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A molecular traffic jam: How overexpression of Pericentrin restricts the movement of IFT20 between the Golgi apparatus and the Primary Cilium

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A molecular traffic jam: How distance affects the movement of IFT20 as it travels in the cell

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Abstract

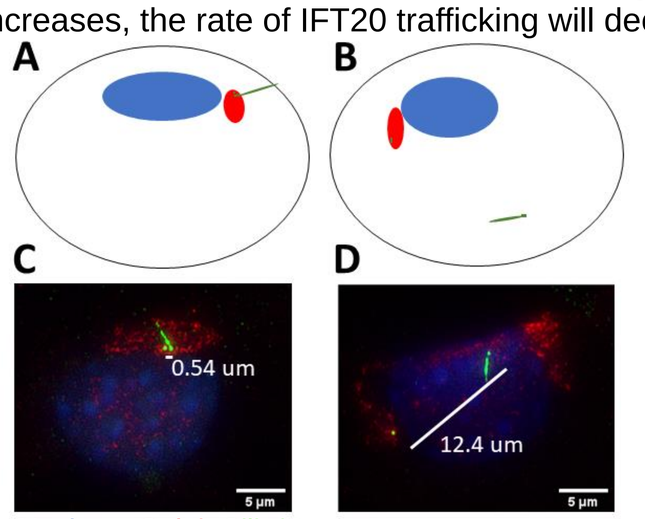
Virtually every cell in the human body has a small antenna projecting from its surface called a primary cilium. When a cilium cannot function properly in an individual, it leads to a range of disorders called ciliopathies. Many genetic mutations that disrupt the primary cilium interfere with a process called protein trafficking, which is the movement of proteins around the cell. Although protein trafficking to and from the primary cilium is known to occur, *the rules that govern protein trafficking within the cell are not known*. The goal of my project is to investigate how the distance between two cellular locations influences the trafficking of a specific protein called IFT20. In order to function, IFT20 must move between two cellular locations, the Golgi complex and the primary cilium. To determine the rules that govern IFT20 trafficking patterns, I will make IFT20 fluorescent so that I can observe it in cultured human cells with a fluorescent microscope. I predict that as the distance between IFT20's cellular origin (the Golgi complex) and its cellular destination (the primary cilium) increases, the rate of IFT20 trafficking will decrease.

Background

Cells must communicate through multiple ways in order to exchange information with each other. One method is through the primary cilium. The primary cilium is essential in cellular communication because organ development is severely affected if cilia are not present in human embryos (Reiter and Leroux, 2018). Cilia defects can lead to a host of disorders called ciliopathies, and impact roughly 1 in 500 live births (Waters and Beales, 2011).

Cilia are formed through the process of intraflagellar transport (IFT) and many IFT proteins facilitate this process. IFT20 is an IFT protein of interest to us because it has been shown to move in and out of primary cilia, be an important player in cilia formation and organization, and move between the Golgi complex and the primary cilium (Follit et al., 2006). Because of its highly important function at the primary cilium, IFT20 will be used as a model to study the conditions that impact protein trafficking near the cilium.

One such cellular condition that can impact protein trafficking is distance between cellular destinations (Figure 1). An increase in distance has been shown to disrupt the cilium's ability to sense the environment (Mazo et al., 2016). However, how this spatial difference affects IFT20 is still unknown. I predict that as the distance between the Golgi complex and the cilium increases, the rate of IFT20 trafficking will decrease.



Nucleus, Golgi, Cilia/Centrosome

Figure. 1. Development of assay to determine trafficking distances. (A and B) Schematic of cellular conditions where the cilium is either near (A) or separated from (B) the Golgi complex. (C and D) Cilium localization near the Golgi complex is not consistent.

Methods

- Molecular subcloning to make fluorescently tagged construct of IFT20.
- Transfect mammalian 3T3 cells with fluorescently tagged IFT20 construct
- Compare localization of fluorescently tagged IFT20 construct and endogenous IFT20
- Induce centrosome separation and cilia formation in 3T3 cells through serum starvation

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Results

Fig. 2. Plasmid Maps of Fluorescent Constructs

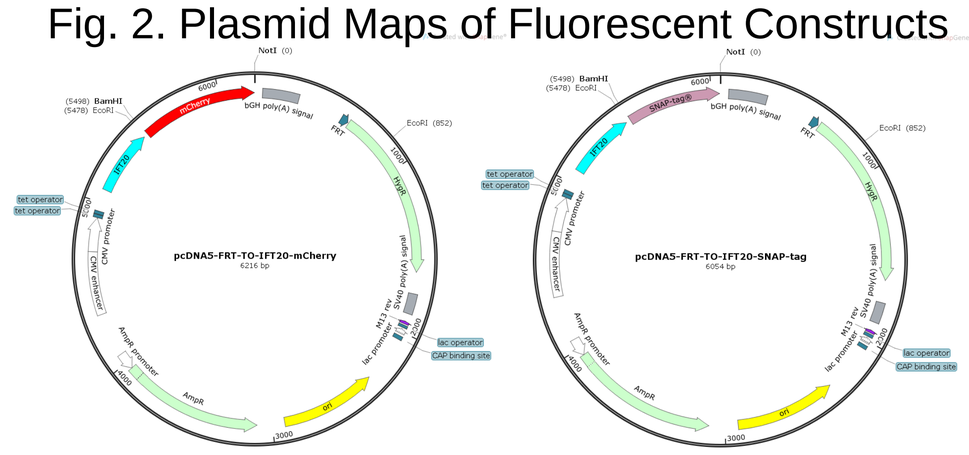
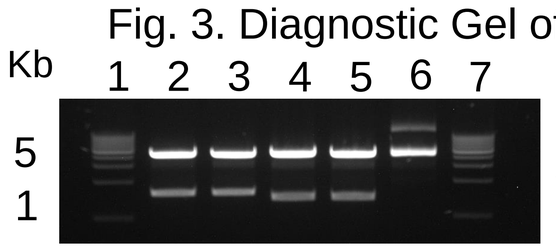


Fig. 3. Diagnostic Gel of Fluorescent Constructs



Legend:
1. Apex 1 Kb Ladder
2. IFT20-mCherry 1
3. IFT20-mCherry 2
4. IFT20-SNAP 1
5. IFT20-SNAP 2
6. Undigested Plasmid
7. Apex 1 Kb Ladder

Fig.3. Fluorescent construct diagnostic digest. Both IFT20-mCherry and IFT20-SNAP were digested with EcoRI restriction enzyme. Expected band sizes, IFT20-mCherry: 4.6 Kb and 1.6 Kb, IFT20-SNAP: 4.6kB and 1.4kB.

Fig. 4. Transfection of pJAF2.13 with jetPRIME reagent

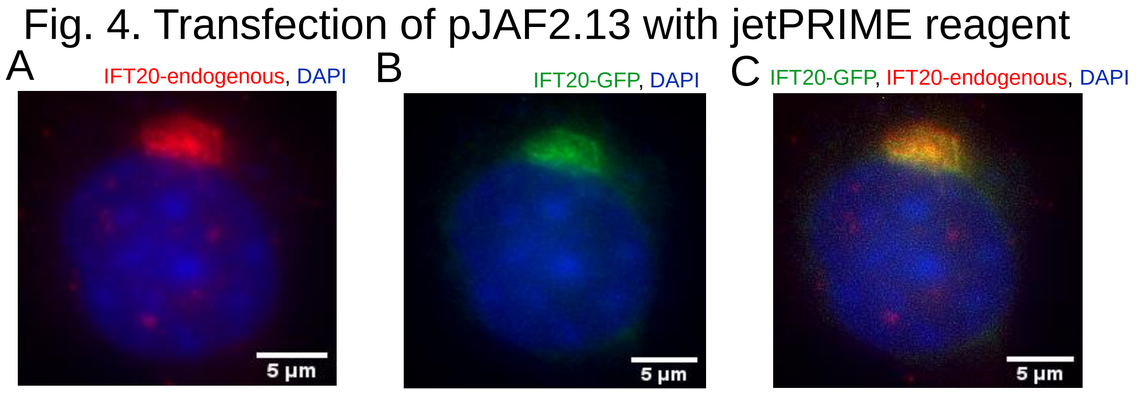
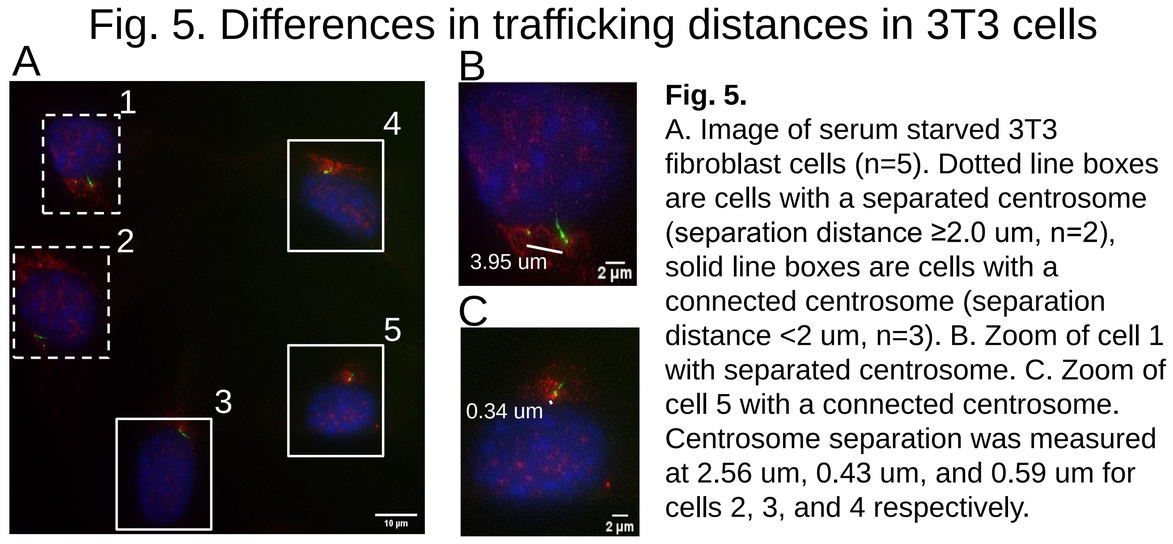


Fig. 4.
A. 3T3 fibroblast cell stained for endogenous IFT20. B. 3T3 fibroblast cell expressing IFT20-GFP fluorescent protein. C. Merged image of A and B showing colocalization of IFT20-GFP with endogenous IFT20. D. Transfection efficiencies used to optimize transfections using jetPRIME reagent. Recommended volume is 2 uL.

Fig. 5. Differences in trafficking distances in 3T3 cells



Nucleus, Golgi, Cilia/Centrosome

Fig. 5.
A. Image of serum starved 3T3 fibroblast cells (n=5). Dotted line boxes are cells with a separated centrosome (separation distance ≥2.0 um, n=2), solid line boxes are cells with a connected centrosome (separation distance <2 um, n=3). B. Zoom of cell 1 with separated centrosome. C. Zoom of cell 5 with a connected centrosome. Centrosome separation was measured at 2.56 um, 0.43 um, and 0.59 um for cells 2, 3, and 4 respectively.

Conclusions

- Subcloning of IFT20 and fluorescent markers was successful. The diagnostic gel shows bands at the expected sizes for both IFT20-mCherry and IFT20-SNAP.
- Transfected IFT20-GFP was shown to localize with endogenous IFT20.
- Transfection efficiency peaked at about 6.5% using 3.0 uL of jetPRIME reagent.
- Preliminary data showed serum starving is not an adequate condition alone to produce separated centrosomes in 3T3 cells.

References

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