

INVITED LECTURE H12

Cell cycle proteomics in *Caulobacter* using a novel 8-plex set of isobaric peptide labels

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Caulobacter crescentus is a non-pathogenic, Gram-negative aquatic bacterium found in lakes, rivers and sea water. Despite its' simplicity, *C. crescentus* cells undergo an unusual asymmetric division to produce two distinct cell types; a mobile 'swarmer' cell, and a stalked cell. The 'swarmer' cell undergoes differentiation into the stalked cell, and it is the stalked cell in which chromosomal replication is initiated to ultimately allow asymmetric division. We have extended the iTRAQ isobaric peptide tagging chemistry to allow for the simultaneous measurement of up to 8 samples. The technology uses an 8-plex set of amine-reactive isobaric tags for peptide derivatization following digestion. When subjected to CID, the tags dissociate to produce strong signature ions in the MS/MS spectrum. Stable isotope labels placed in various positions within the tagging group allow us to generate a set of 8 isobaric tags with signature ions in the region of 113 – 121 Da.. We have used the new 8-plex reagents to measure protein changes in synchronized cell populations at time slices encompassing cellular differentiation up to the point of cell division. We have confirmed the regulation and degradation patterns (vs. Western analysis) of key elements in the cell cycle, including CtrA (cell-cycle transcription regulator) and GcrA (up-regulated through cellular differentiation), which function via a unique out-of-phase pathway. With simultaneous quantitative measurements of over 1500 proteins, we are able to identify novel elements that are expressed to facilitate cellular differentiation and chromosomal segregation in pre-divisional cells. The 8-plex reagents were incorporated into the experimental workflow with minimal changes from the original 4-plex iTRAQ reagents.