

## Using Histidine-Tagged Proteins to Study Eggshell Assembly in *Drosophila*

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The *Drosophila* eggshell provides a system to study extracellular matrix assembly *in vivo*. The eggshell is composed of three major proteinaceous layers, one of which is the vitelline membrane. This layer, located closest to the developing oocyte, is made up of a number of proteins, many of which contain a common 38 amino acid domain known as the VM domain. This domain is unique because it contains three precisely spaced cysteines that have been shown to be involved in the formation of a disulfide network. Using this VM domain, two other putative VM proteins—fc20 and sV58—have been identified. Using mass spectrometry, sV58 was identified as part of the disulfide network of another VM protein, sV23. Furthermore, sV23 and sV58 share the same octapeptide repeating domain, only repeated a different number of times in each. fc20 also contains a repeat; however, this repeat is radically different from the octapeptide found in sV23 and sV58. It is a pentapeptide repeat similar to repeats found in chorion proteins and prions. Adding a histidine tag to these proteins will allow for in-depth study of them as well as any complexes they might form without needed to create a specific antibody.

Thus far, we are in the process of histidine-tagging sV58. Using a BAC clone as a substrate, we have generated a histidine-tagged fragment from the 3' end of the gene. The BAC clone is also being used to subclone the remainder of the gene in order to have sufficient 5' and 3' flanking sequences to ensure proper expression. Having previously histidine-tagged fc20, we have found that its mRNA is expressed at near-endogenous levels and that the protein starts to accumulate at stage 10. Accumulation peaks at stages 11 and 12 and is maintained through stage 14. Furthermore, the protein is cleaved N-terminally in stages 11 and 12 and is found to have a potential furin cleavage site, similar to furin cleavage sites in both sV23 and sV17, other VM proteins. In the future the histidine-tagged version of fc20 will be used in conjunction with affinity chromatography to identify proteins within fc20 disulfide linked complexes.