Genetic and Molecular Dissection of a Circadian Biological Clock: Proteomics and Epigenetics

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Transcription/ translation feedback loops are central to all eukaryotic circadian clocks (Dunlap et al, Cold Spring Harbor Symp. 72: 57 – 68, 2007). In *Neurospora*, the transcription factors WC-1 and WC-2 activate *frq* expression by binding to the Clock Box in the *frq* promoter. FRQ protein then feeds back to inhibit WC activity, becoming phosphorylated at over 75 sites prior to its turnover. Quantitative proteomics and stable isotope labeling have revealed the profile of phosphorylation of each site across the day: Phosphorylation is clustered in time and space; some events speed the clock and some slow it down.

ChIP experiments indicate that the WC proteins do not act only as an obligate complex to activate *frq* expression: *in vivo* binding of WC-2 to the *frq* promoter occurs in a rhythmic fashion coincident with the peak in *frq* transcription, whereas WC-1 is bound continuously. Nucleosomes near the Clock Box are moved in a rhythmic fashion involving the action of two ATP-dependent chromatin-remodeling enzymes, CSW-1 (a homolog of the yeast Fun30, mouse Etl1 and human SMARCAD) and CHD2 (a homolog of the mammalian MI-2 and yeast Chd2 genes). They are required for normal clock function.