Mapping the Interaction of Talin:RIAM Complex in Inside-Out Integrin Activation Pathway

Tejash T. Patel[1,2], Yu-Chung Chang[1], Hao Zhang[1], and Jinhua Wu[1]

[1]Department of Developmental Therapeutics, Fox Chase Cancer Center, Philadelphia, PA 19111

[2]Biomedical Sciences Program, Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131

INTRODUCTION

Integrin receptors are ubiquitously expressed transmembrane adhesion molecules, mediating the attachment between a cell and the tissues surrounding it. Integrin signaling plays important roles in numerous biological processes, including maintenance of normal cellular function and essential homeostasis. In addition, integrin signaling is also involved in the metastasis of cancer cells by changes in normal cell adhesion and migration regulation. The bidirectional signaling capability of integrin receptors allows for both inside-out and outside-in cellular signaling. To date, therapeutic approaches targeting extracellular integrin domains has shown limited success.

Here, we examine a key interaction of a mechanism resulting in adverse integrin activation and changes in regulation mediated via inside-out integrin signaling. Rap1, a member of the Ras superfamily of GTPases, recruits the MRL-family protein, Rap1-interacting adaptor molecule (RIAM), to the plasma membrane. Downstream, RIAM recruits the cytoplasmic protein talin up to the plasma membrane, where the high affinity of talin's F3 region for integrin's β-cytoplasmic tail allows for an interaction and subsequent integrin activation.

Knockdown studies have shown that talin does not translocate to the plasma membrane without RIAM recruitment.[1] Talin is normally cytoplasmic and in an auto inhibitory configuration, where its integrin interacting domain is masked. We hypothesize that the interaction between RIAM and talin induces a steric conflict with the talin auto inhibitory configuration, thus releasing talin from its auto-inhibition state.

The N-terminus of RIAM (residues 1-30) has been shown to bind to talin.[2] In this study, we aim to elucidate the mechanism of the interaction between talin and the RIAM talin-binding (TB) region via structural and molecular studies. In doing so, we will redefine the minimum TB region capable to binding to talin. Our research will also provide a means of manipulating the pathway, and will facilitate the development of anticancer compounds.

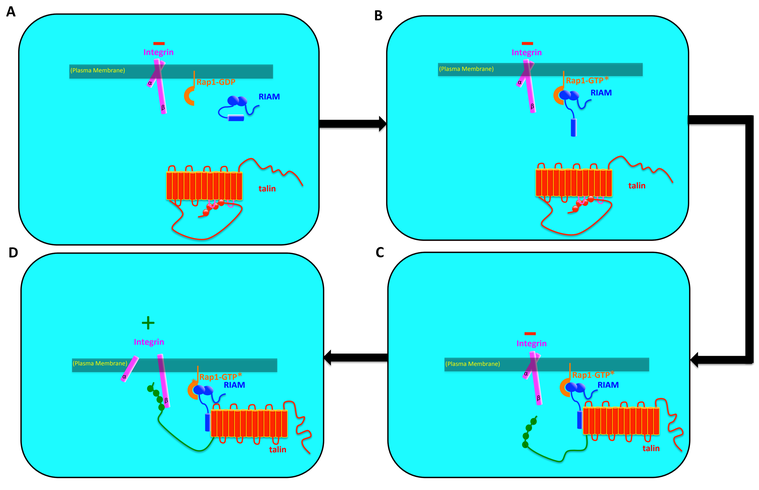

Figure 1. Basic mechanism of Rap-1 induced integrin activation pathway.
(A) Natural state: Inactive Rap1 (GDP-bound), RIAM is cytoplasmic, talin is cytoplasmic and in auto inhibitory configuration, integrin receptor is in low affinity conformation. (B) Active Rap1 (GTP-bound) recruits RIAM to the plasma membrane. (C) RIAM bound to Rap1-GTP and at the plasma membrane recruits talin to the plasma membrane. (D) Talin FERM domain (F0, F1, F2, F3) is released from auto-inhibition state and activates the integrin receptor.

METHODS

- ❖ Plasmid DNA constructs and mutagenesis
- ❖ Protein expression & purification
- ❖ Biochemical assays: Glutathione S-Transferase (GST) pull down, native gel shift, Co-immunoprecipitation
- ❖ Cell culture
- ❖ X-ray crystallography

RESULTS

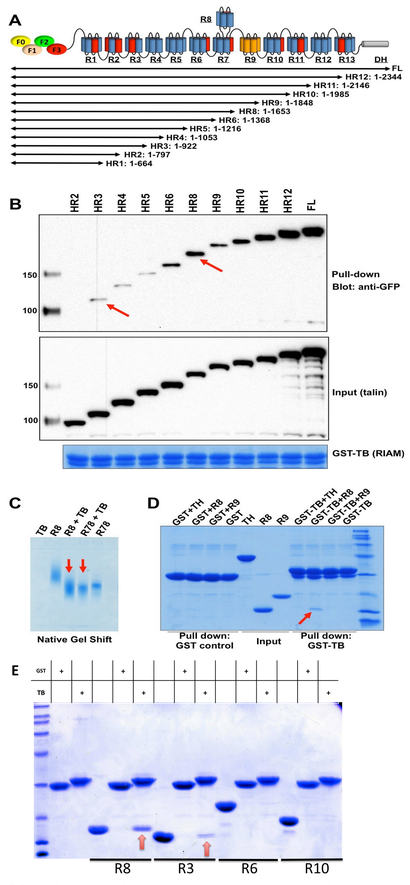

Figure 2. Mapping affinity of talin domains for interaction with RIAM TB region.
It has been demonstrated that the N-terminus 30 amino acid residues of RIAM mediate binding to talin. However, talin domains with highest affinity involved in the interaction with the RIAM TB(1-30) region are unknown. (A) Schematic architecture of talin protein. FL-talin has 2540 residues and is ~270kDa. (B) A series of systematic talin truncations were constructed to map the TB binding domains of talin. In-cell pull down assay was done using GST-tagged RIAM TB as bait protein. R7R8 and R3 were identified to bind to RIAM TB. (C) In-vitro native gel shift assay to verify a direct interaction between RIAM TB(1-30) with R7R8 and R8, using RIAM TB peptide. (D) GST pull down assay to further confirm interaction. Talin head (TH) and T9 domain used as controls. An association is confirmed with the appearance of the respective talin domain band. (E) GST pull down assay of RIAM TB with talin domains R3, R6, R8, R10. Association confirmed as described in (D).

RESULTS

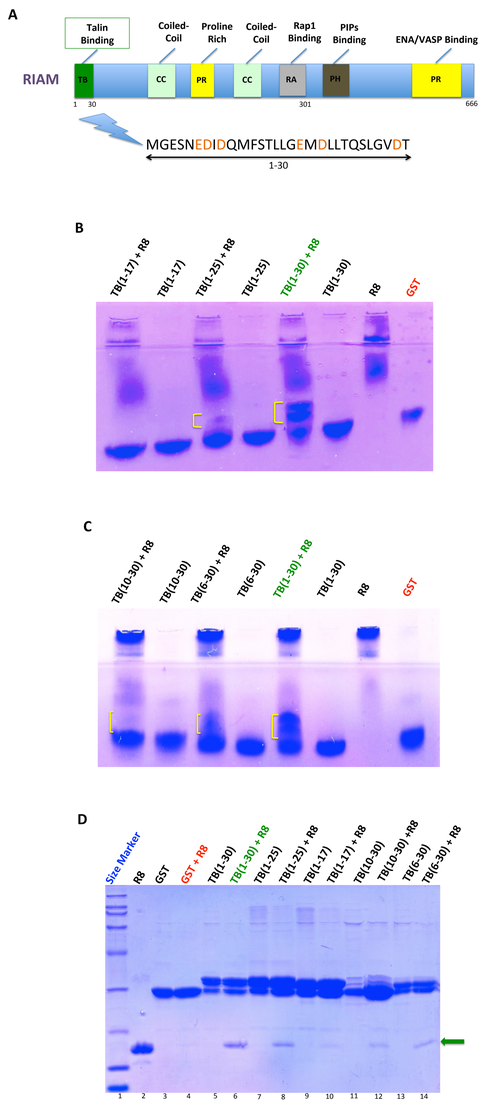

Figure 3. Redefining RIAM minimum TB region. (A) Schematic architecture of RIAM. FL-RIAM has 666 residues and is ~100kDa. Recombinant RIAM TB region truncations were GST-tagged and expressed in *E.coli* BL21; talin R8 was His-tagged. Constructs were purified via affinity chromatography. (B) Native gel shift assay of RIAM C-terminus truncation mutants. TB(1-30) + R8 used as positive control; GST used as negative control. TB(1-25) + R8 show a band shift, which indicates a complex formation. (C) Native gel shift of RIAM N-terminus truncation mutants. Controls as described in (B). TB(10-30) + R8 and TB(6-30) + R8 both show a band shift, indicating a complex formation. (D) GST pull down assay of RIAM TB truncation constructs. Results are visualized via Coomassie strain and an association is confirmed with the appearance of the R8 band. GST + R8 was the negative experimental control and TB(1-30) + R8 was the positive experimental control. Lanes 2,3,5,7,9,11,13 are loading controls.

RESULTS

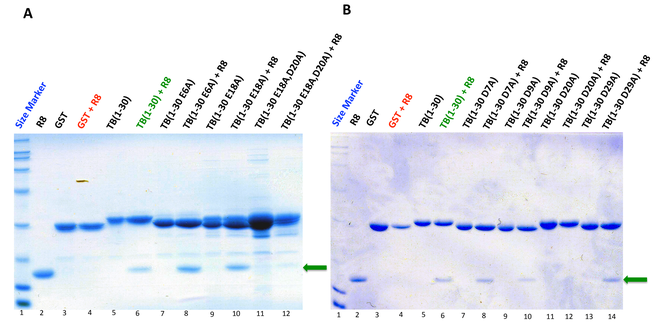

Figure 4. Identifying crucial residues within RIAM TB region.
(A) GST pull down assay of RIAM point-mutants. Results are visualized via Coomassie strain and an association is confirmed with the appearance of the R8 band. GST + R8 was the negative experimental control and TB(1-30) + R8 was the positive experimental control. Lanes 2,3,5,7,9,11 are loading controls. (B) GST pull down assay of RIAM point-mutants. Experimental controls as described in (A). Lanes 2,3,5,7,9,11,13 are loading controls.

CONCLUSIONS

- ❖ The α-helical bundle R3 domain and R8 domain of talin bind to RIAM TB region, with R8 having highest affinity.
- ❖ RIAM TB truncation mutants TB(10-30) and TB(1-25) maintain binding affinity to talin R8. Redefined minimum TB region as TB(6-25).
- ❖ RIAM TB mutation D9A has diminished binding affinity to talin R8; Mutation D20A abolishes binding affinity, thus is a crucial RIAM TB residue for talin recruitment.

FUTURE WORK

- ❖ Charge reversal point-mutant studies to verify importance of charge-charge interactions in formation of talin:RIAM complex.
- ❖ RIAM TB + talin structural studies to examine side-chain interactions at atomic level via X- ray crystallography.
  - ❖ Crystallization trials and conditions optimization
  - ❖ High resolution crystal structure
- ❖ In-cell studies of RIAM TB + R8 in HEK293T cells to compliment in-vitro data with a cell model.
- ❖ Studies of TBII site as described by Goult et al. (*JCB 2013*).
  - ❖ Test TBSII residues with talin R3 as well as R8

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2. H. Lee, C.Lim, W. Puzon-McLaughlinS. Shattil, M. Ginsberg. "RIAM Activates Integrins by Linking Talin to Ras GTPase Membrane-targeting Sequences." *JBC* (2009); 284(8): 5119-5127.