Title: Uncovering Calcium Dynamics in Early Shell Formation of freshwater snail *Biomphalaria Glabrata*: Insights from Trochophore to Veliger Stages

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Calcium waves and oscillation during embryonic development are key elements in the intricate process of molluscan shell formation. However, understanding calcium dynamics in the early embryonic shell formation in gastropod development is still insufficient. The present study explores the role of calcium flux in early shell formation within the embryo of gastropod *Biomphalaria glabrata*. We hypothesized that the role of calcium is not only in providing a critical element for shell formation but also in serving as a signaling molecule for the genetic regulation of calcification. The calcium flux was visualized using the Fura-2 and Fluo-4 calcium indicators through the trochophore (72 hours) and veliger (120 hours) stages of *B. glabrata* development. The dynamics of calcium signals were correlated to the rapid transition from motile trochophore to veliger, marked by cilia-mediated movement and premature shell and foot development. According to our observation, the intracellular calcium signals were attenuated from 72 to 120 hours of embryo development. The expression profiles of genes encoding calmodulin and related protein kinase following the calcium flux in embryos suggested a critical role of the calcium-binding proteins in the early shell development of gastropods. Although the embryonic calcium dynamics and the related signaling pathway of shell formation are under further observation and analysis, the role of calcium in the singling pathway of shell formation has been demonstrated by this preliminary study.

Key words: Shell formation, Calcium dynamics, *In vivo* calcium imaging, *Biomphalaria*

glabrata

Words: 225