

The Effects of Mutations on sV23 Protein Stability: A Comparison of RNA and Protein Accumulation Levels from Mutant sV23 Transgenes

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The *Drosophila melanogaster* eggshell provides a useful means for studying extracellular assembly processes as they occur *in vivo*. Comprised of 3 major proteinaceous layers whose components are secreted by follicle cells, the *Drosophila* eggshell protects the developing oocyte. The vitelline membrane is the first major protein layer to be secreted by the follicle cells and contains 6-8 structural proteins. A conserved feature of all vitelline membrane proteins is a 38 amino acid region known as the VM domain with three characteristic cysteines involved in disulfide bond formation. sV23 was selected for studying the significance of the characteristic cysteines as it is the only vitelline membrane protein gene for which a null mutant exists. sV23 mutant transgenes were used to examine the functional significance of the three cysteines of the VM domain. Protein accumulation data from sV23 cysteine mutant transgenes as well as a transgene carrying a small C-terminal deletion have shown decreases in the amount of sV23 protein relative to wild type. However, lower protein levels could be a consequence of the random insertion of the transgenes into different chromatin environments in the genome. Through reverse transcription and PCR amplification of mRNA from the mutant sV23 transgenes, the mRNA accumulation levels relative to wt sV23 were determined. The ratios of protein to RNA accumulation indicate that: 1.) sV23 with a single cysteine substitution is stable, 2.) the stability of sV23 with two cysteine substitutions or the small C-terminal deletion is slightly compromised, and 3.) the accumulation of sV23 with three cysteine substitutions, despite mRNA levels approaching 50% of wild type, is less than 1%.