

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

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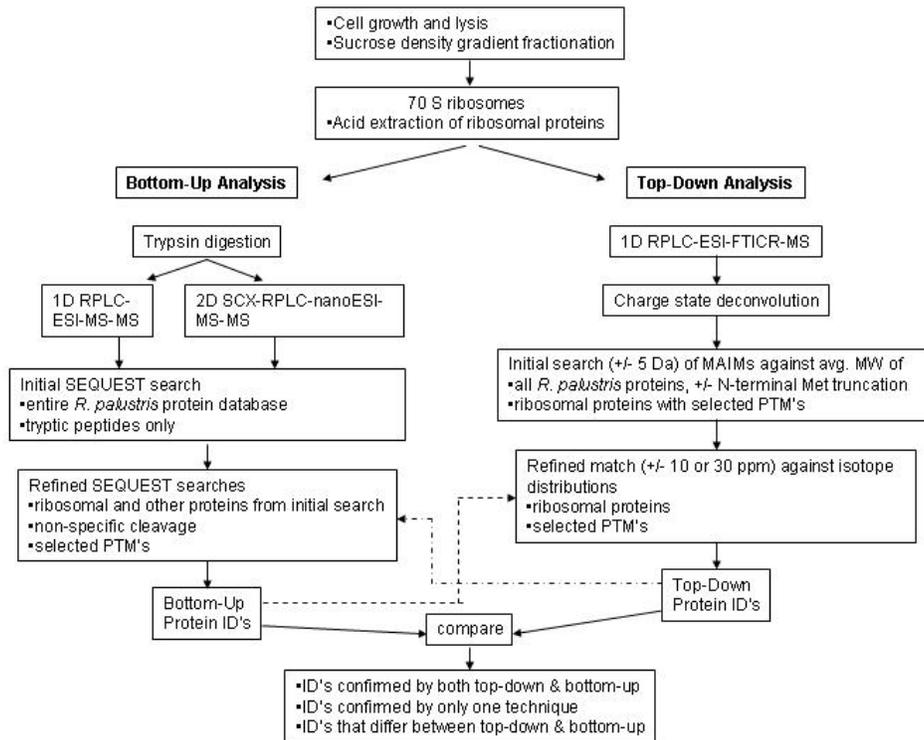
Introduction: *Rhodopseudomonas palustris* is considered to be one of the most metabolically diverse organisms studied to date. This organism can grow in the presence or absence of oxygen and is capable of existing under different growth conditions in response to changes in environment. In order to better 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.

Methods: 70s ribosomes from *Rhodopseudomonas palustris* were purified using sucrose density fractionation. After acidic extraction to remove ribosomal RNA, the ribosomal proteins were analyzed using Top-Down or Bottom-Up mass spectrometric approaches. All Top-down spectra, for intact proteins, were generated using an Ultimate HPLC (LC-Packings/Dionex, Sunnyvale, CA) coupled to a 9.4 T Hres electrospray Fourier transform ion cyclotron resonance mass spectrometer (IonSpec, Irving, CA). From the determined masses, a search of common modifications to bacterial ribosomal proteins was made. For Bottom-Up analysis, trypsinized peptides were subjected to either one dimensional or two dimensional LC-MS/MS using an LCQ-DECA XP ion trap mass spectrometer (Thermo Finnigan, San Jose, CA). The MS-MS spectra identified with the SEQUEST algorithm (Thermo Finnigan) were used for identifying common post-translational modifications of bacterial ribosomal proteins.

Results: Using this integrated technique (see figure) we identified 53 of the 54 orthologues to *Escherichia coli* ribosomal proteins by bottom-up analysis and 42 intact masses from top-down data. Combining both approaches allowed improved accuracy in distinguishing between isoforms and assigning the amino acid positions of several post-translational modifications (See table). For example, we identified two isoforms of RRP-L7/L12. The intact mass for RRP-L7/L12A matched this ribosomal protein with two modifications identified by bottom-up fragmentation spectra (corresponding to di-methylation at K69 and mono-methylation at K70). Without these combined MS approaches we would not have been able to definitively assign these two PTMs to a single isoform of RRP-L7/L12. 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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Integrated “Top-Down” and “Bottom-Up” Mass Spectrometric Approach



Post-translational Modifications of *R. palustris* ribosomal proteins

Protein	Modification	Residue(s)
RRP-L3 ^a	methylation	K155 or K158
RRP-L7/L12 ^b	A: 2 methylations and 1 methylation	K69, K86
	B: trimethylation or acetylation	K86, K89
RRP-L11	Acetylation or trimethylation	K40
RRP-L30	methylation	N-terminus or K3 ^b
RRP-L33	methylation	N-terminus or K3 ^b
RRP-S12 ^d	β-methylthiolation	D88

^a The spectrum localizes the methylation to either K155, K158. ^b Present as two isoforms, A and B.

^c Insufficient data to distinguish between methylation at the N-terminus. ^d Present in modified and unmodified forms.