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Identification of Extracellular Vesicles Involved in the Biomineralization of the Hen Eggshell

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The eggshell is a critical barrier against mechanical stresses and microbial penetration. Its integrity is essential to maintain the hygienic quality of this basic human food and to limit the number of downgraded eggs. In such a context, we are looking for eggshell strength specific markers in order to optimize egg quality.

The eggshell is made of 95% mineral phase (calcium carbonate on calcite form) and an organic matrix (3.5%) mostly containing proteins. Eggshell formation arises from an extra-cellular biomineralization process, which takes place in a fluid that contains eggshell precursors and involves a transient phase of amorphous calcium carbonate (ACC). This work aims at exploring the presence and the role of extracellular vesicles to stabilize ACC and to address it to the mineralization site. In a first approach, we used real time qRT-PCR to assess the expression of vesicular target genes in several tissues. The results confirmed a high expression of vesicular target genes (*edil3*, *anxa1*, *anxa2*, *pdcd6ip*) in oviduct portions where mineralization takes place. In this study, we have also explored the role of EDIL3 and MFGE8 proteins in chicken shell at key stages of shell mineralization, and confirmed they could bind Ca^{2+} and vesicles, thanks to an EGF-like calcium-binding domain and a F5/8C phospholipid-binding domain. It was therefore suggested that both proteins could be involved in the vesicular transport of calcium. In a second approach, electronic microscopy coupled with elementary analysis was used to observe the uterine fluid collected during eggshell biomineralization. Data obtained highlighted the presence of extracellular vesicles (~ 300 nm) containing calcium carbonate. Finally, Western Blot analysis confirmed the presence of EDIL3, a key vesicular protein in the purified vesicle fraction.

The results of this study showed for the first time the involvement of extracellular vesicles in the transport of calcium carbonate for the biomineralization of hen's eggshell. We proposed a model of calcification using vesicles to stabilize ACC and explaining the fast deposition of the crystalline calcite oriented layer in the shell. The proteins described in this study will have to be explored as biological markers for a selection of chicken layers with improved mechanical properties.