Supplementary Information

Functional interaction between autophagy and ciliogenesis

Olatz Pampliega[1], Idil Orhon[4,5], Bindi Patel[1], Sunandini Sridhar[1], Antonio Diaz-Carretero[1] , Isabelle Beau[4], Patrice Codogno[4,5], Birgit Satir[2], Peter Satir*[2], Ana Maria Cuervo*[1,2,3]

Supplementary Video 1-5:

Video 1: **Localization of Vps15 at the axoneme.** Serial images through the Z-stacks of the 3D reconstituted axoneme of kidney epithelial cells after 24h in the absence of serum and co-immunostained for Vps15 (green) and acetylated tubulin (red).

Video 2: **Localization of Atg16L at the axoneme.** Serial images through the Z-stacks of the 3D reconstituted axoneme of kidney epithelial cells after 24h in the absence of serum and co-immunostained for Atg16L (green) and acetylated tubulin (red).

Video 3: **Localization of AMBRA-1 at the axoneme.** Serial images through the Z-stacks of the 3D reconstituted axoneme of kidney epithelial cells after 24h in the absence of serum and co-immunostained for AMBRA-1 (green) and acetylated tubulin (red).

Video 4: **Localization of LC3 at the axoneme.** Serial images through the Z-stacks of the 3D reconstituted axoneme of kidney epithelial cells after 24h in the absence of serum and co-immunostained for LC3 (green) and acetylated tubulin (red).

Video 5: **Localization of GABARAP at the axoneme.** Serial images through the Z-stacks of the 3D reconstituted axoneme of kidney epithelial cells after 24h in the absence of serum and co-immunostained for GABARAP (green) and acetylated tubulin (red).

Supplementary Discussion: expanded discussion and pertinent references.

Supplementary Discussion

Discussion 1

We cannot rule out the possibility that IFT can also contribute to relocation of Atgs to other sites of autophagosome formation but our studies comparing IFT88$^{-/-}$ cells in which only aIFT is altered with IFT20(-) cells in which both aIFT and shuttling from Golgi are altered, support that it is the coincidence of the arrival of Atg16L and signaling events dependent on intact aIFT which leads to maximal induction of starvation-induced autophagy. In addition, most of the changes in the cellular distribution of other Atgs become apparent by just interfering with the aIFT transport. These results support that likely signaling coming through the cilia rather than a direct effect of IFT88 on these proteins, are behind the changes in their cellular distribution.

Different signaling mechanisms described to utilize the primary cilium – pVHL, HIF1a or Aurora A, Par2 complex proteins, LKB1, PDGFRα – may be able to modulate cilia-mediated autophagy. In this study, we report the contribution of Hh signaling to this regulation, one of the best-characterized cilium dependent signaling pathways. Dynamic movement of proteins into and out of the cilium is well documented for this signaling pathway. Because proteins exiting the cilium find themselves at the basal body where most of the regulatory Atgs reside, we propose that the arrival of these signaling proteins may act as a trigger for autophagosome formation. In support of this idea, constitutive upregulation of Hh signaling in permanently ciliated Ptc1-/- cells enhances association of Atgs to the basal body, elongation of the Atg5 hook-like structures and abundance of LC3 along the primary cilium. In addition to these local changes, constitutive activation of Hh is enough to reproduce changes in the overall cellular distribution of Atgs, such as Atg7, further reinforcing the likelihood of an amplification-effect of the signal that originates from the cilium to activate the autophagic program.

Peripheral ciliary membrane, matrix proteins and transmembrane receptors transport into the growing cilia through the ciliary transition zone in association with the assembly of aIFT particles. We propose that the four Atgs located in the primary cilium - Vps15, AMBRA, LC3 and GABARAP – also reach this compartment in an IFT-dependent manner. Interestingly, Vps15 and AMBRA have short amino

acid sequences found to be ciliary localization signals for proteins such as polycystins or opsin, while GABARAP and LC3 do not. LC3 and GABARAP may gain access to the cilium by lateral diffusion after reaching the plasma membrane since as we have shown in this work, nutrient starvation induces the recruitment of these two proteins towards the plasma in an IFT-dependent manner. Transport of some or all of these molecules into the cilium may depend on their lipid conjugation, a property shared by the four of them.

Discussion 2

Atg were, for the most part, absent in the catalogue of primary cilia proteins generated recently using proteomic approaches in isolated primary cilia [1]. We attribute the absence of Atg in that study to the fact that we found most Atg in the basal body, and this structure was not recovered in the isolated cilia (judging by the absence of basal body proteins in the proteomic analysis). Failure to detect the Atg proteins that we found associated to the axoneme could have been due to solubility issues, as these Atg are all lipid binding proteins and they failed to detect many known ciliary membrane proteins, such as polycystins (attributed by the authors to their reduced solubility under the extraction conditions used in their analysis).

Discussion 3

As previously reported, we found that part of IFT20 is degraded through the proteasome both under nutrient-rich and -deprived conditions (data not shown), whereas autophagic degradation of IFT20 seems to be more prominent in the presence of nutrients. The mechanism(s) by which IFT20 evades autophagic degradation during starvation requires further investigation. It is possible that conformational changes resulting from its interaction with cargo ciliary membrane proteins protect IFT20 from degradation under those conditions and allow for its full engagement in ciliogenesis.

Discussion 4

Hh signaling seems to exert opposite effects on autophagy depending on the experimental setting and cell type [2-5]. These differences could be in part determined by the relative contribution of downstream Gli proteins in each cell type (i.e. some of them have opposite effects in other cellular processes, and for example, we show here that overexpression of Gli1 alone is capable to rescue the autophagic phenotype, whereas the inhibitory effect on autophagy has been more closely linked to changes in Gli2). However, considering our current findings, it is also possible that the opposite effects of Hh signaling on autophagy could be related to its ciliary origin or not. In this respect, ciliary Hh signaling may exert its stimulatory effect on autophagy by directly influencing Atgs located at the base of this organelle, and thus be different from the signaling originating from other cellular locations.

References

1 Ishikawa, H., Thompson, J., Yates, J. R., 3rd & Marshall, W. F. Proteomic analysis of mammalian primary cilia. *Curr Biol* **22**, 414-419,(2012).

2 Li, H. *et al.* Sonic hedgehog promotes autophagy of vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* **303**, H1319-1331,(2012).

3 Jimenez-Sanchez, M. *et al.* The Hedgehog signalling pathway regulates autophagy. *Nat Commun* **3**, 1200,(2012).

4 Wang, Y., Han, C., Lu, L., Magliato, S. & Wu, T. Hedgehog signaling pathway regulates autophagy in human hepatocellular carcinoma cells. *Hepatology*,(2013).

5 Petralia, R. S. *et al.* Sonic hedgehog promotes autophagy in hippocampal neurons. *Biology open* **2**, 499-504,(2013).