Investigating the regulation of the metalloprotease ADAM10 by tetraspanins in colon cancer, a multidisciplinary approach

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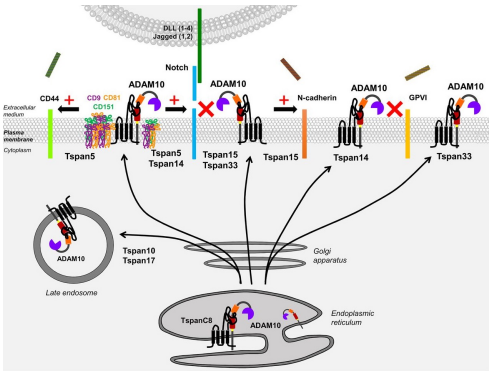
Abstract

ADAM10 is a transmembrane metalloprotease, an enzyme embedded in the cell membrane, which cleaves several substrates that play an important role in tumor progression. This makes ADAM10 a promising therapeutic target in cancer. Notably, ADAM10 plays a critical role in the Notch signaling pathway, which is essential for cell communication and development.

Our previous work has shown that ADAM10 interacts directly with a specific group of tetraspanins, known as TspanC8, which includes Tspan5 and Tspan15. These proteins act as molecular organizers at the cell membrane, regulating ADAM10 in several ways, including intracellular trafficking, substrate specificity and Notch signaling.

The main goal of the project is to elucidate the molecular mechanisms involved in the regulation of ADAM10 by TspanC8, using advanced single molecules fluorescence techniques. We are using a strategy combining a genetic code expansion and single molecule FRET (fluorescence resonance energy transfer) to test the hypothesis that TspanC8 regulates the action of ADAM10 at different distances from the plasma membrane. We are also investigating the impact of TspanC8 on ADAM10 membrane compartmentalization using single-molecule localization microscopy such as PAINT (point accumulation for imaging in nanoscale topography). This study will shed new light on the role of ADAM10 and its associated tetraspanins in colon cancer, and pave the way for potential targeting of ADAM10 function in this cancer.

Background: ADAM10, tetraspanins and cancer



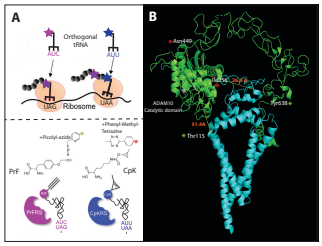
ADAM10 is expressed as a zymogen, the prodomain of which is removed in the Golgi apparatus through the action of proprotein convertases. At the plasma membrane, ADAM10 cleaves off the ectodomain of its transmembrane substrates, leading to the release of a soluble extracellular fragment and a remnant transmembrane CTF. The latter is then processed by the γ-secretase complex, yielding an intracellular domain which is fated to either degradation or has a signaling activity, as described for Notch.

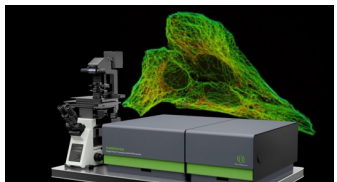
The tetraspanin web is hierarchically organized in several levels of interactions: primary complexes composed of a tetraspanin directly associated with a so-called specific primary partner protein. These primary complexes associate with one another through tetraspanin to tetraspanin interactions to generate a network of interactions named tetraspanin web (Charrin, 2014)). In line with this partner/pair model, the next step after demonstrating that ADAM10 associates with tetraspanins was to identify those that directly associate with ADAM10.

The interaction of a subset of the tetraspanin family, named TspanC8, interact with ADAM10, allowing its exit from the ER and its accumulation in either late endosomes (Tspan10 and Tspan17) or at the plasma membrane (Tspan5, Tspan14, Tspan15 and Tspan33). At the plasma membrane, Tspan5, Tspan14 and Tspan15 differentially associate with the tetraspanin web and have a different impact on Notch signalling (Dornier, 2012; Jouannet, 2016).

Methodology: a single molecule approach

- Single molecule FRET (fluorescence resonance energy transfer) to test the hypothesis that TspanC8 regulates the action of ADAM10 at different distances from the plasma membrane



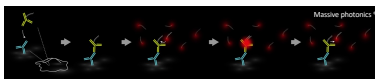

Luminosa (Single Photon Counting Confocal Microscope) - Picoquant

A) Upper panel: schematic representation of cotranslational incorporation of non canonical AAs and chemoselective fluorescence modification using genetic code expansion and bioorthogonal click chemistry (Quast, 2019). Coexpression of the orthogonal synthetase together with its cognate amber suppressor tRNACUA or tRNAUUA in the presence of the noncanonical amino acid (colored stars) allows the specific incorporation of non canonical aminoacids; Lower panel: incorporation of p-propargyloxy-l-phenylalanine (PrF) and/or cyclopropene- l-lysine (CpK) is mediated by the orthogonal synthetase (PrFRS and/or CpKRS) together with its cognate amber suppressor tRNA (the tyrosyl-tRNA synthetase /tRNACUA pair and/or the pyrrolysyl-tRNA synthetase/tRNAUUA for PrF and CpK, respectively). PrF (purple) and/or CpK (blue) are in a second step labelled using click-chemistry with respectively picolyl azide or phenyl-tetrazine conjugated with a fluorescent dye. B) 3D representation of ADAM10/TSPAN15 complex adapted from Lipper 2023. ADAM10 and TSPAN15 are displayed in green and blue, respectively. The dashed orange line represents the distance between 2 aminoacids in white. The stars indicate the fluorophores.
Fluorescence spectroscopy will be performed using a single photon counting confocal microscope (Luminosa, Picoquant).

Expected outcomes: from our combination of molecular biology, biochemistry and smFRET, we expect to get accurate distances between ADAM10 and Tspan5 or Tspan15 from experiments in solution. They will be very informative to explain the differential substrate activity of ADAM10 on its substrate (we will use TMEM132a) and describe the molecular membrane landscape of this key enzyme.

- PAINT (point accumulation for imaging in nanoscale topography) to study ADAM10 membrane partitioning upon tetraspanin expression

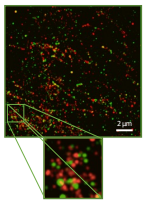
DNA-PAINT (DNA-Point Accumulation for Imaging in Nanoscale Topography) is a localization based super-resolution microscopy method (Jungmann, 2010) that uses short, dye-labelled single stranded DNA (ssDNA strands) that transiently bind to their complements. It is based on the use of an Imager strand – a dye-labelled DNA oligo present in imaging buffer - and a Docking strand – a DNA oligo complementary to the imager strand that is delivered to the target of interest via binders (e.g. antibodies, single domain antibodies).



When a dye-labelled imager transiently hybridizes to a docking strand in DNA-PAINT imaging buffer, fluorescent signal or "blinking" occurs. To create a super-resolution image, a camera captures blinking over a period of time (10 – 50 min) and the resulting image is post-processed. A 10-15 nm lateral resolution can be achieved in intact cells with PAINT, also enabling unlimited target multiplexing. Indeed, it is simply possible to couple different docking strands (*e.* different DNA sequences) to the target binders and image each target sequentially using the same laser line with its corresponding imager strand.

Expected outcomes: Molecular mapping obtained with DNA Paint experiments will provide information about the distances between ADAM10 and its substrates on a nanometer scale in intact cells, which should reflect the probability of the enzyme to cleave a given protein in the presence of Tspan5 and or T15C5, a chimeric molecule of Tspan5 and Tspan15.

The images on the right correspond to single molecule localization microscopy experiments of the tetraspanins CD9 (green) and CD81 (red) and illustrate the expected results with Tspan5 and Tspan15.



Research landscape

All the experiments will be carried out in the "Integrative Biophysics of Membrane (IBM) team (https://integrativebiophysicsofmembranes.wordpress.com/) at the CBS (https://www.cbs.cnrs.fr/index.php/en/), an interdisciplinary research institute with the general objective to conduct cutting-edge research in structural biology, biophysics and bioengineering in order to describe and understand the physico-chemical mechanisms underlying biological processes, from the molecular and cellular level to supramolecular assemblies in the context of living organisms. The team gathers researchers and engineers with training in physics, biophysics and cell biology.

The Master's student will be co-supervised by Nicolas Kuszla and Pierre-Emmanuel Milhiet. Depending on the project's progress and his/her preferences, he/she will be involved in one of the two methodologies.

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