RESULTS

Ga1 and 3 proteins interacts with RASA3, 5-HT1A and TNFAIP8

To address the interactions between Gαi 1 and 3 proteins with the novel proteins in living cells, BRETs were conducted (Figure 2). Based on the BRET ratios, RASA3, 5-HT1A and TNFAIP8 appears to be significantly greater than the negative control potassium channel HCN4-GFP2 which has been documented as having no interaction with G-proteins (Whitaker and Accili, 2008). In comparison to 5-HT1A and RASA3, a strong interaction between TNFAIP8-GFP2 and the Gui proteins were seen. The optimal position (largest BRET ratio) of the rLuc in the Gui proteins with RASA3 and TNFAIP8 seemed to be at position 122 and 91 respectively. No significant difference in the BRET ratios was seen in the different positions of the rLuc for the 5-HT1A –Goi interaction.

A) RASA3

B) 5-HT1A

C) TNFAIP8

Figure 2: BRET ratios of Ga1 and Ga3 wt and QL constructs with A) RASA3, B) 5-HT1A, C) TNFAIP8. BRET ratio is calculated using the corrected GFP signal (at λ = 480 nm) divided by the corrected luciferase signal (at λ = 530 nm). A GFP-HCN4 plasmid was used as a control. In B) and C), the agonist used was 4 µM 5-HT, 10 ng/µL TNF respectively. The position of the rLuc gene was indicated with the HT-1A, HCN4 and RASA3 constructs. HCN4 was used as a negative control to obtain background, since it does not interact with G-proteins.

Figure 3: β-galactosidase assay of TIP1 and 2, and TNFAIP8 with Ga3 wt and QL. Yeast strains were transformed with pAS2-1-Ga3 wt or QL and pACT2-TNFAIP8, TIP1E or TIP2E, and then assessed by β-galactosidase assay. The β-gal activities are normalized to the positive control vector pCL1-1, and presented as mean ± SE (N = 3).

Characterization of the interaction between TNFAIP8 with Ga3 proteins using Yeast-two hybrid and β-galactosidase assay

In order to further investigate the specificity of the TNFAIP8-Ga3 interaction, a yeast two-hybrid was conducted. Selection upon –Leu, -His, - Trp revealed growth on TNFAIP8 with Ga3 wt and QL, and TIP1 and TIP2 with Ga3QL. To quantify the strength of the interaction, a β-galactosidase assay was conducted (Figure 2). TIP1 E and TIP2E, both of which are TNFAIP8-related proteins found in mice displayed minimal β-gal activity with both Ga3 wt and QL, while TNFAIP8 was observed to have a high β-gal activity.

DISCUSSION

Ga1 and 3 proteins interacts with RASA3, 5-HT1A and TNFAIP8

Since all the BRET ratios were quite significantly different from the control (HCN4-GFP2), BRET results (Figure 2) demonstrate that Ga1 Ga3 both interact with RASA3, 5-HT1A, and very much so with TNFAIP8. There was no preference for Ga3, which may be due to the over-expression of both proteins required to detect BRET signals. The optimal positioning of the rLuc in the Gui proteins is most likely 122 for RASA3, and 91 for TNFAIP8 since the highest BRET ratio was obtained at those positions. Meanwhile, the position of rLuc did not affect the interaction between Gui proteins and 5-HT1A.

The specificity of the TNFAIP8-Ga3 interaction was investigated further using a yeast two-hybrid system followed by a β-gal assay. In the Yeast-two hybrid, the conjugated protein system allows for the production of His, and thus allowing yeast containing both proteins that interact with each other to survive. The β-gal assay further reinforces the idea that TNFAIP8 is specific for Ga3 proteins, TIP1E and TIP2E proteins that are TNFAIP8 related proteins in mice show virtually no β-galactosidase activity and thus, minimal interactions between proteins.

The next step is to move the position of rLuc in the Ga3 construct since only position 91 was examined for the Ga3. The optimal position of rLuc for investigation of the RASA3-Ga3 interaction may be 122 as seen with Ga3 i1.