Heterotrimeric G Protein βγ Subunits Interact with the Cytoplasmic Dynein Light Chain Tctex-1

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Introduction

Cytoplasmic dynein is a microtubule-based molecular motor involved in a diverse array of cellular functions, including intracellular transport, regulation of Golgi dynamics, mitotic spindle assembly, and retrograde axonal transport. The dynein motor is a multi-subunit protein with two heavy chains containing the ATPase and motor activities, two intermediate chains (DICs), multiple light intermediate chains (LICs), and one or more of each of three groups of light chains (DLCs; 8, 14, and 22 kDa). Cytoplasmic dynein light chain, Tctex-1, which belongs to the 14 kDa family of DLCs has been found to interact with numerous cellular proteins, including the light transducing visual pigment Rhodopsin[1]. Tctex-1 was also identified as an activator of G protein signaling in the absence of an upstream G protein-coupled receptor (GPCR)[2]. Heterotrimeric G proteins consist of α-, β-, and γ-subunits which, in their unstimulated state, exist as the GDP-bound α-subunit complexed with the βγ-heterodimer. GTP-binding results in the dissociation of the α and the βγ subunits which are then free to signal to their respective effector molecules. More than 20 different Gα subunits, 6 different Gβ subunits and 12 different Gγ subunits are known. In this study, we describe a novel role for the dynein light chain, Tctex-1. Using co-immunoprecipitation, *invitro* binding assay and immunofluorescence, we showed that Tctex-1 can preferentially interacts with the βγ subunit of the heterotrimeric G proteins and that the c-terminus of Tctex-1 was important for this interaction. We also showed that the interaction between Tctex-1 and Gβ may be a common mechanism shared by several Gβ isoforms. In an effort to understand the significance of this interaction we analyzed the endogenous localization Tctex-1 and Gβ and found that endogenous Tctex-1 and Gβγ can co-localize in distinct cellular compartments in multiple cell types. The universal nature of this interaction suggests an important regulatory role for Tctex-1 in Gβγ function and/or cellular compartmentalization.

Abbreviations

IP: Immunoprecipitation
IB: Immunoblotting
TCL: Total cell lysate
$G\alpha_t$: Transducin alpha

References

1. Tai, A. W., Chuang, J. Z., Bode, C., Wolfrum, U., and Sung, C. H. (1999) *Cell* **97**, 877-887.
2. Takesono, A., Cismowski, M. J., Ribas, C., Bernard, M., Chung, P., Hazard, S., 3rd, Duzic, E., and Lanier, S. M. (1999) *J Biol Chem* **274**, 33202-33205.

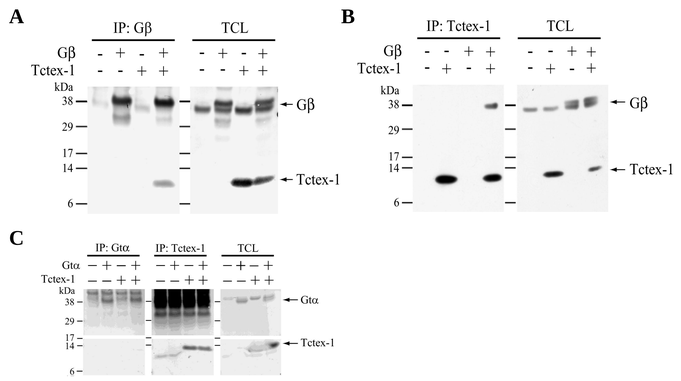

Figure 1: **Tctex-1 interacts with G protein beta subunit**. A-B) HEK cells were co-transfected with Gβ1γ1 and Tctex-1. Cells were harvested 48h post-transfection and cell lysates were IP with anti-Gβ antibody (A), anti-Tctex-1 antibody (B) and IB with anti-Gβ (top) and anti-Tctex-1 (bottom) antibodies. Right half of each panel is a direct western blot using 20 μg of TCL to show protein expression levels. (C) HEK cells co-transfected with $G\alpha_t$ and Tctex-1 were IP with anti-$G\alpha_t$ (left panel), anti-Tctex-1 (middle panel) and IB with anti-$G\alpha_t$ (Top) and Tctex-1 (bottom). The right panel is a direct western using 20 μg of TCL to check protein expression levels

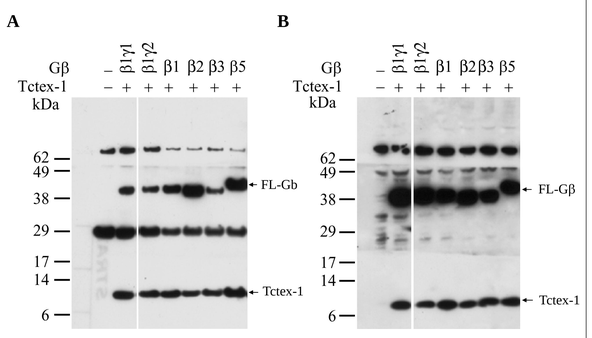

Figure 2: Tctex-1 interacts with several Gβ isoforms. HEK cells were co-transfected with Flag-tagged Gβ1, β2, β3, or β5 and Tctex-1 expression plasmids. The cells were harvested 48h post-transfection and cell lysates were IP with an anti-Tctex-1 antibody (A). 20 μg of total protein was analyzed by western blot to check protein expression levels (B). The filters were blotted with an anti-DIC antibody (Top), anti-Flag antibody (middle), and anti-Tctex-1 antibody (bottom).

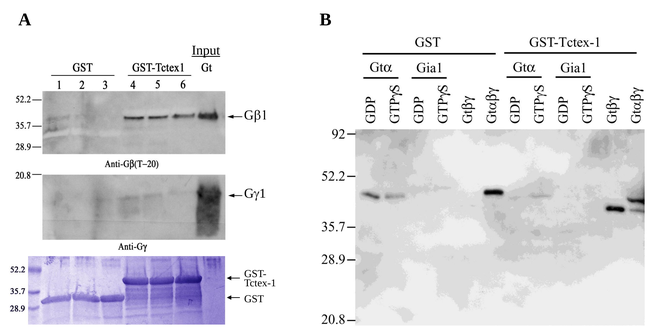

Figure 3: Interaction of Tctex-1 with G protein Transducin. GST (lanes 1-3) or GST-Tctex-1fusion protein (lanes 4-6) (300 nM) were incubated with holotransducin (Gt) (40 nM) (lanes 1,4), plus GDP (10 μM) (lanes 2,5), plus GTPγS (10 μM) and $MgCl_2$ (5mM) (lanes 3,6) in a total volume of 250 μl for 2h at 4°C. 20 μl of 50% Glutathione matrix was added to the protein mixture and the retained G protein subunits were identified by IB following gel electrophoresis (A). The filters were probed with an anti-Gβ antibody (Top) and anti-Gγ1 antibody (middle). Input, 20 μl of the incubation mixture. A Coomasie Blue stained gel in the bottom panel shows equal amount of the GST and GST-Tctex-1 were used in the assay. Purified Gαt and Gai1(40 nM) were incubated with GST or GST-Tctex-1 in the presence of GDP (10 μM) or GTPγS (10 μM) (B).

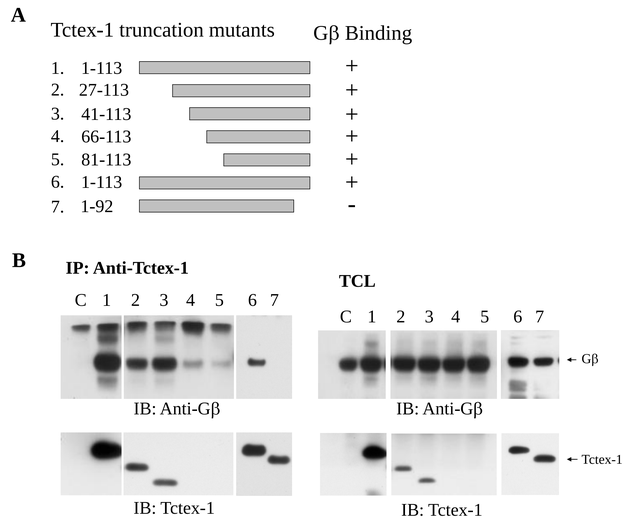

Figure 4: **Mapping of the Gβ binding domain of Tctex-1**. (A) Schematic diagram showing the various truncation mutants of Tctex-1 used to map the Gβ binding domain of Tctex-1. The ability of each mutant to co-IP Gβ is also summarized in the figure. (B) HEK cells were co-transfected with the indicated truncation mutants of Tctex-1 along with Gβ. Cells were harvested 48h post-transfection and IP with anti-Tctex-1 antibody and IB with anti-Gβ antibody (Top) and anti-Tctex-1 antibody (Bottom). The expression levels of each protein was analyzed by direct western using 20 μg of TCL. The lane number in (B) corresponds with the respective construct number in (A). Truncation of the c-terminal 21 amino acids (construct #7 (1-92aa)) results in loss of the ability of Tctex-1 to co-IP Gβ suggesting that the c-terminal of Tctex-1 is important for this interaction.

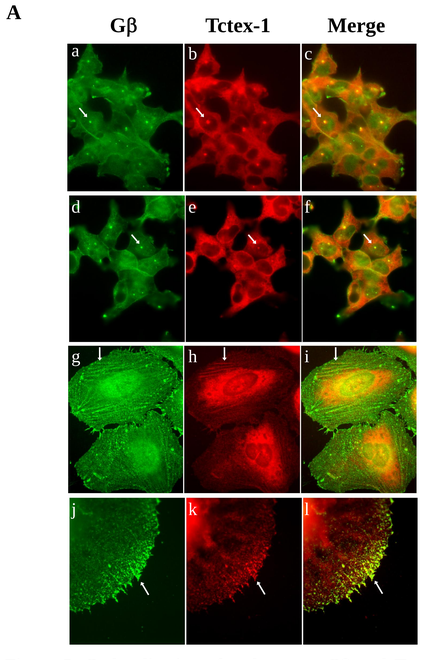

Figure 5: **Co-localization of endogenous Gβ and Tctex-1 in HeLa and HEK cells.** Gβ co-localizes with p150-Glued (a,b,c) and Tctex-1 (d,e,f) at the MTOC in HEK cells. Endogenous Gβ1 can be detected on stress fibers (g,h,i) and at the cell periphery with Tctex-1 in methanol-fixed HeLa cells. Green:Gβ; Red:Tctex- 1.

Results and Conclusions

1. Tctex-1 can co-immunoprecipitate Gβ but not Gα in overexpressed HEK mammalian cells.

2. In an *invitro* binding assay, GST-Tctex-1 can directly interact with transducin Gβγ subunit but not transducin Gα or Gαi1.

3. Tctex-1 can interact with all the various Gβ isoforms examined in this study, specifically, β1, β2, β3 and β5.

4. The mapping of the Gβ binding domain on Tctex-1 using the truncation mutants of Tctex-1 suggests that the c-terminus of Tctex-1 is important for this interaction.

5. Gβ and Tctex-1 can be localized in specific cellular compartments and cytoskeletal structures in mammalian cells.

6. The universal nature of this interaction suggests an important regulatory role for Tctex-1 in Gβγ function and/or cellular compartmentalization.