Expression of a GFP-tagged histone H1 gene during ascus development in *Neurospora crassa*. N.B. Raju¹, M. Freitag² and R.L. Metzenberg¹,³. ¹Stanford Univ, ²Univ Oregon, ³UCLA.

A GFP-tagged histone H1 gene (hH1-GFP) under the control of the ccg-1 promoter was inserted at the his-3 locus (his-3+:hH1+-eGFP+), hereafter called H1-GFP. We have examined the expression of H1-GFP in developing asci of homozygous and heterozygous crosses. In homozygous crosses (H1-GFP x H1-GFP), nuclei fluoresce brightly from karyogamy until the production of mature, multinucleate ascospores. In heterozygous crosses (H1-GFP x wild type), expression of H1-GFP is completely silenced, at least until after ascospores are delimited. Silencing does not extend into the autonomously developing ascospores. Nuclei in four of the eight spores begin to fluoresce 18 to 24 h after ascospore delimitation. Fluorescence intensity increases for an additional 24 h as the ascospores form striations and begin to pigment. Additional mitoses occur in mature, black ascospores, and more than 60 nuclei can readily be seen in each of the four H1-GFP ascospores. The four ascospores without H1-GFP do not show fluorescence. Sad-1, which suppresses meiotic silencing of ectopically inserted genes (Shiu et al. 2001, Cell 107:905-916), also suppresses the silencing of H1-GFP in heterozygous asci. In H1-GFP x Sad-1 crosses, expression resembles that of a homozygous H1-GFP cross: Nuclei fluoresce until after ascospores are delimited. The residual H1-GFP in non-H1-GFP nuclei gradually fades while nuclei in the four ascospores that contain H1-GFP continue to fluoresce brightly. These observations provide a clear visual demonstration of meiotic silencing by unpaired DNA. (Support: NSF grant 9728675 to D. Perkins, NIH grant GM35690 to E. Selker and NIH grant GM08995 to RLM.)