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Quantitative Proteomic Analysis of Histone Exchange and Chromatin Dynamics

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Genome-wide studies use techniques, like chromatin immunoprecipitation, to purify small chromatin sections so that protein-protein interactions, protein-DNA interactions and histone posttranslational modifications (PTMs) can be analyzed for their roles in modulating gene transcription. A balanced level of chemical cross-linking is required to preserve the native chromatin state during immunopurification, while still allowing for solubility and interaction with affinity reagents. We used an isotopic labeling technique combining affinity purification and mass spectrometry called transient isotopic differentiation of interactions as random or targeted (transient I-DIRT) to identify the amounts of chemical cross-linking required to prevent histone exchange during chromatin purification. Bioinformatic analyses revealed that histones containing transcription activating histone PTMs exchange more rapidly than those correlated to gene silencing and therefore require a higher level of cross-linking to preserve the in vivo chromatin structure. Using these approaches, we have performed a purification of a single loci from budding yeast and identified specifically associated proteins and PTMs. This approach will be widely applicable to studying the dynamics of chromatin metabolism.

Biography

Alan Tackett completed Ph.D. from University of Arkansas for Medical Sciences, Little Rock, AR and Post doctoral study from The Rockefeller University, New York, NY .Now working as an Associate Professor of Biochemistry and Molecular Biology in University of Arkansas for Medical Sciences, USA. My laboratory focuses on technology development for the high resolution analysis of chromatin and the elucidation of protein-protein interactions that direct the posttranslational modification of histones. Additionally, I direct the UAMS Proteomics Facility and have extensive experience in collaborative research projects involving various areas of mass spectrometry. My group has expertise in using mass spectrometry to identify proteins in complex mixtures as well as quantifying protein abundance using various procedures (e.g., label-free, spectral counting, stable isotope tags & SILAC). We also perform various quantitative analyses of posttranslational modifications.

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