Determination of protein synthesis on a proteomic scale

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Summary

Understanding of the highly complex regulation of cellular physiology has greatly benefited from various genome-wide approaches measuring transcripts, proteins and metabolites. Genome-wide studies have shown that for a large number of proteins, transcript levels do not directly reflect cellular protein levels, as one might dogmatically have expected. Protein expression can be regulated at various points going from transcript to protein. Cellular protein levels are governed by protein half-life as well. *Chapter 1* gives an overview of post-transcriptional regulation that affects either translation or degradation of proteins. Determination of protein synthesis and degradation for many proteins simultaneously, will be invaluable to pinpoint where post-transcriptional regulation takes place. Currently, genome-wide determination of protein synthesis as well as half-life is difficult. Especially when transient changes in synthesis or degradation need to be detected during cellular adaptation. Classically, protein synthesis and degradation are determined by pulse-chase labelling using radio-isotopes. Although this approach offers high temporal resolution, it is difficult to integrate within a mass spectrometry based proteomics approach. However, the use of stable-isotopes in stead of radio-isotopes, although supremely compatible with mass spectrometry, offers only limited temporal resolution. The latter is caused by the bulk of unlabelled protein, which obscures labelled species, especially if labelling times are short.

Non-natural amino acids are an alternative to stable-isotopes. They combine mass spectrometric compatibility with (much) higher temporal resolution. The methionine analogue azhal is such a pulse-label that has been shown to be incorporated into proteins by *E. coli,* mammalian cell lines and cultured insect cells. Non-natural amino acids achieve higher temporal resolution in the face of short labelling times through selective enrichment of labelled proteins or peptides from the unlabelled background. The azhal-labelled molecules are enriched by covalent attachment to affinity-resins through various forms of ‘click chemistry’ directed against the azide-moiety of azhal. This enables sensitive mass spectrometric detection of low abundant azhal-labelled peptides or proteins.

Chapter 2 describes the physiological response of two prokaryotic model organisms (*E. coli* and *B. subtilis*) to azhal. Furthermore, the effects of azhal incorporation on protein structure and function are investigated in different recombinant proteins. *E. coli* grows equally well on azhal as on methionine during the first 30 minutes upon substitution of the latter, after which growth arrest gradually sets in. In contrast, *B. subtilis* grown on the analogue has an initial lag phase and a considerably lower growth rate. Three photo-active proteins (PYP, AppA and YtvA) labelled with azhal do not show evidence of aberrant folding. Upon illumination however, these proteins display somewhat altered recovery rates from signalling to ground state, compared to their methionine containing counterparts. Azhal labelled LacZ, however, cannot be produced, probably due to misfolding and rapid degradation of this protein caused by azhal incorporation.

In *Chapter 3* an alternative approach to affinity enrichment of azhal-labelled peptides is presented. This enrichment is based on a change in retention-time induced by the selective reaction of the azide-moiety with tris(2-carboxy-ethyl)-phosphine. This reaction induces no less than four different reaction products in azhal-containing peptides or proteins, three of which are described here for the first time. Selectively modified peptides enriched by the retention-time shift are subsequently identified by tandem-MS. Following a pulse-labelling period of only 15 minutes, 527 proteins representative of all major Gene Ontology categories are identified in *E. coli* using this enrichment approach.

Chapter 4 describes the quantitative application of the enrichment approach. iTRAQ is used to quantitatively compare azhal-labelled peptides between growth conditions, representing relative changes in new protein formation. The initial phase after a change in growth temperature from 37 ºC to 44 ºC in *E. coli* is studied. Measurement of the relative amounts of 344 proteins newly synthesized in 15 minutes upon a switch in growth temperature showed that nearly 20% in- or decreased more than two-fold. Most regulated proteins detected have functions as chaperones or proteases, in accordance with this change in growth conditions, while the changes in new protein formation are highly similar to those found in earlier radiolabelling studies. In addition, the analytical strategy is extended to determine changes in total protein levels on the same time-scale, using quantitative data from the non-shifted peptides not containing azhal. Collation of changes in protein levels with changes in newly synthesized proteins enables identification of ‘stable’ and ‘labile’ proteins. The vast majority of proteins were found to be stable, only a subset of 5 proteins having a higher turnover rate under these growth conditions.

The extended strategy is also used in *Chapter 5* to determine average relative translation rates for 10 minutes immediately following a switch from aerobiosis to anaerobiosis. The majority of proteins with increased synthesis-rates upon an anaerobic switch are involved in glycolysis and pathways aimed at preventing glycolysis grinding to a halt by a cellular redox-imbalance. Newly formed proteins, quantified following heat shock (*Chapter 4*) and a switch to anaerobiosis (*Chapter 5*), are also compared with microarray data from literature obtained under similar conditions. Surprisingly, this reveals -for the first time- that regulation following a temperature increase is predominantly transcriptional, whereas for a substantial number of proteins translational regulation seems to be used upon sudden anaerobiosis. This illustrates the utility of azhal-based pulse-labelling to probe translational regulation.

Chapter 6 deals with strong points as well as remaining caveats in pulse-chase labelling using azhal, as presented in the previous chapters. The approach is compared to pulse-labelling with radio- or stable-isotopes and other approaches attempting to identify

genes that are regulated post transcription. Future technical developments in both affinity enrichment as well as chromatographic enrichment are introduced. Together with the further reduction in labelling times, these improvements could provide analyses of synthesis-rate and stability of hundreds of proteins, truly adding new layers to the analysis of cellular proteome dynamics.