JCB
Online Supplemental Material

Sheff et al. http://www.jcb.org/cgi/doi/10.1083/jcb.200111048

Description of the mathematical modeling equations and curve fits.

Modeling of Tfn recycling traffic was based on experimental data for internalization and recycling of Tfn. ^{125}I Tfn was bound on ice to adherent CHO-hTfnR cells in 6-well corning culture dishes for 45 min in PBS supplemented with Ca^{2+} and Mg^{2+}. Cells were washed on ice to remove nonspecifically bound Tfn. More nonspecifically bound Tfn was removed by washing the cells for 0 s with DME at 37°C. Cells were allowed to internalize Tfn in DME with 0.5 mg/ml unlabeled Tfn and the media fully exchange at each time point. Counts in the media were determined at each time point and counts remaining in the cells at the finish of each experiment. For internalization rates, cells were warmed for 0, 2, 4, 6, or 8 min and then plunged into 4°C PBS. Surface counts were removed with washes of PBS brought to pH 2.9 and PBS pH 7.4.

All bound counts for each well were normalized to the total counts specifically bound in that well.

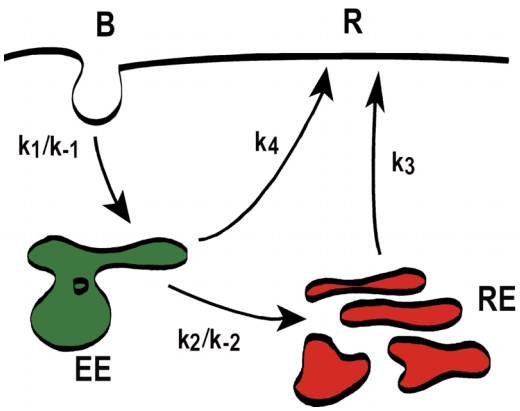
Models were based on first order rate constants between compartments as described previously. Variables were defined as:

B = material bound to basolateral membrane	EE = basolateral early endosomal compartment
RE = recycling endosome compartment	R = recycled ligand in the media
k_1 = rate of transport of ligand from B to EE	k_{-1} = rate of transport of ligand from EE to B
k_2 = rate of transport of ligand from EE to RE	k_{-2}= rate of transport of ligand from RE to EE
k_3 = rate of transport of ligand from RE to R	k_4 = rate of transport of ligand from EE to R

Note that k_{-1} differs from k_4 in that ligand returning to the surface along the k_{-1} pathway has not passed through an acidic compartment and is not released into the media.

The assignment of rate constants is graphically displayed (right). When a pathway is absent, the value k_n is set to zero for that pathway. Negative subscripts indicate retrograde pathways. The equations describing transit of ligand were integrated to yield the following algebraic forms for calculation:

$$B = B_0 e^{-k1t} + EE_0(1-e^{-k-1t})$$
$$EE = EE_0 e^{-(k-1+k4+k2)t} + B_0(1-e^{-k1t}) + RE_0(1-e^{-k-2t})$$
$$RE = RE_0 e^{-(k3+k-2)t} + EE_0(1-e^{-k2t})$$
$$R = R_0 + EE_0(1-e^{-k4t}) + RE_0(1-e^{-k3t})$$



Modeling and curve fitting was performed using Microsoft Excel 98 solving for all equations at 0.25-min time intervals and comparing to data at experimental time points (Microsoft) as described (Sheff, D.R., E.A. Daro, M. Hull, and I. Mellman. 1999. *J. Cell Biol.* 145:123–139). Goodness of fit was determined by sum squared error where:

$$SSE = \Sigma([\text{cumulative Tfn recycled at time I}] - [\text{Predicted value from model at time I}])^2$$

Statistical comparison of fits was performed by Fischer's F test as described (Motulsky, H.J., and L.A. Ransnas. 1987. *FASEB J.* 1:365–374).

$$F = \frac{(SSE_1 - SSE_2)/(df_1 - df_2)}{SSE_2/df_2}$$

Where SSE is sum square error and df is the degrees of freedom for each model. p values were derived from standard table for the calculated value of F and the degrees of freedom for the more complex (more parameters) model being compared.