac{[C_e]^N_{SS} + [E_{RC}]^N_{SS}}{[C_e]^C_{SS}} )</td>
</tr>
<tr>
<td>( \tau )</td>
</tr>
</tbody>
</table>
even at low cargo concentrations (Fig. 2, A and B); nuclear and cytoplasmic concentrations equilibrate due to the passive permeability. Starvation for exportin receptors also leads to loss of nuclear depletion (Fig. 2, E and F), with a transition at \( |C_e|^{CSS} = (a/p)E_0^{SS} \) that moves progressively to lower cytoplasmic cargo level. Conversely, increase of exportin or RanGTP supports nuclear exclusion under higher cargo load.

The righthand panels in Fig. 2 depict kinetic rate predictions using the same parameters as above. Time constants appear as a function of cytoplasmic concentration in Fig. 2, C and G. These could be considered predictions for FRAP measurements. The most notable feature is that the dominant time constant depends on the cytoplasmic cargo concentration, in sharp contrast to simple passive diffusion, in which the time constant for exchange between two reservoirs is determined solely by the permeability of the channel that joins them. This unusual dependence arises from the implicit dependence of \(|ER|^N\) on \(|C_e|^C\) via Eq. 5. The smaller the NES-cargo concentration, the faster will be the exchange.

Pseudolinearity of \(N/C\) and \(\tau\)

In comparing Fig. 2, B and C and F and G, both the steady-state ratio and exchange rate saturate for \(|C_e|^{CSS}\) in the same shape. To examine their relation in equations, each was approximated as a function of \(|C_e|^{CSS}\). Where \(|C_e|^{CSS} < (a/p)E_0^{SS}, N/C = p/a\) and \(\tau = (p/a)\tau_0\). Where \(|C_e|^{CSS} > (a/p)E_0^{SS}\), we find approximate solutions \(N/C = ((|C_e|^{CSS} - (a/p)E_0^{EN})/|C_e|^{CSS})\) and \(\tau = \tau_0(|C_e|^{CSS} - (a/p)E_0^{EN})/(|C_e|^{CSS} - (a/p)E_0^{EN})\) (see section 3 in the Supporting Material).

Note that for very large cargo concentrations, the time constant and \(N/C\) ratio saturate to their passive values, \(\tau_0\) and 1, respectively, whereas at low concentrations both values are smaller by the same factor, \(p/a\). This is reflected in a pseudolinear relation between the kinetic rate and the steady-state \(N/C\) ratio, shown in Fig. 2, D and H. Such a relation could not be anticipated from qualitative arguments. Further, the relation is insensitive to both RanGTP and exportin concentrations and can be expected to be universal. This prediction should provide a salient test of the model.

Steady-state \(N/C\) ratio in permeabilized cells

Permeabilized cell assays allow measurement of molecular distributions by fluorescence intensity after titrating a specific quantity or concentration of cargo into a cell extract, all other factors remaining equal. Fig. 3 shows the...
steady-state distribution of GFP-NES as a function of the cytoplasmic concentration. The curve shows two saturations, at both high and low concentrations. According to the theory, the lower saturation represents the ratio of passive to active permeabilities of the nuclear envelope, \( p/a \). The passive permeability could be measured by FRAP for signal-free GFP in the same system, which gave a time constant of 194 s (see section 4-2 in the Supporting Material). Taking the nuclear volume at \( v_N = 800 \times 10^{-18} \text{ m}^3 \), we can estimate that \( p \approx 4 \text{ m}^3/\text{s} \). The numerical value of 0.4 for the ratio \( p/a \) indicates that the receptor-mediated translocation is 2.5 times faster than the passive, or \( a \approx 10 \text{ m}^3/\text{s} \). The crossover from low to high concentration behavior occurs at a cargo concentration of \( [a/p]E_{SS}^N \). In the measurement, this crossover appears at \( 0.7 \mu M \), from which we estimate the steady-state nuclear exportin concentration at \( \sim 300 \text{ nM} \), double the reported total in \( S. \text{ cerevisiae} (17) \). These values from the permeabilized system, using \( Xenopus \) egg extract as a source of cytosol, may be compared to the live-cell assays described below.

**Kinetic measurement of nuclear export**

To test the model prediction of a linear increase in recovery time with increasing concentration of NES-cargo, we performed FRAP measurements in both permeabilized and living cells. It is important to recall that the FRAP measurement is performed in a preexisting steady state, where flux in equals flux out. Therefore, measuring the recovery of nuclear fluorescence in permeabilized cells is equivalent to measuring the molecular exchange rate. As described above, GFP showed a recovery time constant of 194 s in permeabilized cells. Turning to GFP-NES, at low cytosolic concentrations recovery was very fast, 50 s or even less. Results are summarized in Fig. 4 (full data set results are given in section 4-1 of the Supporting Material). Note the clean, single-exponential fit and the strong dependence of the time constant on cytoplasmic concentration of export substrate. The extracted time constants are compiled and plotted as a function of cytosolic concentration (Fig. 4 C) or \( N/C \) ratio (Fig. 4 D). Fast recovery occurred under conditions in which the steady-state \( N/C \) ratio was low, indicating active export. At the higher cytosolic concentrations, where export failed and the \( N/C \) ratio reached 1, recovery was slow and in fact comparable to that of the signal-free GFP, as predicted.

FRAP measurements were then repeated in live cells. We first established the rate of passive permeation by expressing a signal-free GFP substrate. Bleaching of either the nucleus or the cytoplasm yielded identical time constants in each cell tested (nine cells), \( \sim 40 \text{ s} \) (Fig. S7). It is simple to show that the time constant should be \( p(1/[v_N] + [1/v_C])^{-1} \) according to simple diffusion theory, from which we obtain a permeability of \( p = 20 \text{ m}^3/\text{s} \). (This is larger than the value obtained in permeabilized cells with Xenopus egg extract, but of similar order.) FRAP was then repeated for cells expressing GFP-NES (17 cells). Several examples are shown in Fig. 5 B for different \( N/C \) ratios. In comparison with the permeabilized-cell assay, higher cytoplasmic concentrations were supported before saturation of the export system occurred, yet the trend is identical to that seen in permeabilized cells. When \( N/C < 1 \), recovery is very fast, but when \( N/C = 1 \), it is equal to the passive time constant seen with signal-free GFP. These results appear in Fig. 5, C and D, plotted versus cytoplasmic concentration, \( [C_{e1}]_{SS}^C \), and \( N/C \) for comparison to the theory. The scatter is much larger than in the permeabilized-cell assay, but the same trend of a time constant increasing in proportion to the steady-state \( N/C \) ratio is apparent. In addition, the CRM1 inhibitor leptomycin B (21) leads to equal concentrations (\( N/C = 1 \)) and gives the same recovery time constant, \( \tau_0 \), as seen for the passive transport of GFP (six cells tested; see section 4-5 in the Supporting Material).

**CONCLUSIONS**

In this work, we have formulated receptor-mediated nuclear export in a simple mathematical model of coupled transport equations. The model encompasses both active

![Biophysical Journal 105(9) 1997–2005](image)
export and passive diffusion in one. Accounting for both modes is especially important in the study of nuclear export. As translation occurs in the cytoplasm, in principle nuclear exclusion would be sufficient to ensure cytoplasmic localization, and experimentally the two may be difficult to distinguish. The model is formally similar to a pump-leak model, proposed earlier for nuclear import (22), though it differs from our reversible import paradigm. Our model is solved self-consistently by standard methods and makes straightforward predictions for both steady state and dynamics. Although the complete analytical solutions involve complicated combinations of all the relevant input parameters, the model predictions simplify greatly in relevant limits and are in agreement with the experimental results. In particular, saturation of the \(N/C\) ratio at low concentration yields a measure of the permeability ratio, \(pl/\), whereas the crossover concentration to export failure occurs at \([a/p]E_N^N\).

The model and comparison with experiment highlight fundamental differences between mechanisms of receptor-mediated nuclear import and export. The first is that transport via exportin receptors is essentially irreversible, because of the enzymatic disruption of the exportin-cargo-RanGTP complex. Export kinetics are dominated by the permeability of the nuclear envelope, in contrast to import, where receptor binding represents the rate-limiting parameter. The export system steady-state shows a clearer saturation behavior and transition point than the import system, where it is asymptotically a Langmuir binding shape (6). Second, the influence of nonfluorescent competing cargoes, i.e., dark matter, is fundamentally different for export than for import in a tracer-based experiment such as FRAP. In the case of export, these cargoes effectively reduce the available RanGTP, whereas in import, their competition for transport receptors buffers the transport properties explicitly (6). The third difference is that the nuclear export system is very easily saturated in comparison with the import machinery. We find that export failure occurs even at submicromolar cargo concentration, to be compared with saturation of NLS-mediated import at several-micro- molar concentration (9).

**SUPPORTING MATERIAL**


The plasmid pKW430 was a kind gift of Karsten Weis. Both authors designed experiments, analyzed results, and wrote the article jointly. S.K. performed the experiments and developed the mathematical model.

This work was supported in part by a grant from the Israel Science Foundation and by the Gerhardt M. J. Schmidt Minerva Center for Supramolecular Architecture. The lab has benefited from the historical generosity of the Harold Perlman family.

**REFERENCES**