Reevaluation of the role of the small host cell GTPase Rab6 in the HIV-1 replication cycle

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Summary

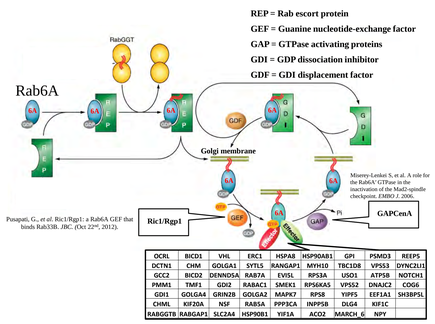
Background: The HIV-1 genome consists of only 9 genes and thus, depends highly on host cellular factors to replicate. Recent studies, through genome-wide siRNA screens, have identified many possible "HIV-1 dependency factors" HDFs. However, there lies a large inconsistency between these analyses and the majority of potential hits have not been verified in primary human target cells. Therefore, the goal of this proposal is to validate a recently identified possible HIV-1 Env-mediated host factor, Rab6A GTPase.

Methods: We utilized siRNA mediated knockdown in multiple cell lines and primary human monocyte-derived macrophages (MDMs) coupled with a HIV-1 single-round infection assay to define the mechanism by which this protein facilitates HIV-1 infection. Additionally, because Rab6A is ubiquitously expressed in two closely related isoforms, Rab6A and Rab6A', that have similar but distinct roles in the cell, both isoforms were investigated. Given that Rab6A is always cycling between active and non-active states, we examined the activity state needed for HIV-1 infection through dominant and constitutively active mutants of Rab6A, as well as knocking down the Rab6A-specific guanine nucleotide exchange factor (GEF), Ric1/Rgp1.

Results: siRNA knockdown of both isoforms of Rab6 in the U87 cell line confirmed its role in the viral replication cycle. Moreover, down regulation of both Rab6 isoforms in 293T cells used to generate virus demonstrated no effect on the overall production or infectivity of viruses, demonstrating that the protein facilitates processes only in the early stage of the replication. Contrary to previous reports, no dependency of the effect of knock down of Rab6 on the HIV-1 envelope was observed. Specific knock down of the individual isoforms, Rab6A and Rab6A', demonstrated that both were utilized by the virus but HIV-1 had a greater dependency upon Rab6A. Similar results were obtained with knockdown studies using primary human monocyte-derived macrophages. Diminishing Ric1/Rgp1 decreased infectivity revealing this Rab6A-specific GEF as an additional host factor for HIV-1.

Conclusions: Taken as a whole, these results demonstrate that Rab6 is co-opted by HIV-1 to facilitate a post entry step in the infection process and this is dependent on Ric1/Rgp1 activity. Further investigations may reveal a novel therapeutic pathway to combat HIV-1/AIDS, as well as help dissect the fundamental biological role of Rab6A(A/A') in primary human macrophages.

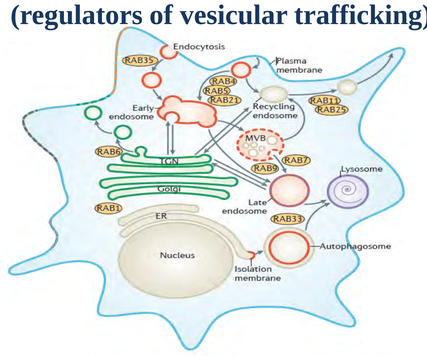
Rab6A - cycling



OCRL	BICD1	VHL	ERC1	HSPA8	HSP90AB1	GPI	PSMD3	REEP5
DCTN1	OHM	GOLGA1	SYTL5	RANGAP1	MYH10	TBC1D8	VPS53	DYNC2LI1
GCC2	BICD2	DENND5A	RAB7A	EVIL	RPS3A	USO1	ATP5B	NOTCH1
PMM1	TMF1	GDI2	RABAC1	SMEK1	RPS6KA5	VPS52	DNAJC2	COG6
GDI1	GOLGA4	GRIN2B	GOLGA2	MAPK7	RPS8	YIPF5	EEF1A1	SH3BP5L
CHML	KIF20A	NSF	RAB5A	PPP3CA	INPP5B	DLG4	KIF1C	
RABGGTB	RABGAP1	SLC2A4	HSP90B1	YIF1A	ACO2	MARCH_6	NPY	

Alex Hutagalung and Peter Novick. Role of Rab GTPases in Membrane Traffic and Cell Physiology. *Physiol Rev.* 91, 119-149 (2009).

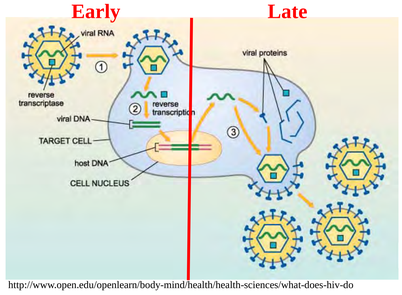
Rab GTPases (regulators of vesicular trafficking)



Steve Jean and Amy Kiger. Coordination between Rab GTPase and phoshoinositide regulation and functions. *Nature Reviews* 13, 436-470 (2012).

HIV-1 Life Cycle

Using a pseudoviral single-round infection assay, the HIV-1 life can be divided into two distinct phases; early (1) and late (2). (1) All steps between attachment of the virus to the host cell and integration of the viral genome. (2) All events from post-integration to maturation of newly synthesized virions.

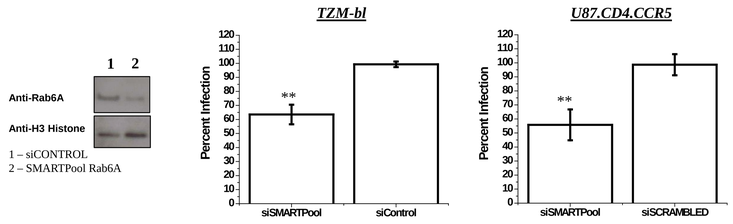


http://www.open.edu/openlearn/body-mind/health/health-sciences/what-does-hiv-do

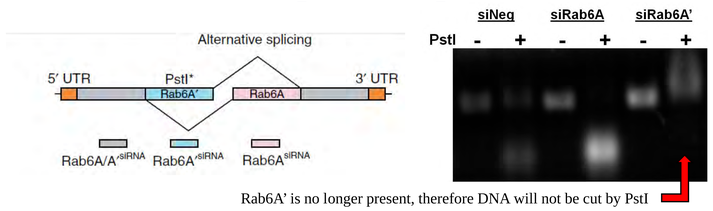
Hypothesis

We hypothesize that activated Rab6A(A/A') is required for efficient HIV-1 infection of MDMs and activation of Rab6A(A/A') is dependent on Ric1/Rgp1 activity.

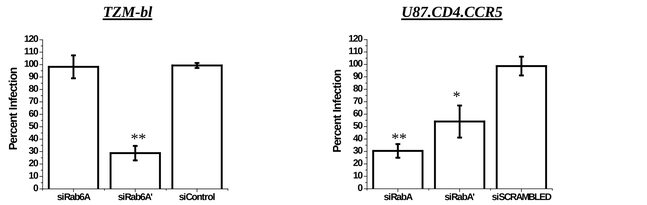
Double knockdown of Rab6A(A/A') in the multiple cell lines restricts HIV-1 infection



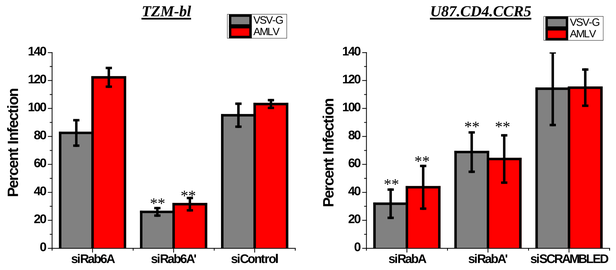
Rab6A isoform specific depletion via siRNA



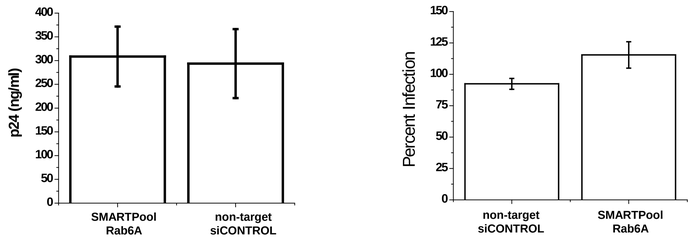
Individual silencing of either Rab6A or Rab6A' inhibits HIV-1 infection



Effect of Rab6A or Rab6A' specific depletion is not HIV-1 Env dependent in a normalized infection

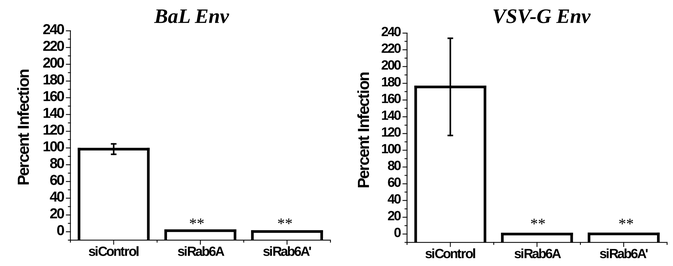


Double knockdown of Rab6(A/A') has no effect on the amount or infectivity of virus produced [293T cells]

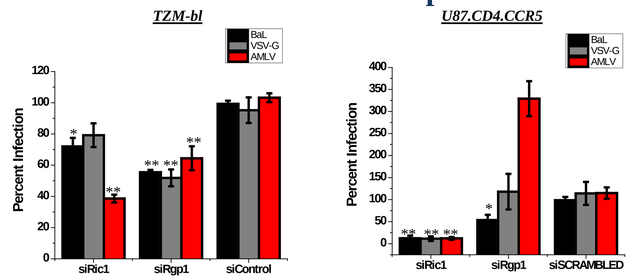


Data represents at least three independent experiments done in triplicate with * p<0.05 & ** p<0.005

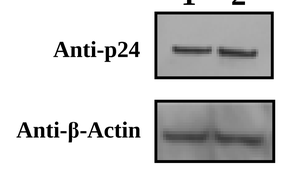
Depletion of Rab6A and Rab6A' inhibits HIV-1 infection regardless of Envelope in primary macrophages



Silencing of the Rab6A-specific GEF (Ric1/Rgp1) reduces HIV-1 infection irrespective of Env

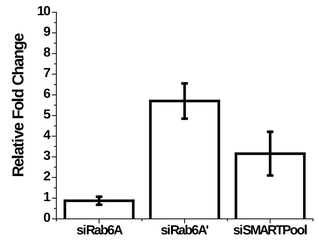


Knockdown of Rab6A/A' does not effect overall amount of CA entering the cell



Above: U87.CD4.CCR5 cells were challenged with HIV-1 Bal Env for 2hrs post a 72hr knockdown of Rab6A/A'. Excess viruses was washed away and whole cell lysates were collected 3hrs post infection and analyzed via Western.

Analysis of late viral DNA reverse transcription products in Rab6(A/A') silenced cells



Above: Whole cell cDNA was collected from U87.CD4.CCR5 cells post 72hr knockdown and RT-PCR was performed to detect for viral U5Ψ late reverse transcription products.

Conclusions

We have proven our hypothesis by showing the following:

1. ***Rab6A(A/A') are required for efficient HIV-1 infection of cell lines and primary human macrophages.***
2. ***Rab6A(A/A') functions in the early stage of HIV-1 infection and this is not dependent on HIV-1 ENV***
3. ***Rab6A-specific GEF (Ric1/Rgp1) is required for efficient HIV-1 infection in cell lines.***
4. ***While both function in a post-entry step, Rab6A and Rab6A' seem to have non-overlapping roles during the early stage of viral replication.***

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