Ph.D. Thesis project in proteomics

Large scale proteins turnover measurement to investigate the impact of maize genetic variability on proteostasis.

KEYWORDS:

Plant, proteomics, synthesis, degradation, metabolic labelling, nitrogen 15 stable isotopes, bio-computing, modelling, genotype, system biology, algorithm

PROCEDURE:

Candidates must apply on adum.fr (Reference 45782). Additionally, please send a motivation letter with a CV and the name of at least one references to willy.bienvenut@universite-paris-saclay.fr

PROJECT

Protein homeostasis (or proteostasis) is crucial to finely tune the abundance of the proteins in a cell by maintaining the equilibrium between protein synthesis and degradation, thus allowing an efficient biological activity [1]. The associated processes to maintain this equilibrium are constantly in action during organism's development [2] or responses to environmental stress [3]. Proteostasis is a key element among the molecular mechanisms required to maintain cell vital functions through tight regulations from gene transcription to post-translational proteins modifications. It is also responsible for the moderate correlation repeatedly observed between the abundance of proteins and the level of the corresponding transcripts [2]. The protein turnover rate (PTR) is a main parameter of proteostasis and it is usually associated to protein synthesis (K_s) and degradation (K_d) rates. Then, the accurate measurements of these kinetics constants, at the proteome level, are required to better understand the mechanisms of proteostasis regulations and their impact on the development or responses to environmental stresses.

PTR measurement methodologies based on pulse SILAC metabolic labelling are now frequently used for unicellular organisms to small animals model [4] but are not compatible with autotroph species like plants. Hence, this is a great difficulty to shed a new light on the mechanisms regulating plant protein abundance. The Millar group (Uni. West. Australia) [5-7] had some success with a method based on suboptimal ^{15}N metabolic labelling to define the K_d / K_s for a few hundreds of proteins [5]. Unfortunately, this methodology remains difficult to handle due to the complexity of the experimental design and the lack of integrated data processing tools that severely hampered its implementation.

The main objectives of this project are **to develop an alternative metabolic labelling strategy** for large scale PTR measurement then, to **apply it on maize to evaluate the impact of genetic variability [8] on plant proteostasis**. We propose to setup a methodology based on **low ^{15}N metabolic labelling** in plant combined with **standard proteomics approach** to determine **proteins synthesis and degradation constants at the proteome level**. This novel approach takes advantage of recent **improvements in sensitivity and accuracy of the mass spectrometers** to measure the peptide isotopic distribution [9]. Then, p**ost-acquisition data-processing** will take advantage of our **locally developed proteomics tools** [10, 11] to determine the K_s and K_d for the characterised proteoforms. This development and the proposed application will provide a novel element in multiomics investigations to better the regulation mechanisms of protein abundance and how they affect plant phenotypes.

The detailed version of this project is available on the adum.fr web site (reference 45782)

REFERENCES

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