Barriers and molecular trains trap Joubert syndrome protein in cilia

December 10 2013

(Medical Xpress)—A cilia disease protein causing Joubert Syndrome, ARL-13, is actively trafficked across the base of cilia and molecular diffusion barriers prevent its exit, according to new research from the UCD Conway Institute published in *PLoS Genetics*.

Primary cilia are hair-like projections extending from the surfaces of most human cell types that serve as important antennae to relay external sensory and signalling information back to the cell. Disrupting cilium structure or function leads to a wide range of diseases, termed ciliopathies, linked to multiple symptoms including cystic kidneys, blindness, bone formation defects, mental retardation and obesity.

An important feature of the cilium is its compartmentalised nature. This allows many proteins, including those involved in ciliopathies to become specifically enriched within the structure where they function. Although not well understood, the process of ciliary compartmentalisation is thought to involve active transport systems such as intraflagellar transport (IFT) and molecular diffusion barriers at the ciliary base (transition zone).

Using a combination of genetics and live imaging approaches, Dr Oliver Blacque and his team in the UCD Conway Institute have recorded the movement of molecules across the base of cilia in real-time, which provided kinetic information about the barrier itself. They believe this is the first time fluorescent recovery after photobleaching (FRAP) has been used in an 'in vivo' setting to assess protein diffusion into and out of

cilia.

The team showed that proteins causing Meckel syndrome and nephronophthisis, which are symptomatically related to Joubert syndrome, comprise a barrier at the transition zone that prevents leakage of ciliary membrane-associated ARL-13 out of cilia. In contrast, they found that IFT proteins play no role in barrier formation but instead are required for actively transporting ARL-13 across the barrier.

Commenting on the significance of the research, Dr Blacque said, "We have been able to directly test 'in vivo' the interplay of active transport and membrane diffusion barrier mechanisms in restricting proteins to cilia. We found that Joubert syndrome-associated ARL-13 can act as a cargo of intraflagellar transport (IFT) trains as they move from the periciliary membrane into the cilium, across a transition zone barrier directly regulated by MKS and NPHP (cilia disease) proteins.

These findings extend our previous work that suggested a diffusion barrier at the ciliary base, and again show how nematode genetics and imaging serve as powerful allies for uncovering basic principles of cell biology and human disease gene pathomechanisms."

The Blacque team, who led the entire study, worked in conjunction with the UCD Conway Imaging Core facility to develop the FRAP technique. They also collaborated with colleagues in the Universities of Radboud and Tuebingen to identify the composition of human Arl13b complexes using semi-quantitative and quantitative (SILAC) affinity proteomics.

Dr Blacque and his team now hope to use their FRAP assay to uncover more genes directly responsible for establishing diffusion barriers at the ciliary base and define their precise kinetic contributions to barrier function.

At a wider level, this work will help to shed light on how important signalling processes are confined to small regions of the cell's plasma membrane.

More information: "Active transport and diffusion barriers restrict Joubert Syndrome-associated ARL13B/ARL-13 to an Inv-like ciliary membrane subdomain." Sebiha Cevik, Anna A. W. M. Sanders, Erwin Van Wijk, Karsten Boldt, Lara Clarke, Jeroen van Reeuwijk, Yuji Hori, Nicola Horn, Lisette Hetterschijt, Anita Wdowicz, Andrea Mullins, Katarzyna Kida, Oktay I. Kaplan, Sylvia E. C. van Beersum, Ka Man Wu, Stef J. F. Letteboer, Dorus A. Mans, Toshiaki Katada, Kenji Kontani, Marius Ueffing, Ronald Roepman, Hannie Kremer,Oliver E. Blacque. *Plos Genetics* Dec. 05 2013

Provided by University College Dublin