Identification of uterine ionic transport proteins involved in providing the mineral materiel for eggshell formation in hens

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612

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The bird eggshell is a complex bioceramic material formed in the uterus (uterine shell gland) segment of the chicken oviduct. The shell is made of 95% calcium carbonate (calcite) and 3.5% organic matrix, which is a complex mixture of proteins, glycoproteins and proteoglycans[1,2]. In other species forming CaCO3 shell, it has been postulated that metastable Amorphous calcium Carbonate is produced intracellularly then this solid form is secreted at site of biomineralisation[3]. In hens, the uterine fluid where calcification takes place contains large level of soluble calcium (Ca^{2+}) and bicarbonate (HCO_3^-) and is largely hyper-saturated relative to Calcite. A prerequisite for shell mineralization is therefore the supply of large amounts of Ca^{2+} and HCO_3^- in this uterine fluid. Both ions (Ca^{2+}, HCO_3^-) are secreted in the uterus from the blood via trans-epithelial transport, in association with transfers of other ions including Na^+, H^+ and Cl^- net absorption and K^+ secretion. The whole process requires diverse ion's channels, pumps and exchangers[4,5]. A general model, constituted of more than thirty transporters and describing ion transfers across the uterine tubular gland cells during eggshell formation has been recently proposed using the transcriptomic data measuring over-expression of uterine gene expression when chicken eggshell calcification is in formation [6,7].

Calcium is not stored in uterus but is continuously supplied at high rate through the uterine glandular cells from blood. A large amounts of calcium passively penetrate the cells through a Ca channel then is secreted into the uterine lumen against the concentration gradient by the calcium pumps ATP2B1 and ATP2B2, which can extrude calcium from the cytosol to an extracellular fluid against a strong electrochemical gradient[5]. The low level of intracellular calcium (<0.0002 mM) is maintained thanks to the Calbindin D28K which protects cells from apoptotic degradation which will be induced in case of high intracellular Ca^{2+}. In addition, low free calcium levels in the cell are kept by calcium uptake in the endoplasmic reticulum via ATP depending calcium pumps (ATP2A2, ATP2A3).

Carbonate of the eggshell is not deriving from blood HCO_3^- but rather from the plasma CO_2, which is hydrated in the uterus. Carbonic anhydrases (CA2) are responsible for the transformation of CO_2 to HCO_3^- and is expressed at high level during calcification. CA2 is also present in the uterine fluid for conversion of HCO_3^- and carbonate. Anion exchanger (SLC26 family members) seems to be involved in the secretion of bicarbonates. Others transporters have been identified and are involved in the maintenance of the cellular and body homeostasis through Na^+, K^+ and Cl^- movement in the glandular cells, or participate to the reabsorption of H^+ protons generated during the eggshell formation.

Recently, we further enriched the model of ion transfers by identifying novel ion transporters by quantifying gene expression at the different initial steps of shell calcification using transcriptomic RNAseq. This approach reveals more than 150 additional proteins which are potentially involved in the transport of minerals. More specifically, we observed a large increased expression for 66 genes associated with calcium, 6 with the bicarbonates, 61 potentially implicated for the cellular homeostasis, including 3 in phosphate transport, and 15 in proton transport. To establish a hierarchy amongst these numerous candidates, we studied the hourly kinetic of their uterine gene expressions in hens for which we suppressed or re-induced the calcification process and in hens, the egg calcification of which has been disturbed by low dietary calcium supply. This quantitative analysis allows underlining the main uterine transporters amongst the numerous candidates to complete the initial model.

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