RNAi of rpS3a suggests a link between this gene and arrested ovarian development during adult diapause in Culex pipiens (#1138)

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ABSTRACT

In mosquitoes that do not enter diapause, rpS3a is consistently expressed, but in mosquitoes programmed for diapause, expression of rpS3a is intermitted during early diapause, but is then consistently elevated in females 1 month and older. RNAi was performed to evaluate a possible function for rpS3a related to the arrested ovarian development during diapause. dsRNA injected into non-diapausing females suppressed ovarian development. Decreased expression of rpS3a following dsRNA injection was confirmed by Northern blot hybridization. Topical application of JHIII, an endocrine trigger for diapause termination in this species, yielded an almost complete recovery from the RNAi effect. We propose that rpS3a is involved in the shut down of ovarian development that characterizes the adult diapause of Cx. pipiens.

INTRODUCTION

• Culex pipiens, the Northern house mosquito, is known as one of main vectors of West Nile virus.
• Female mosquitoes in Cx. pipiens enter an adult diapause with the following characteristics:
  - Programmed by the short day length of autumn (Sanburg and Larsen, 1973)
  - Fat reserves as the energy source (Robich and Denlinger, 2005)
  - Shutdown of JH (juvenile hormone) (Spielman, 1974)
  - Arrested ovarian development during diapause (Christophers, 1911)
• Ribosomal protein S3a is a highly conserved protein that contributes to numerous physiological functions including:
  - Inhibited ovarian development by suppressing rpS3a in Drosophila melanogaster (Reynaud et al., 1997)
  - Up-regulation during oogenesis in Anopheles gambiae (Zurita et al., 1997)
  - Down-regulation during diapause in Cx. pipiens (Robich et al., 2007)

METHODS

• Insect rearing:
  Nondiapause mosquitoes at 18°C with a 15Light:9Dark cycle
  Diapause mosquitoes at 18°C with a 9Light:15Dark cycle
• Northern blot hybridization:
  monitor rpS3a expression patterns
• RNAi procedure
  (after Sim and Denlinger, 2008):
  - dsRNA synthesis and injection: inject dsRNA of rpS3a (400ng) to nondiapause females
  - Measure the primary follicle size: check the effect of RNAi
• Topical application of JHIII:
  confirm the role of JHIII

REFERENCES