Rab5 is a signalling GTPase involved in actin remodelling by receptor tyrosine kinases

Lanzetti L, Palamidessi A, Areces L, Scita G, Di Fiore PP
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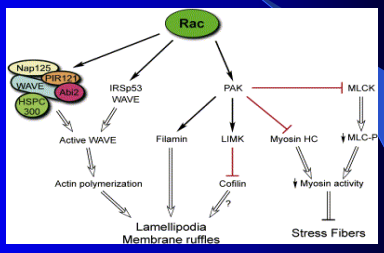
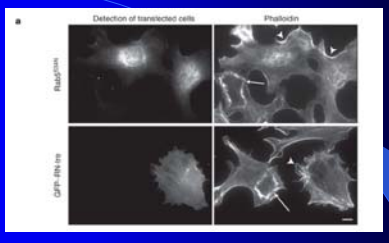
Signaling from Rac to the Cytoskeleton



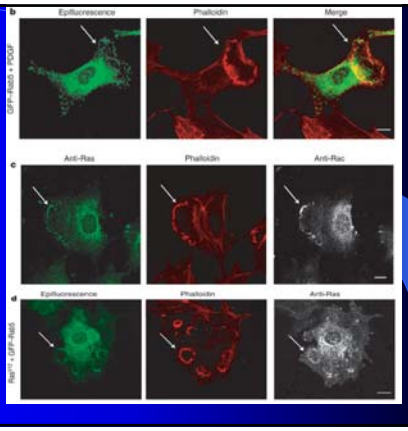
- cell edge ruffles (lamellipodia) : RTK → Ras → Rac
- dorsal surface ruffles (circular ruffles):
- Active mutants of Ras or Rac do not induce circular ruffles
- Dominant negative mutants of Ras and Rac inhibit circular ruffles formation
- Concomitantly with the activation of Ras/Rac, additional RTK-triggered pathways may be required for circular ruffling.

Hypothesis

- Rab5 is a candidate regulator in the circular ruffle pathway.
- Rab5 can be activated by receptor tyrosine kinases
- An active Rab5 mutant induced actin remodelling



- Dominant negative Rab5 mutant ($Rab5^{S34N}$) inhibited the induction of circular ruffles by PDGF, whereas cell edge ruffles were unaffected
- Overexpression of the Rab5-specific GAP RN-tre also inhibited circular ruffles.



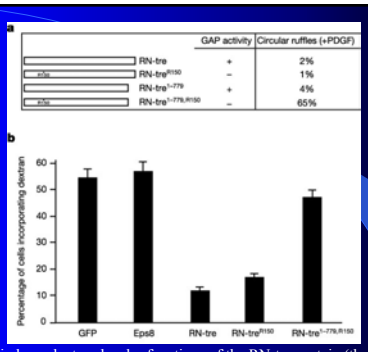
Three independent signals, originating from Rab5, PI(3)K and Rac, are simultaneously required for circular ruffling.

Table 1 **Actin cytoskeleton remodelling activity of Rab5 in combination with various GTPases and effector proteins**

Transfection or PDGF treatment	Membrane ruffles (%)*	Circular ruffles (%)*
Ras^{V12}	+*(89‡)†	−(0)
$Rab5^{wt}$	−(2)	−(0)
$Rab5^{Q79L}$	−(3)	−(0)
Ras^{V12} + $Rab5^{wt}$	+(76‡)	+(23‡)
Ras^{V12} + $Rab5^{Q79L}$	+(88‡)	+(14‡)
$Ras^{V12,S35}$	−(2)	−(0)
$Ras^{V12,S35}$ + $Rab5^{wt}$	−(4)	−(0)
$Ras^{V12,C40}$	+(93‡)	−(0)
$Ras^{V12,C40}$ + $Rab5^{wt}$	+(90‡)	+(30‡)
$PI(3)K^{CD2p110}$	+(76‡)	−(0)
$PI(3)K^{CD2p110}$ + $Rab5^{wt}$	+(81‡)	+(22‡)
Rac^{Q61L}	+(88‡)	−(0)
Rac^{Q61L} + $Rab5^{wt}$	+(90‡)	−(0)
PDGF	+(91‡)	+(78‡)
PDGF + Rac^{N17}	−(3)	−(0)
Ras^{V12} + $Rab5^{wt}$ + Rac^{N17}	−(3)	−(0)
$PI(3)K^{CD2p110}$ + $Rab5^{wt}$ + Rac^{N17}	−(2)	−(0)

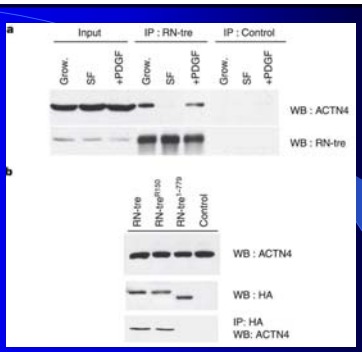
Raf/MAPK

PI(3)K

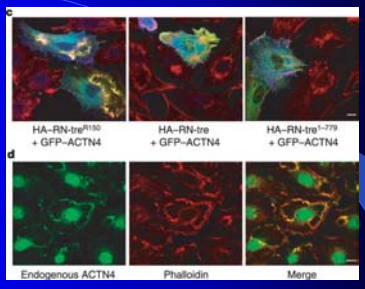


Two independent molecular functions of the RN-tre protein (the GAP activity and another residing in the C terminus) can be linked to both the processes of circular ruffling and macropinocytosis.

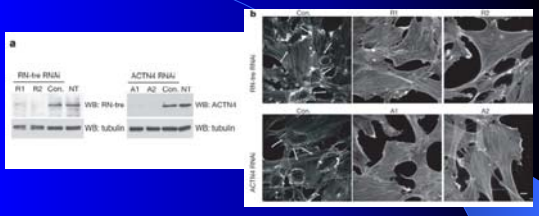
- What's the C-terminus function?
- Mass spectrometry was used to identify proteins that interact with the carboxyl terminus of RN-tre, ACTN4 — an actin-binding protein that crosslinks filamentous (F)-actin was identified.



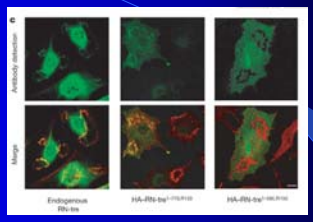
PDGF dependent RN-tre and ACTN4 in vivo association is dependent on the presence of the last 49 amino acids of RN-tre.



- Overexpression of ACTN4 rescued the inhibitory effect of the RN-tre^{R150} mutant on PDGF-induced circular ruffling, not of the RN-tre^{wt} or of the RN-tre$^{1–779}$ mutant. ACTN4 functions downstream of Rab5 and RN-tre in the RTK-dependent induction of circular ruffling.
- Endogenous ACTN4 localized to PDGF-induced circular ruffles.



- RNA interference-mediated silencing of either RN-tre or of ACTN4 resulted in a marked impairment of PDGF-induced circular ruffles, with cell edge ruffles unaltered.



- Endogenous RN-tre and the RN-tre$^{1–779,R150}$ mutant localized to circular ruffles, whereas a further deletion mutant, RN-tre$^{1–580,R150}$, did not.

RN-tre has the potential to interact simultaneously with actin and ACTN4.

- at least two regions in RN-tre (amino acids 507–686 and 716–828) interacted directly with F-actin, whereas the ACTN4-interacting site (amino acids 779–828) did not

Model

- Rab5 participates to the formation of circular ruffles through its effector RN-tre.
- RN-tre establishes a three-pronged connection with Rab5, ACTN4 and actin, aid crosslinking of actin fibres into actin networks at the plasma membrane.

a novel role for the Rab-family small GTPase Rab5

- Rab5 functions in a receptor tyrosine kinase signaling pathway that promotes actin remodeling and the formation of circular ruffles, in addition to its well known role in endocytic trafficking.