Measurement of *in vivo* specific translational activity of mRNA UTRs by microinjection of luciferase reporter genes in sea urchin eggs.

Florian Pontheaux
Station Biologique Roscoff, CNRS, Sorbonne Université, UMR 8227
fpontheaux@sb-roscoff.fr

Translation is the last key event for the expression of the coding genome. As in previous steps, many regulations drive translation. Thus, investigating those regulations is essential. Upon fertilization, sea urchin eggs directly start their mitotic division. These divisions require a strong increase of translation (without any transcriptional activity) upon fertilization regulated by mTOR and MAPK pathways (Chassé et al. 2016 ; Mulner-Lorillon et al. 2017). Thereby the sea urchin is the perfect model to experimentally learn about physiological and *in vivo* translational regulations. The translatome at the egg-to-embryo transition of the sea urchin (Chassé et al. 2018) revealed that some mRNAs (especially cell cycle and translation regulators) are specifically recruited into the translational machinery. The translatome also revealed that these mRNA recruitments to the translational machinery do not respond in the same way to inhibition of the mTOR (mechanistic Target Of Rapamycin) pathway. Specific translation of mRNAs is regulated upon fertilization. The developed technique allows us to investigate the *in vivo* translational activity brought by specific untranslated regions (UTRs). UTRs of interest are cloned flanking the coding region of Luciferase reporters (Firefly or Renilla luciferases). *In vitro* transcribed mRNAs are then microinjected into sea urchin eggs. Using the Dual-Glo® Luciferase Assay System (Promega®), we were able to follow the *in vivo* translation of luciferases. We showed that mRNA translation is rapidly detectable in first divisions with only 5 microinjected eggs. Effects of different signalling pathway inhibitors were tested by incubation. Using an mRNA construction with the Renilla luciferase as a control of translation and a Firefly luciferase mRNA under the control of 5'UTR of different mRNAs, we revealed different translational activities dependent on the 5'UTR. These experiments provide new insight on how translational control is affected by mRNA UTRs in sea urchins after fertilization. The technique allows us to analyse the *in vivo* effect of cis/trans regulators and signalling pathways on mRNA translational activity during the first mitotic division of sea urchin development. Moreover, physiological and pathological conditions can be modulated to look at differential responses.

Key words: Translation, Microinjection, mRNA, 5'UTR, Luciferase, Sea urchin