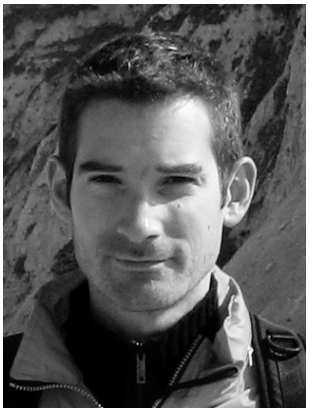

2000 - 2006	Ph.D. – BZH
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Julien Béthune

Interplays between endomembranes, RNA-binding proteins and cellular polarization

Goal

We are interested in the mechanisms of post-transcriptional gene silencing by miRNAs, and their interplays with endomembranes. This includes the development of new techniques for the identification of protein-protein interactions. We are also characterizing the function of COPI transport vesicles during the differentiation of pluripotent cells using CRISPR/Cas9-mediated genome editing.

Background

In the past decade, microRNAs (miRNAs) have emerged as key regulators of most cellular functions by potentially post-transcriptionally repressing at least 50% of expressed mRNAs. Not surprisingly, misregulation of miRNAs leads to diseases including cancer. Argonaute (Ago) proteins are the central players in miRNA-mediated silencing, using miRNA as guide to find their mRNA targets. Interestingly, Ago largely associates with endomembranes, notably with the endoplasmic reticulum. Data from different laboratories using various model systems suggest that membrane-associated Ago have specific functions that we try to characterize. Membrane-enclosed transport vesicles mediate protein and lipid transport within the secretory pathway. In fungi, co-transport of mRNAs and endomembranes was shown to regulate cell polarization. More generally, in higher eukaryotes, a functional role of the early secretory pathway, notably COPI vesicles, during polarization events such as neurogenesis has been suggested but is still hardly studied.

Research Highlights

Novel proteomics approaches for the analysis of specific functional complexes

To identify additional regulating factors of the miRNA-mediated silencing pathway, we have performed BioID on the Ago2 protein. BioID is a proximity-dependent labeling technique that leads to the biotinylation of proximal proteins within live cells. Proteomics analysis revealed that, when compared to co-immunoprecipitation (co-IP) approaches, BioID identified numerous known interacting proteins that are required for miRNA-mediated silencing but were absent in co-IP. Like many other proteins, Ago2 is part of several protein complexes corresponding to distinct functional steps of the miRNA pathway. To address the general challenge of assigning novel identified protein-protein interactions to specific functional protein complexes, we have developed a split-BioID assay that allows the conditional bi-

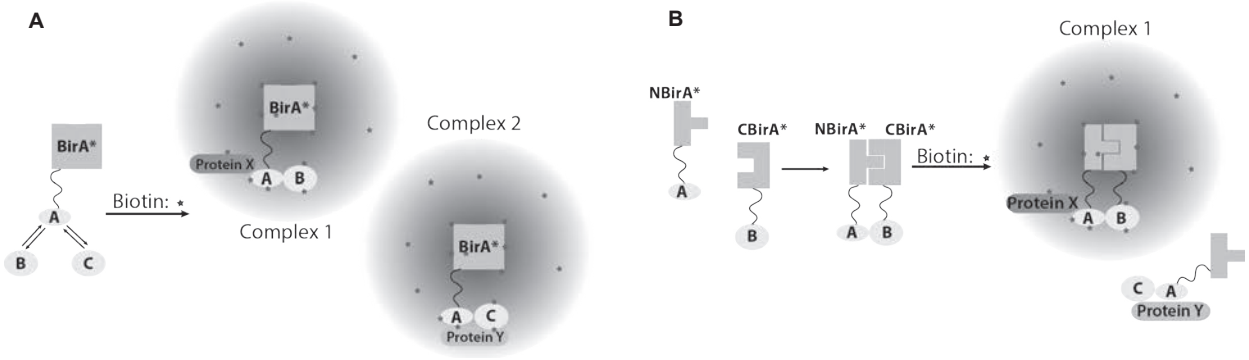

Figure 1: Split-BioID allows probing the proteome of spatiotemporally defined protein complexes, A: *Classical BioID applied to protein A that is part of two distinct complexes when interacting with protein B or C. Both protein complexes are labeled.* ***B:*** *Split-BioID is only activated upon interaction of proteins A and B, allowing conditional proteomics of complex 1.*

otinylation of proximal proteins upon the interactions of two known interacting partners (Fig. 1). With this assay we could successfully probe the proteomes of two functionally distinct protein complexes of which Ago2 is part of (Schopp et al., submitted). These combined approaches led to the identification of novel regulating factors of the miRNA pathway that we are currently characterizing.

Functional characterization of ER-associated Ago2

To understand the functional roles of membrane-associated Ago2, we make use of a mouse embryonic stem cell line in which a knockout of all four Ago proteins is inducible. Using a PiggyBac transposon-based system, we are generating rescue cell lines with various variant of Ago2 that are trapped to different endomembranes. The phenotypes of the rescue cell lines will then be analyzed and help us understanding why Ago2 associate with endomembranes.

Function of COPI-coated vesicles during stem cell differentiation

To investigate a proposed functional role of Golgi-derived vesicles during neurogenesis, we have used murine pluripotent cell lines as a model system for cellular differentiation. Using the CRISPR/Cas9 approach, we have knocked out the γ1-COP or γ2-COP coatomer paralog subunits. We found that removal of γ1-COP leads to defective embryoid bodies formation, which is the first step of differentiation. As a consequence, neurite extension is then later strongly impaired. These data are the first evidence for a paralog specific function of the COPI pathway (see report Wieland group) and suggests that COPIγ1 is critical during early steps of stem cell differentiation. We are currently characterizing the molecular mechanisms underlying this observation.

Selected Publications

Schopp, I., Amaya Ramirez, C., Kreibich, E., Skribbe, M., Wild, K., and Béthune, J. (2016). Split-BioID: a conditional proteomics approach to monitor the composition of spatiotemporally defined protein complexes. Submitted

Béthune, J., Artus-Revel, C.G., and Filipowicz, W. (2012). Kinetic analysis reveals successive steps leading to miRNA-mediated silencing in mammalian cells. EMBO Rep 13, 716-723.

Beck, R., Sun, Z., Adolf, F., Rutz, C., Bassler, J., Wild, K., Sinning, I., Hurt, E., Brugger, B., Béthune, J., and Wieland, F. (2008). Membrane curvature induced by Arf1-GTP is essential for vesicle formation. Proc Natl Acad Sci U S A 105, 11731-11736.

Sun, Z., Anderl, F., Frohlich, K., Zhao, L., Hanke, S., Brugger, B., Wieland, F., and Béthune, J. (2007). Multiple and stepwise interactions between coatomer and ADP-ribosylation factor-1 (Arf1)-GTP. Traffic 8, 582-593.

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