



**HAL**  
open science

## Autophagy discriminates between Alix and ESCRTs.

Anne Petiot, Rémy Sadoul

► **To cite this version:**

Anne Petiot, Rémy Sadoul. Autophagy discriminates between Alix and ESCRTs.. *Autophagy*, 2009, 5 (1), pp.106-7. inserm-00450116

**HAL Id: inserm-00450116**

**<https://inserm.hal.science/inserm-00450116>**

Submitted on 19 Mar 2012

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## **Autophagy discriminates between Alix and ESCRTs.**

Anne Petiot<sup>1,2</sup> and Rémy Sadoul<sup>1,2</sup>

<sup>1</sup>INSERM U836, Grenoble Institute of Neuroscience, F-38042 Grenoble, France.

<sup>2</sup>Université Joseph Fourier, Grenoble Institute of Neurosciences (GIN), F-38042 Grenoble, France.

Corresponding author: Rémy Sadoul

**Phone: +33 4 56 52 05 44**

**Fax: +33 4 56 52 05 54**

**Email: [remy.sadoul@ujf-grenoble.fr](mailto:remy.sadoul@ujf-grenoble.fr)**

Addendum to: Petiot A, Strappazon F, Chatellard-Causse C, Blot B, Torch S, Verna JM, and Sadoul R. Alix differs from ESCRT proteins in the control of autophagy. *Biochem Biophys Res Commun* 2008; **375**: 63-68.

## **SUMMARY**

**Alix and ESCRT proteins are required for membrane fission during viral budding and egress and during the abscission stage of cytokinesis. These common roles have suggested that Alix functions as an ESCRT protein, a conclusion challenged by the finding that unlike ESCRTs, which control the formation of multivesicular endosomes, Alix does not influence the degradation of the EGF receptor. We previously showed that Alix controls neuronal death by an unknown mechanism, but dependent on its interaction with ESCRT proteins. Since then, numerous reports have shown that ESCRTs participate in macroautophagy. Given the direct interaction between ESCRTs and Alix, together with the known contribution of autophagy to cell death, it was hypothesized that Alix controls autophagy and thereby cell death. Our recent published results show that this is not the case. ESCRT protein activity therefore needs Alix for viral budding and cytokinesis but not for autophagy. The function of ESCRT can thus be clearly be disconnected from that of Alix.**

**KEY WORDS:** Alix; Autophagy; ESCRT; endocytosis; MVB.

**ACKNOWLEDGEMENTS:** The authors would like to thank Fiona Hemming for critical reading of the manuscript. This work was supported by Inserm, the University Joseph Fourier, and grants from the Association Française contre les Myopathies, (AFM), the Association pour la Recherche sur la Sclérose Latérale Amyotrophique (ARS), the Association pour la recherche sur le cancer (ARC), the Fondation pour la Recherche Médicale (FRM) and region Rhone Alpes.

The endosomal system is composed of a series of intracellular compartments within which endocytosed molecules and redundant cellular material are hydrolyzed. Endocytosed material tends to flow vectorially through the system from early

endosomes to late endosomes and then into lysosomes, whereas phagocytosis and autophagy provide alternative entry points. Endocytosed membrane proteins meant for degradation are selectively sequestered within vesicles budding from the membrane into the lumen of endosomes. This process leads to the formation of endosome intermediates called multivesicular endosomes (MVEs), filled with intraluminal vesicles, which are hydrolyzed after fusion of the MVEs with lysosomes.<sup>1</sup> Intracellular protein and organelle degradation occurs via autophagy after MVEs or endosomes fuse with autophagosomes to form amphisomes.<sup>2</sup> The autophagic process ends with the fusion of amphisomes with lysosomes.

After endocytosis, the cytoplasmic domains of receptors pointing out into the cytosol trigger the sequential building onto the endosomal membrane, of protein complexes called the *endosomal sorting complex required for transport* (ESCRT I, ESCRT II, ESCRT III). ESCRT III complexes, made of CHMP proteins in mammalian cells, entrap transmembrane proteins and contribute to the deforming of the endosomal membrane into vesicles.<sup>3</sup>

The role played by ESCRTs is central to the replication of enveloped viruses, which recruit them via their GAGs in order to bud off membranes.<sup>4</sup> Both retroviruses and vesicles of MVEs bud away from the cytoplasm, and CHMP proteins of ESCRT-III seem instrumental for the fission of vesicles/virus from membranes. More recently, ESCRT proteins were shown to function in the last step of cytokinesis, which allows separation of the daughter cells after cell division.<sup>5-6</sup> These events all require fission of a thin membrane tubule from within. CHMP proteins make helical tubular structures possibly inside deforming membranes. Fission would occur upon disassembly of the helical structures by the AAA-type ATPase Vps4/SKD1, which is known to be required for MVE genesis and viral abscission.<sup>7</sup>

Alix is another protein that might be involved in membrane deformation/fission in tight relation with ESCRT proteins. This cytoplasmic protein binds to both Tsg101 and SNF7/CHMP4B, members of the ESCRT I and III, respectively,<sup>8-9</sup> and to

lysobisphosphatidic acid (LBPA), a phospholipid which is highly concentrated inside MVEs and may help the intraluminal vesiculation of endosomes.<sup>10</sup> In both cases of cytokinesis and virus budding, Alix represents a way to recruit the ESCRT machinery necessary for membrane fission. The situation is far less clear regarding a role of Alix in MVE genesis. A first hint that it might play a role in this process stems from its homology with a yeast protein, Bro1, required for the sorting of mono-ubiquitinated transmembrane proteins into intraluminal vesicles of MVEs.<sup>11</sup> Furthermore, in HeLa cells knockdown of Alix results in a loss of intraluminal vesicles inside endosomes.<sup>10</sup> Surprisingly, however, neither we nor other labs have found any significant effect of Alix on endocytosis and degradation of the EGF receptor, a process known to require ESCRT proteins.<sup>12-13</sup> Thus, the final consequence of Alix activity in MVEs remains largely unclear.

Originally, Alix was characterized in a screen for proteins capable of binding the calcium binding protein ALG-2 (Apoptosis Linked Gene-2),<sup>14</sup> first reported to be required for T cell apoptosis.<sup>15</sup> Alix expression is up-regulated in degenerating neurons<sup>16</sup> and overexpression is sufficient to activate caspases and thereby neuronal death.<sup>17-18</sup> In contrast, dominant negative mutants of the protein block neuronal death. In particular, we found that several Alix mutants electroporated into chick embryo motoneurons *in ovo* rescue them from cell death occurring naturally during development. The death inhibiting activity of the mutants was tightly dependent on their capacity to bind to ESCRT proteins.<sup>18</sup> Programmed cell death of motoneurons is known to involve caspases, even though inhibiting the proteases only blocks nuclear destruction without allowing motoneuron survival.<sup>18-19</sup> Death might thus occur through a caspase-independent mechanism, which is inhibited by Alix mutants.

Caspase-independent cell death remains ill defined, although autophagy has often been designated as responsible for cytoplasmic destruction.<sup>20-21</sup> Because of the tight relationship between Alix and the endo-lysosomal system we reasoned that its capacity to block caspase-independent neuronal death might relate to a possible role

in the process of autophagy. In a first set of experiments we found that autophagosomes decorated with the microtubule-associated protein 1 light chain 3 LC3 accumulated in cultured post-mitotic neurons expressing Alix or deprived of trophic support. Furthermore, an Alix dominant negative mutant inhibited accumulation of such vacuoles in neurons deprived of trophic support (Strappazzon et al., unpublished observation). These results could suggest that Alix drives autophagy and thereby neuronal death, or simply reflect the fact that autophagy accompanies neuronal death, which is itself controlled by Alix through an unrelated mechanism. In our recent paper<sup>22</sup> we used BHK-21 cells to discriminate between these interpretations by uncoupling autophagy from cell death. In these cells Alix overexpression has no apparent effect on cell survival, and autophagy can be induced independently of cell death by depletion of essential amino acids. Using this paradigm, we found that Alix depletion or expression of Alix mutants did not affect LC3 localization or bulk protein degradation. These results were unexpected in view of recent reports implicating ESCRT proteins in autophagy. In particular depletion of Tsg-101 of ESCRT-I and SNF7/CHMP4B of ESCRT-III, which both interact with Alix, were shown to lead to accumulation of autophagosomes in mammalian and insect cells, respectively.<sup>23</sup> Thus, our findings demonstrate that Alix control of neuronal death is unrelated to autophagy. They also clearly discriminate between the functions of Alix and ESCRT proteins and suggest that during autophagy Alix does not intervene in the recruitment/activation of ESCRTs as in the case of viral budding or cytokinesis.

## REFERENCES

1. van der Goot FG, and Gruenberg J. Intra-endosomal membrane traffic. *Trends Cell Biol* 2006; **16**: 514-521.
2. Liou W, Geuze HJ, Geelen MJ, Slot JW. The autophagic and endocytic pathways converge at the nascent autophagic vacuoles. *J Cell Biol*. 1997; 1:61-70.
3. Williams RL, and Urbe S. The emerging shape of the ESCRT machinery. *Nat Rev Mol Cell Biol* 2007; **8**: 355-368.
4. Morita E and Sundquist WI. Retrovirus budding. *Annu Rev Cell Dev Biol* 2004; **20**: 395-425.
5. Morita E, Sandrin V, Chung H Y, Morham SG, Gygi SP, Rodesch CK, and Sundquist WI. Human ESCRT and ALIX proteins interact with proteins of the midbody and function in cytokinesis. *Embo J* 2007; **26**: 4215-4227.
6. Carlton JG, and Martin-Serrano J. Parallels between cytokinesis and retroviral budding: a role for the ESCRT machinery. *Science* 2007; **316**: 1908-1912.
7. Lata S, Schoehn G, Jain A, Pires R, Piehler J, Gottlinger HG, and Weissenhorn W. Helical structures of ESCRT-III are disassembled by VPS4. *Science* 2008; **321**: 1354-1357.
8. Strack B, Calistri A, Craig S, Popova E, and Gottlinger HG. AIP1/ALIX is a binding partner for HIV-1 p6 and EIAV p9 functioning in virus budding. *Cell* 2003; **114**: 689-699.
9. von Schwedler UK, Stuchell M, Muller B, Ward DM, Chung HY, Morita E, Wang HE, Davis T, He GP, Cimbora DM, Scott A, Krausslich HG, Kaplan J, Morham SG, and Sundquist WI. The protein network of HIV budding. *Cell* 2003; **114**:701-713.
10. Matsuo H, Chevallier J, Mayran N, Le Blanc I, Ferguson C, Faure J, Blanc N S, Matile S, Dubochet J, Sadoul R, Parton RG, Vilbois F, and Gruenberg J. Role of LBPA and Alix in multivesicular liposome formation and endosome organization. *Science* 2004; **303**: 531-534.

11. Odorizzi G, Katzmann DJ, Babst M, Audhya A, and Emr SD. Bro1 is an endosome-associated protein that functions in the MVB pathway in *Saccharomyces cerevisiae*. *J Cell Sci* 2003; **116**: 1893-1903.
12. Cabezas A, Bache KG, Brech A, and Stenmark H. Alix regulates cortical actin and the spatial distribution of endosomes. *J Cell Sci* 2005; **118**: 2625-2635.
13. Doyotte A, Mironov A, McKenzie E, and Woodman P. The Bro1-related protein HD-PTP/PTPN23 is required for endosomal cargo sorting and multivesicular body morphogenesis. *Proc Natl Acad Sci* 2008; **105**: 6308-6313.
14. Missotten M, Nichols A, Rieger K, and Sadoul R. Alix, a novel mouse protein undergoing calcium-dependent interaction with the apoptosis-linked-gene 2 (ALG-2) protein. *Cell Death Differ* 1999; **6**: 124-129.
15. Vito P, Lacana E, and D'Adamio L. Interfering with apoptosis: Ca<sup>2+</sup>-binding protein ALG-2 and Alzheimer's disease gene ALG-3. *Science* 1996; **271**: 521-525.
16. Hemming FJ, Fraboulet S, Blot B, and Sadoul R. Early increase of apoptosis-linked gene-2 interacting protein X in areas of kainate-induced neurodegeneration. *Neuroscience* 2004; **123**: 887-895.
17. Trioulier Y, Torch S, Blot B, Cristina N, Chatellard-Causse C, Verna JM, and Sadoul R. Alix, a protein regulating endosomal trafficking, is involved in neuronal death. *J Biol Chem* 2004; **279**: 2046-2052.
18. Mahul-Mellier, A-L, Hemming FJ, Blot B, Fraboulet S, and Sadoul R. Alix, making a link between apoptosis-linked gene-2, the endosomal sorting complexes required for transport, and neuronal death in vivo. *J Neurosci* 2006; **26**: 542-549.
19. Oppenheim RW, Flavell RA, Vinsant S, Prevette D, Kuan CY, and Rakic P. Programmed cell death of developing mammalian neurons after genetic deletion of caspases. *J Neurosci* 2001; **21**: 4752-4760.
20. Clarke PG. Developmental cell death: morphological diversity and multiple mechanisms. *Anat Embryol* 1990; **18**: 195-213.

21. Bursch W. The autophagosomal-lysosomal compartment in programmed cell death. *Cell Death Differ* 2001; **8**:569-581.
22. Petiot A, Strappazon F, Chatellard-Causse C, Blot B, Torch S, Verna JM, and Sadoul R. Alix differs from ESCRT proteins in the control of autophagy. *Biochem Biophys Res Commun* 2008; **375**: 63-68.
23. Rusten TE and Simonsen A. ESCRT functions in autophagy and associated disease. *Cell Cycle* 2008; **7**:1166-1172.