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Supporting Material

Title: Multiple Cellular Proteins Modulate the Dynamics of K-ras Association with the Plasma Membrane

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Supporting Material for Bhagatji et al., 'Multiple Cellular Proteins Modulate the Dynamics of K-ras Association with the Plasma Membrane'

Kinetic Analysis of Prenylated Peptide Transfer between Lipid Vesicles

We assume that an amphiphile (here, a fluorescent prenylated peptide) is initially distributed symmetrically between the inner and outer leaflets of a population of 'donor' large unilamellar vesicles, under conditions in which all but a negligible fraction of the peptide is bound to the vesicles. A large excess of 'acceptor' vesicles is added at time zero, and the amount of peptide remaining associated with the donor vesicles, P(t), is monitored subsequently as a function of time. If $P_o(t)$ and $P_i(t)$ denote the concentrations of peptide associated with the outer and the inner leaflets of donor vesicles, respectively, the appropriate kinetic equations are

$$dP_o/dt = -k_d P_o + k_{flip} P_i \qquad [1a]$$

and

$$dP_i/dt = k_{flip} P_o - k_{flip} P_i \qquad [1b]$$

where k_d and k_{flip} are the first-order rate constants for dissociation of the peptide from the bilayer and for transbilayer diffusion of the peptide, respectively.

Solving this system of equations yields the general equation

$$P(t) = A_1 \exp(\beta_1 t) - A_2 \exp(\beta_2 t) \qquad [2]$$

where

$$\beta_1 = (-1/2)\left(k_d + 2k_{flip} - \sqrt{k_d^2 + 4k_{flip}^2}\right)$$

$$\beta_2 = (-1/2)\left(k_d + 2k_{flip} + \sqrt{k_d^2 + 4k_{flip}^2}\right)$$

$$A_1 = \left(P^0\left(b_1 + 2k_{flip}\right) + k_d P_i^0\right) \Big/ \sqrt{k_d^2 + 4k_{flip}^2}$$

and

$$A_2 = \left(P^0\left(b_2 + 2k_{flip}\right) + k_d P_i^0\right) \Big/ \sqrt{k_d^2 + 4k_{flip}^2}$$

where P^0 and P_i^0 are respectively the total concentration of peptide in the donor vesicles and the concentration of peptide in the inner leaflet of the donor vesicles at t = 0.

In the case where $k_{flip} >> k_d$, the time course adopts the simpler monoexponential form

$$P(t) = P^0 \exp(-f_{ext} k_d t) \qquad [3]$$

where f_{ext} is the fraction of total bilayer lipids exposed at the outer surface of the donor vesicles; in this case the value of k_d can be determined from the effective rate constant for (monoexponential) transfer process and the value of f_{ext}, which can be determined independently as described in the main text.

In the more general case in which the value of k_{flip} does not exceed k_d by a very large factor, the value of k_d is most easily determined from the initial slope of the progress curve for P(t); differentiating Eq. [2] above shows this slope to be $-k_d P_o^0$ where $P_o^0 = (P^0 - P_i^0)$ is the concentration of peptide present in the outer leaflets of the donor vesicles at time zero. If the donor vesicles are initially labeled 'symmetrically' (in both leaflets) with the peptide, $P_o^0 = f_{ext}P^0$ and the initial rate of transfer of peptide to the acceptor vesicles is $f_{ext}k_dP^0$. The value of k_d can then be determined as

$$k_d = \left.\frac{dF(t)}{dt}\right|_{t=0} \Big/ \Delta F_{max} \qquad [5]$$

where F(t) represents the sample fluorescence as a function of time and ΔF_{max} is the difference between the plateau ('infinite time') and initial fluorescence values in a given run. It can be shown that Eq. [5] is applicable even when the concentration of acceptor vesicles exceeds that of the donor vesicles by only a finite ratio, such that at equilibrium a nonzero proportion of the fluorescent peptide is associated with donor vesicles.

Legends for Supplementary Figures S1-S3

1. Confocal microscopic images of HeLa cells expressing CFP-Kras or FRB_2-CFP-Kras and transfected with siRNAs or plasmids to alter cellular levels of PDEδ, PRA1 and/or galectin-3. Panel (A) - Control cells expressing CFP-Kras. Panels (B), (C) – Cells expressing CFP-Kras and treated with the siRNAs designated as species 1 in Materials and Methods to knock down endogenous (B) PDEδ or (C) PRA1. Panel (D) - Cells treated with siRNAs to knock down both endogenous PDEδ and PRA1, and coexpressing CFP-Kras (upper portion) and the Golgi-localized marker GalT(1-80)-YFP (lower portion). Panels (E), (F) – Cells cotransfected with a CFP-KRas-encoding plasmid and a bicistronic plasmid encoding (E) PDEδ or (F) PRA1 together with YFP (as an expression marker, shown in the inset panels). Panel (G) - Control cells expressing FRB_2-CFP-Kras. Panels (H), (I) – Cells expressing FRB_2-CFP-Kras and overexpressing PDE (panel H) or PRA1 (panel I) from a bicistronic plasmid also encoding YFP (insets = YFP fluorescence). Panel (J) - Cells expressing FRB_2-CFP-Kras(G12V) and overexpressing galectin-3 from a bicistronic plasmid also encoding YFP (inset). Space bar = 10 μm. Experimental conditions were as described in Materials and Methods.

2. Panel (A) – qPCR-based determination of the extent of knockdown of mRNAs encoding galectin-3, PDEδ and PRA1 at the indicated times after treatment of wild-type HeLa cells with the second siRNA species indicated for each protein target in Materials and Methods. Panel (B) – Immunoblotting-based measurements of the level of endogenous galectin-3 in wild-type HeLa cells at the indicated times after treatment with the galectin-3-directed siRNAs designated as species 1 (black bars) and species 2 (hatched bars) in Materials and Methods.

3. Panel (A) - HeLa cells stably transfected to express HA-PRA1 were fractionated to obtain high-speed pellet (P) and supernatant (S) fractions as described previously (Roy et al., *Biochemistry* 39 (2000), 8298-8307), and equal portions of the two fractions were separated by SDS-PAGE and immunoblotted using anti-HA primary antibody. Panels (B) - (D) – Confocal images of a HeLa cell co-expressing (B) YFP-PRA1 and (C) the Golgi marker GalT(1-80)-CFP (panel C). While the distributions of the two markers appear grossly similar, as the merged image (D) illustrates (red = YFP-PRA1), only a fraction of the PRA1-YFP truly colocalizes with the Golgi marker (Pearson correlation coefficient = 0.60 for the two markers in the image pair shown). (E) - (G) – Confocal images of a cell co-expressing (E) YFP-PRA1 and (F) the late endosomal marker CFP-rab7 (plasmid generously provided by Dr. Scott Kiss, McGill University). Panel G – merged image (red = YFP-PRA1). The inset in the lower right-hand shows a second merge of the region enclosed by the dashed box in the main image, in this case with the component images shifted vertically by two pixels with respect to each other. The quality of the overlap in the inset is significantly poorer, illustrating that the overlap shown in the main image is not an artifact arising from the somewhat more diffuse distribution of the CFP-rab fluorescence. Space bar = 10 μm. Experimental details were as described in Materials and Methods.

Figure S1

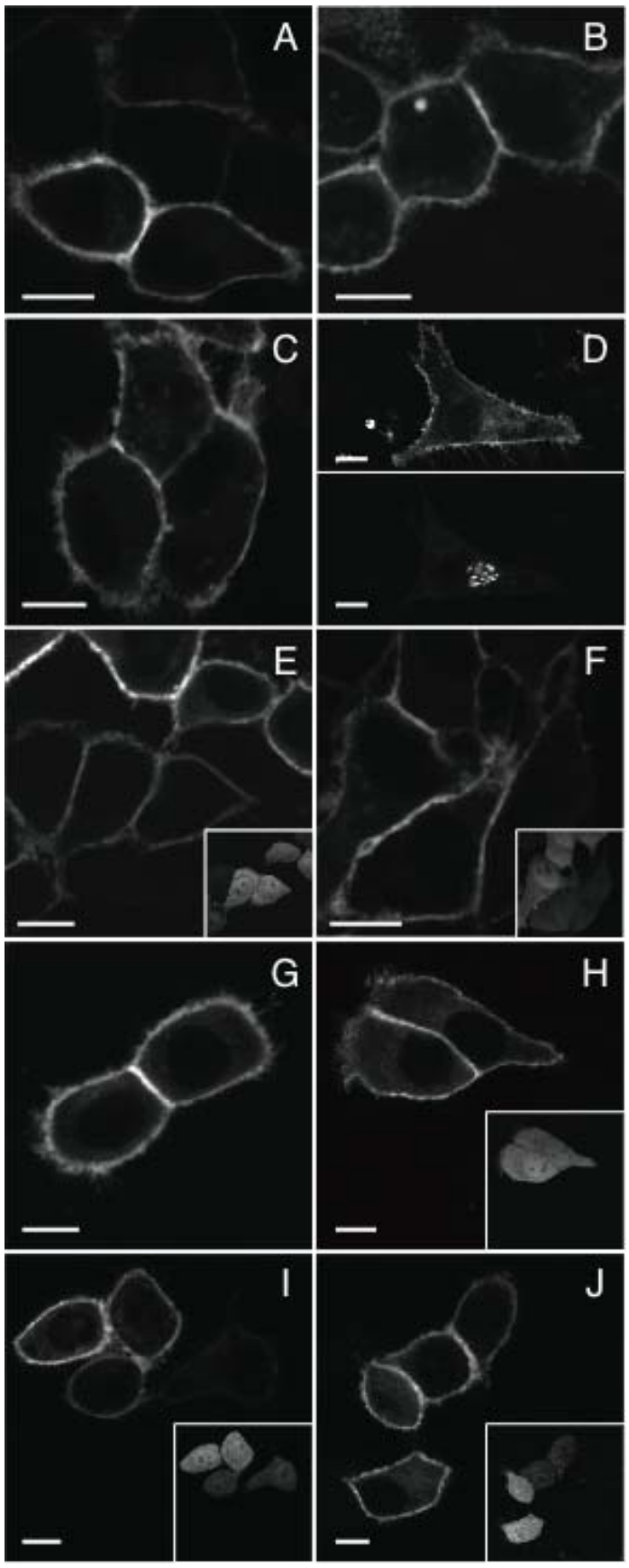
A
B
C
D
E
F
G
H
I
J

Figure S2

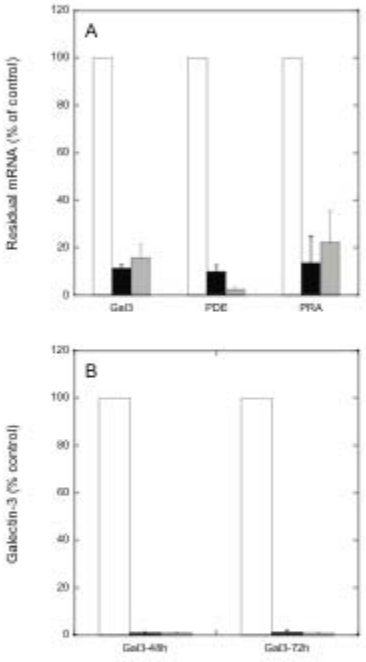
A
Residual mRNA (% of control)
120
100
80
60
40
20
0
Gal3
PDE
PRA
B
Galectin-3 (% control)
120
100
80
60
40
20
0
Gal3-48h
Gal3-72h

Figure S3