Solving the Structure of Collagen IV in Drosophila

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Almost one million people in the world will be diagnosed with a genetic kidney disease occurring in the collagen IV within the glomerular basement membrane (GBM). I wanted a less-complex vivo model for studying mutations within this collagen IV. Drosophila collagen IV is promising because it is less-complex and inexpensive, therefore, it could help advance research of these mutations. My research is to gain knowledge on the structure of collagen IV in Melanogaster Drosophila. Human collagen IV is encoded by six genes (a1-a6). These genes make up a trimeric protomer in three different constructs, but in the human GBM the main construct is a1a1a2. Drosophila collagen IV is a much simpler collagen encoded by only 2 genes: Cg25c (a1) and VKG (a2). I used artificially constructed protomer chains of the two possible compositions. They were transfected into Expi-CHO cells. I then took native Drosophila material and homogenized it down to only the NC1 Domain. The artificial constructs and the native material were analyzed using size-exclusion chromatography, circular dichroism, and crystallization. By comparing the constructs to the native Drosophila material, I determined that the Drosophila protomer is a1a1a2, which is similar to mammalian collagen IV. However, I discovered Drosophila collagen IV assembles slightly differently. The artificial constructs were analyzed in the presence of chloride, which is responsible for the assembly of human collagen IV into hexamers. The Drosophila collagen IV did not form hexamers in the presence of chloride. This means that the assembly process is driven by the presence of a different ion. So, the next step in determining if flies are an accurate vivo model is to determine what drives the assembly process and the exact protein structure

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