Screen for Histone Mutations that Allow Activation of the HO Gene in the Absence of SW15 Activator

Julie C. Tran (David Stillman)
Department of Pathology
University of Utah

Many human diseases arise from problems in gene regulation. Research of the transcriptional regulatory machinery in yeast can contribute to an understanding of new general principles that govern transcriptional regulation in cells of multicellular organisms. The yeast HO gene is an excellent model to utilize when studying eukaryotic gene regulation. HO is activated by the transcription factor Swi5. Yeast also have a similar transcription factor Ace2, with an identical DNA-binding domain, but nonetheless can activate a different gene. Ace2 binds to the HO promoter with the same affinity as Swi5 in vitro, but in vivo Ace2 fails to activate HO transcription. There are multiple mechanisms that may prevent Ace2 from activating the HO promoter. Swi5 enters the nucleus slightly before Ace2, and we hypothesize that there may be a change in chromatin structure during this time. If the chromatin structure is modified at HO after Swi5 binds, this altered chromatin may prevent Ace2 from binding. To examine this hypothesis, we are screening for histone mutations that allow yeast to activate HO in the absence of the normally required Swi5 activator. The screen uses an HO-ADH2 reporter, in which the ADH2 adenine biosynthetic gene is inserted in place of the HO open reading frame, so that ADH2 is regulated by the HO promoter. HO expression can then be monitored by growth on media lacking adenine. In the swi5 HO-ADH2 strain used for the screen, the cells can only grow in the presence of added adenine. Thus, we have used genetic selection to look for mutations that allow this strain to grow in the absence of adenine, requiring expression of HO-ADH2 despite the absence of the normally required Swi5 activator. This strain also has all of the histone genes knocked out, but carries a plasmid with wild type histone genes. PCR mutagenesis under error prone conditions has been performed on a second plasmid containing the histone genes, and then transformed into this strain, selecting for mutations that allow growth in the absence of adenine. Strains carrying mutant histone plasmids that allow expression of HO-ADH2 are being isolated from yeast and sequenced to identify the mutations. The mutations in the histones found in the screen can help to determine what chromatin structure changes may be occurring. One possibility is that a factor bound to HO recruits an inhibitor, such as a histone deacetylase, and Ace2 has difficulty binding to this modified chromatin.