Novel machines that control *cyclin A* and the cell cycle of B cells through chromatin remodeling and transcription factor recruitment

Paul Romesser, Anupama Sinha, David H. Perlman, Hua Huang, Mark E. McComb, Gerald V. Denis and Catherine E. Costello
Cancer Center; Cardiovascular Proteomics Center; Mass Spectrometry Resource; Boston University School of Medicine, Boston, MA

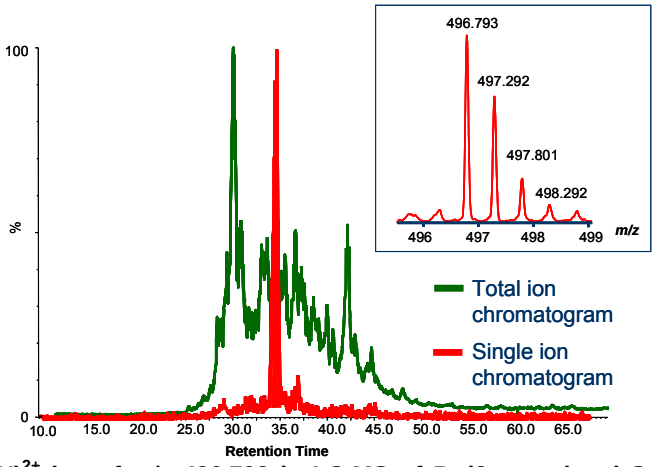
Introduction: Brd2 is a double bromodomain-containing nuclear-localized transcription factor kinase, related to the basal transcription factor TAF$_{II}$250, that participates in an incompletely described multiprotein transcriptional complex involved in the cell cycle. Its double bromodomain, a motif often found in various regulators of transcription such as co-activators, histone acetylases (HATs), histone deacetylases (HDACs), and other chromatin remodeling machines, consists of a 110 amino acid motif that binds to acetylated ε-amino functional groups of lysine residues in nucleosomal histone proteins. Brd2 complexes recruit E2Fs and histone H4–directed histone acetyltransferase to the *cyclin A* promoter, contributing to cell cycle control in normal B cells. B cell-restricted constitutive expression of Brd2 in transgenic mice transcriptionally activates *cyclin A*, leading to B cell leukemia and lymphoma. We hypothesize that Brd2 provides a "scaffold" for transcription or chromatin remodeling machinery and that the time-ordered identity of Brd2-associated proteins will help to reveal the components and functions of this machinery. Therefore, we have undertaken a mass spectrometric (MS) proteomic analysis of Brd2-containing protein complexes purified from B cells through immunoaffinity chromatography.

Methods: Mouse splenic B cells were isolated by MACS-based magnetic bead separation with anti-CD43 negative selection. Cytoplasmic and nuclear extracts were pooled and subjected to Brd2 immunoaffinity column chromatography, consisting of Protein A agarose that was covalently linked to anti-Brd2 antibodies to minimize antibody/complex co-elution. Extracts were passed over the column, washed extensively with ice-cold buffer and subjected to a pH drop to elute the complex. After pH neutralization, complexes were dried down, or subjected to intact protein MALDI-TOF MS or in-solution tryptic digestion followed by peptide MALDI-TOF MS. Dried samples were reconstituted and subjected to further separation by SDS-PAGE. Discrete protein bands visualized by Coomassie staining were excised and digested with trypsin in-gel. Eluted peptides were analyzed by MALDI-TOF MS and by LC-MS/MS. MALDI-TOF MS was conducted with a Bruker Reflex IV™ MS. On-line capillary LC-MS with automatic tandem mass spectrometry (MS/MS) was performed using a Waters QTOF-API-US™ coupled to a Waters capillary LC system. MALDI-TOF MS data was analyzed using the software M/Z™ and web-based MASCOT™ peptide mass fingerprinting (PMF). LC-MS/MS data were analyzed using Waters MassLynx™ and ProteinLynx Global Server™ software suites employing Swiss-Prot /TREMBL and user-programmed databases, as well as, NCBI BLAST analysis.

Results: Using immunoaffinity chromatography, we have purified Brd2-containing transcription factor complexes from B cells and have subjected them to MS analyses. Through peptide mass fingerprinting and LC-MS/MS peptide sequencing, we have identified several known and many novel components of these complexes, including basal transcription factors, chromatin and chromatin-remodeling proteins (Swi/Snf), nuclear kinases and other nucleic acid-associated proteins.

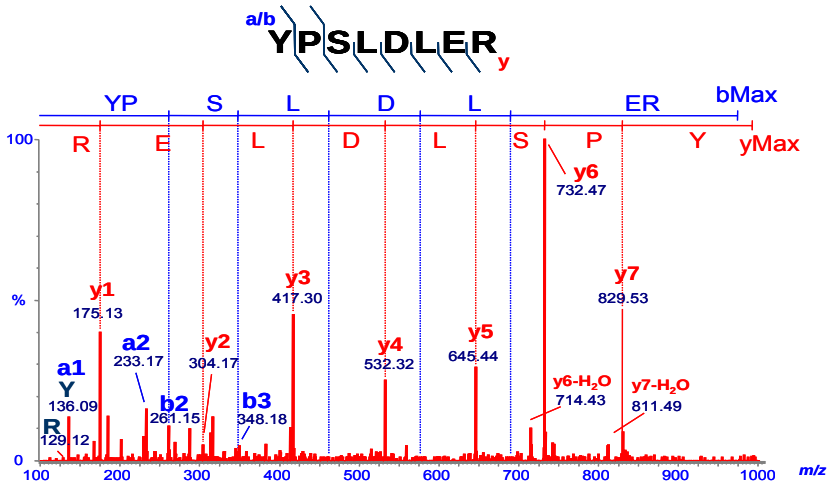
Conclusion: The Brd2-associated set of Swi/Snf components we report may define a specific Brd2-dependent chromatin-remodeling complex that regulates transcription. Ongoing studies of these complexes throughout the cell cycle will reveal their dynamic changes and provide great insight into their functionality. Comparative analyses of the Brd2 complexes in B cell lymphoma are likely to be informative of mechanisms of proliferation and lymphomagenesis. The results will have broad significance for our understanding of adaptive immunity and B cell malignancy.

TIC and SIC of $(M+2H)^{2+}$ precursor ion at *m/z* 496.79



Detection of an $(M+2H)^{2+}$ ion of m/z 496.793 in LC-MS of Brd2-associated Swi/Snf complex. Brd2-associated proteins were isolated from mouse B cell lysate by immunoaffinity chromatography, resolved by SDS-PAGE and digested in-gel with trypsin. Purified peptides were analyzed by LC-MS using a capillary LC-coupled QTOF MS (Waters). The total ion chromatogram (TIC) from a single Coomassie-stained gel band is shown in green; the single ion chromatogram (SIC) for the $(M+2H)^{2+}$ ion of m/z 496.793 (MS inset) is shown overlaid in red.

Tandem MS of *m/z* 496.79 precursor ion



HP1-BP74/3 protein identified through sequencing the m/z 496.79 precursor ion by LC-MS/MS. Tandem MS of the m/z 496.79 precursor ion was obtained by CAD fragmentation during the LC-MS run using the QTOF MS in IDA mode. Detected a/b and y ions are shown labeled in blue and red, respectively. The deduced sequence marked with the detected fragmentation ions is shown above the spectrum.

Acknowledgements: This project was supported by American Cancer Society grant RSG-05-072-01 (GVD) and NIH grants CA075107, CA102889 (GVD); P41-RR10888, S10-RR15942 and NHLBI contract N01-HV-28178 (CEC).

References:

Denis, G. V. (2001). Bromodomain motifs and “scaffolding”? *Front. Biosci*. **6**: 1065-1068.

Greenwald, R., J. R. Tumang, A. Sinha, N. Currier, R. D. Cardiff, T. L. Rothstein, D. V. Faller and G. V. Denis (2004). Eμ-*BRD2* transgenic mice develop B cell lymphoma and leukemia. *Blood* **103**: 1475 – 1484.

Sinha, A., D. V. Faller and G. V. Denis (2005). Bromodomain analysis of Brd2-dependent transcriptional activation of *cyclin A. Biochem. J.* **387**: 257 – 269.