WNT stimulation dissociates a Frizzled 4 inactive state complex with $G\alpha_{12/13}$

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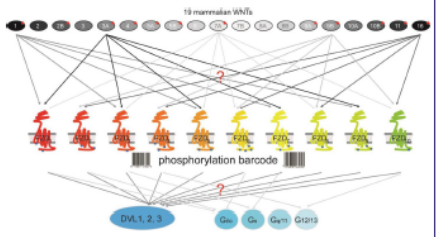
BACKGROUND

- Frizzled (FZD) receptors constitute the Class Frizzled of unconventional G-protein-coupled receptors (GPCRs)
- In mammals, there are 19 WNTs and 10 FZDs
- Mechanisms of signal initiation and FZD–G protein coupling remain poorly understood
- Kilander et al. (2014) found that FZD_6 interacts with heterotrimeric G proteins Ga_{i1} and Ga_q
  - FZD_6-G protein complex dissociates after WNT binding
  - Intracellular DVL levels play a crucial role for complex formation
- FZD_4 plays an important role in retinal vascular development and is frequently mutated in Norrie disease or familial exudative vitreoretinopathy

RESEARCH QUESTIONS

1. Investigate functional interaction of FZD_4 & heterotrimeric G proteins
2. Characterize the novel FZD_4-$G\alpha_{12/13}$ complex assembly
3. Assess the involvement of DVL in the complex formation between FZD_4 & $G\alpha_{12/13}$
4. Define specific WNTs interacting with the FZD_4-$G\alpha_{12/13}$ complex
5. Elucidate downstream signaling cascades initiated by the activated $G\alpha_{12/13}$
6. Study the physiological role of FZD_4 - $G\alpha_{12/13}$

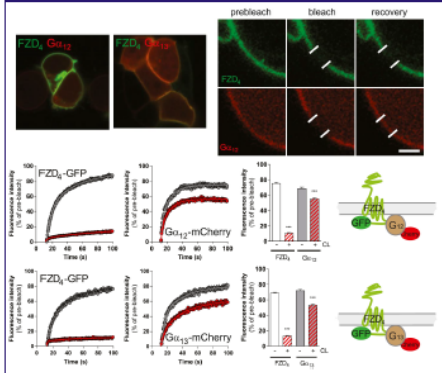


Challenges in studying WNT/Frizzled signaling:

- There are 10 different FZDs and 19 different WNTs in mammals, creating a gigantic pool of possible interactions
- Possible interactions with:
  - Several postulated FZD co-receptors (LRP5/6, ROR, TSPAN12, etc.)
  - 3 DVL isoforms
  - 4 different families of heterotrimeric G protein
- Lack of a clear overview of WNT/Frizzled pharmacological interactions → Many different WNTs bind many and/or the same FZD receptors
- A majority of commonly available cell lines express a minimum of 7-8 different FZDs

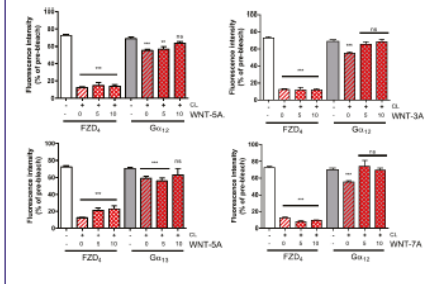
RESULTS

FZD_4 interacts with heterotrimeric $G\alpha_{12/13}$



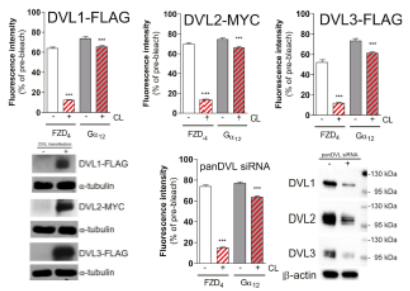
- A live-cell fluorescence imaging technique called **fluorescence recovery after photobleaching (FRAP)** allows to study the mobility and diffusion properties of FZDs and G proteins, or the interaction between them
- After biotin-avidin crosslinking, which immobilizes all membranous and membrane-bound proteins, **the FZD_4-bound $G\alpha_{12/13}$ shows retarded mobility**
- In contrast, members of the G protein family $G\alpha_{i/o}$, $G\alpha_s$, $G\alpha_q$ do not show any changes in mobility (not shown)

FZD_4- $Ga_{12/13}$ complex dissociates upon WNT stimulation



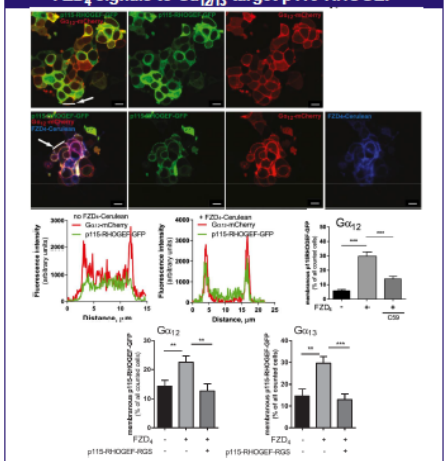
- **Agonist treatment** with purified, recombinant WNTs (300ng/ml) **disrupts** the observed **FZD_4-$G\alpha_{12/13}$ complex**
- **FRET** measurements (not shown) indicate that energy transfer from FZD_4-GFP to $G\alpha_{12/13}$-mCherry occurs at a baseline and decreases with WNT-7A stimulation (300 ng/ml, 5 minutes) indicative of a **receptor–G protein dissociation or rearrangement**

FZD_4- $Ga_{12/13}$ complex is independent of DVL



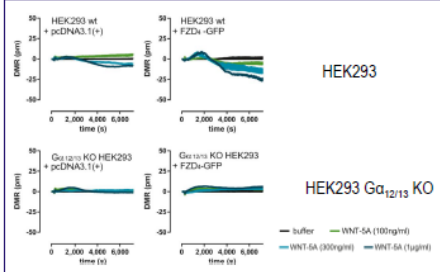
- We created **defined cellular levels of DVL** in HEK293 cells through **siRNA** or independent **overexpression** of all 3 DVL isoforms
- Neither depleting nor enriching the cells with DVL influences the formation of a FZD_4-$G\alpha_{12/13}$ complex (contrary to FZD_6 → see Kilander et al. 2014)
- The scaffold protein **DVL is dispensable** for a **FZD_4-$G\alpha_{12/13}$ inactive state complex formation**

FZD_4 signals to $Ga_{12/13}$-target p115-RHOGEF



- **p115-RhoGEF** is a known **$G\alpha_{12/13}$ target** that physically interacts with the active, GTP-bound Gα subunit and stimulates its GTPase activity
- **FZD_4** in combination with **$G\alpha_{12/13}$ recruits p115-RhoGEF** to the membrane, indicating an **active $G\alpha_{12/13}$ signal pathway**
- Endogenously secreted **WNTs signal through FZD_4-$G\alpha_{12/13}$** to contribute to p115-RhoGEF recruitment (not shown)

WNT-Induced Dynamic Mass Redistribution in HEK293 depends on $Ga_{12/13}$



- Label-free methods such as DMR allow the analysis of **ligand-induced changes in living cells**
- **Negative DMR** after WNT-5A stimulation in **wild-type cells decreases even further in FZD_4-transfected cells**
- The observed changes in DMR are **sensitive to $G\alpha_{12/13}$ protein knockout**, indicative of **FZD_4 receptor signaling via $G\alpha_{12/13}$**

CONCLUSION

- FZD_4 and $G\alpha_{12/13}$ **functionally interact**
- **WNTs** can **dissociate $G\alpha_{12/13}$** from an inactive-state complex with FZD_4
- **DVL is dispensable** for FZD_4-$G\alpha_{12/13}$ inactive-state complex formation
- FZD_4-$G\alpha_{12/13}$ inactive-state complex mediates **RHO signaling** through membrane recruitment of **p115-RhoGEF**

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