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VMO-II Mediates the Binding of the Chalaziferous Layer with the Vitelline Membrane in Quail Eggs

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Electron microscopic observations have revealed an electron density gradient in the chalaziferous layer of avian egg. The density is highest in the innermost sub-layer, which is bound with the vitelline membrane, and is thus traditionally referred to as the vitelline membrane outer layer (VMO). After high-concentration salt treatment, two proteins, VMO-I and VMO-II, are liberated from the egg envelopes of quail (*Coturnix japonica*), which results in the separation of the chalaziferous layer from the vitelline membrane. VMO-I and VMO-II were purified through gel-filtration and subjected to antisera produced in rabbits. The molecular sizes of these two proteins were estimated to be 9 to 15kDa for VMO-II and 18kDa for VMO-I. Their amino acid sequences were very similar to those of chickens. Immunofluorescent and immunoelectron micrographs showed that VMO-II was produced by the luminal epithelium and VMO-I by both the luminal and glandular epithelia of the infundibulum. Immunogold particles for the labeling of both proteins were distributed throughout the chalaziferous layer, as well as in the chalazae, with a density gradient similar to that mentioned above. In salt-treated envelopes, immunogold labeling was completely absent when stained with anti-VMO-II antiserum and weak with anti-VMO-I antiserum. Immunohistochemical studies revealed that purified VMO-II bound with vitelline membranes of salt-treated envelopes, and ligand blotting tests revealed that ZP1 and ZP3 were the molecules bound by VMO-II; the binding was inhibited by various kinds of sugars.

VMO-II might play a role in the binding of the chalaziferous layer with the vitelline membrane, a process that leads to the anchoring of the chalaza cord.

Keywords: chalaziferous layer, egg envelopes, immunohistochemistry, infundibulum, quail

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