

Characterization of the 70S Ribosome from *Rhodopseudomonas palustris* using an Integrated “Top-Down” and “Bottom-Up” Mass Spectrometric Approach

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Rhodopseudomonas palustris is considered to be one of the most metabolically diverse organisms studied to date. This organism can grow in the presence or absence of oxygen and is capable of existing by any one of the four major modes of metabolism which include photoheterotrophic (energy from light and carbon from organic compounds), photoautotrophic (energy from light and carbon from carbon dioxide), chemoheterotrophic (carbon and energy from organic compounds) and chemoautotrophic (energy from inorganic compounds and carbon from carbon dioxide). Interestingly *R. palustris* has the capabilities of interchanging within these modes in response to drastic changes in environment. In order to better understand the network of complexes responsible for this metabolic diversity, a detailed understanding of each complex is needed. To this end, we present a proteomic study involving a comprehensive mass spectrometric approach that integrates intact protein molecular mass measurement (“top-down”) and proteolytic fragment identification (“bottom-up”) to characterize one of the most highly conserved and well studied complexes, the 70S ribosome. Using this integrated technique we identified 53 of the 54 orthologues to *Escherichia coli* ribosomal proteins, distinguished between isoforms and assigned the amino acid positions at which particular post-translational modifications occurred. Our mass spectrometry data also allowed us to check and validate the gene annotations for three ribosomal proteins predicted to possess extended C-termini. In particular, we identified a highly repetitive c-terminal “alanine tail” on L25. This type of low complexity sequence, common to eukaryotic proteins, has previously not been reported in prokaryotic proteins. To our knowledge, this is the most comprehensive protein complex analysis to date that integrates two MS techniques.

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