Separating newly synthesized histone H3 from mature H3: analysis of H3 maturation in *Saccharomyces cerevisiae* and *Ustilago maydis*. J. H. Waterborg (Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64110)

Newly synthesized histone H3 in fungi like the smut *U.maydis* and the yeast *S.cerevisiae* elutes as a minor peak about 0.7 % acetonitrile later than the main, mature H3 protein in reversed-phase hplc. The basis for this delayed elution was identified. New H3 (nH3) with ~2.5 acetylated lysines per molecule, without non-acetylated H3 forms (AU/AUT gel analysis), and with increased methylation levels, matures with a half-life of 0.5 and 3 hours in smut and yeast, respectively, into the mature H3 (mH3) forms. mH3 retains, on average, only 1 acetylated lysine. Preparative amounts of mH3 and nH3 from smut and yeast were analyzed. Western blotting of yeast mH3 and nH3 revealed site-specific decreases in acetylation during maturation. V8 protease peptide analysis by hplc revealed an overall reduction in 1-50 peptide heterogeneity caused by acetylation and methylation. Upon maturation, the quantitative acetylation of K56 in nH3 was completely lost from mH3. The hplc mobility shift caused by this change mimicked the difference between the SHIMA H3K56A alanine substitution mutant and wild-type H3 in yeast. The delayed hplc elution of new H3 is caused by the quantitative K56 acetylation which affects the histone-fold-based hydrophobic character of histone H3 in reversed-phase hplc solvents. Acknowledgments: Supported by the Missouri Life Sciences Research Board, award 13254-2007 to JHW.

Contributed lecture at the 32nd Annual International Asilomar Chromatin and Chromosomes Conference, December 9-12, 2010, Asilomar Conference Grounds, Pacific Grove, CA.
Will appear in the February 2011 issue of Biochemistry and Cell Biology, volume 89.