Examination of wnt signaling mediated melanin transport and shell color formation in Pacifc oyster (*Crassostrea gigas*)

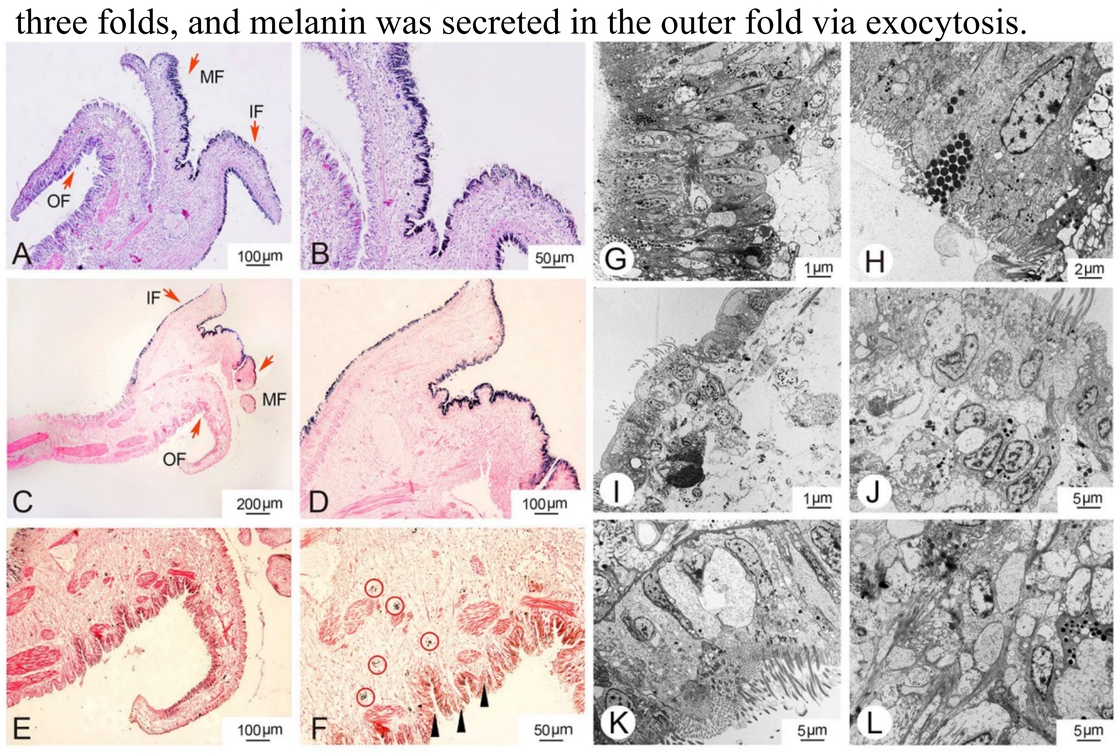
Yue Min [1], Qi Li[1,2]

1. Key Laboratory of Mariculture, Ministry of Education, and College of Fisheries, Ocean University of China, Qingdao 266003, China； 2. Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

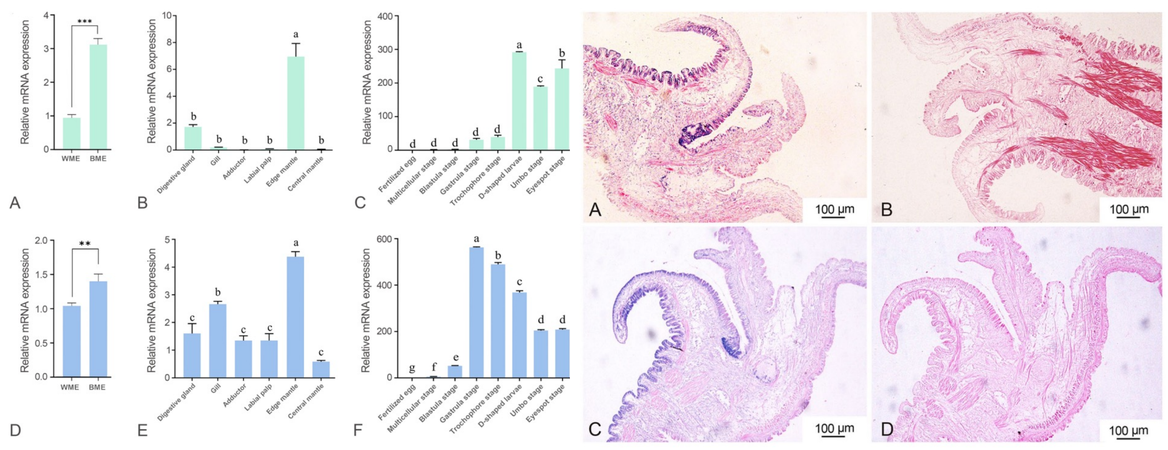
Abstract:

Mollusca exhibit diverse shell coloration primarily due to melanin, which plays roles in mechanical strengthening, antioxidation, and thermoregulation. However, the regulatory network for melanogenesis and melanin transport in molluscs is not well understood. This study systematically analyzes melanin distribution and transport in the Pacific oyster (*Crassostrea gigas*) using light and high-resolution transmission electron microscopy. We characterized the *CgWnt1* and *CgWnt2b-a* genes, finding them predominantly expressed in the mantle of black-shelled oysters, especially in the outer fold. By employing RNA interference and inhibitors to inhibit Wnt signaling both in vivo and in vitro, we observed impaired melanogenesis and reduced tyrosinase activity. These results highlight the crucial role of Wnt ligands and downstream factors in melanogenesis. Our findings provide valuable insights into the regulatory mechanisms of shell pigmentation in *C. gigas,* demonstrating that Wnt signaling modulation promotes melanogenesis and contributes to the understanding of molluscan melanin production and shell coloration.

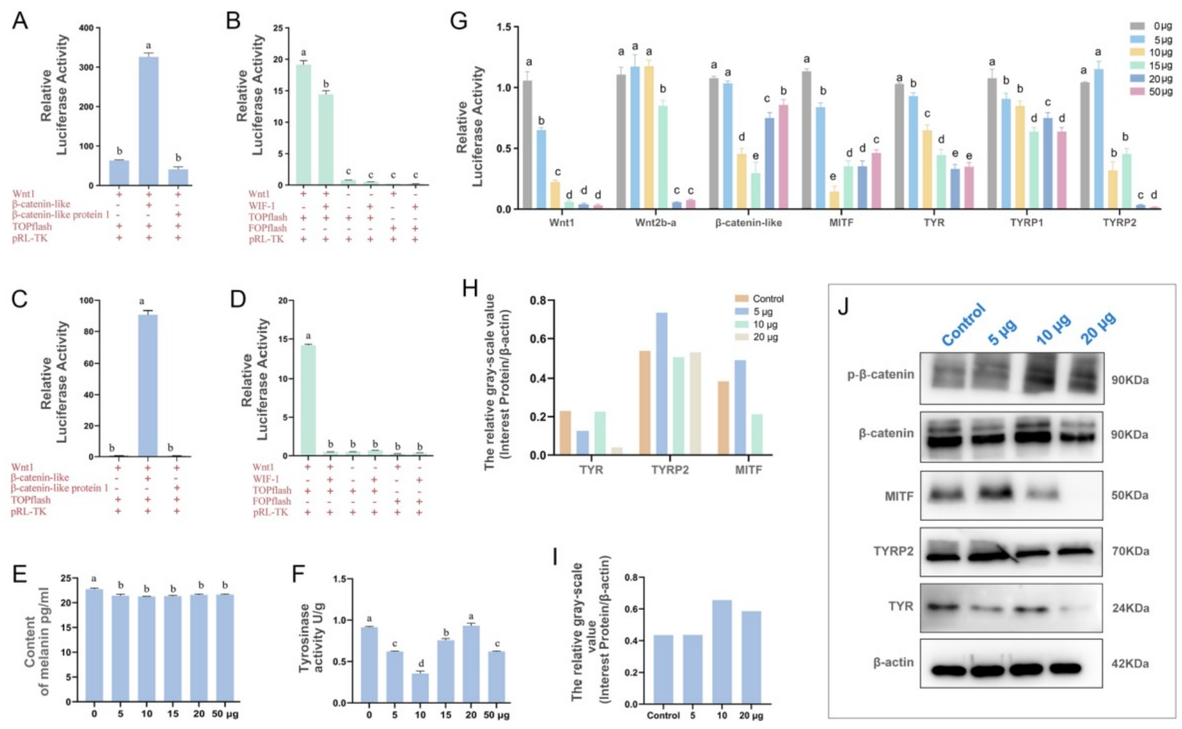
Result 1: Melanin-containing melanosomes were clearly distributed in all three folds, and melanin was secreted in the outer fold via exocytosis.



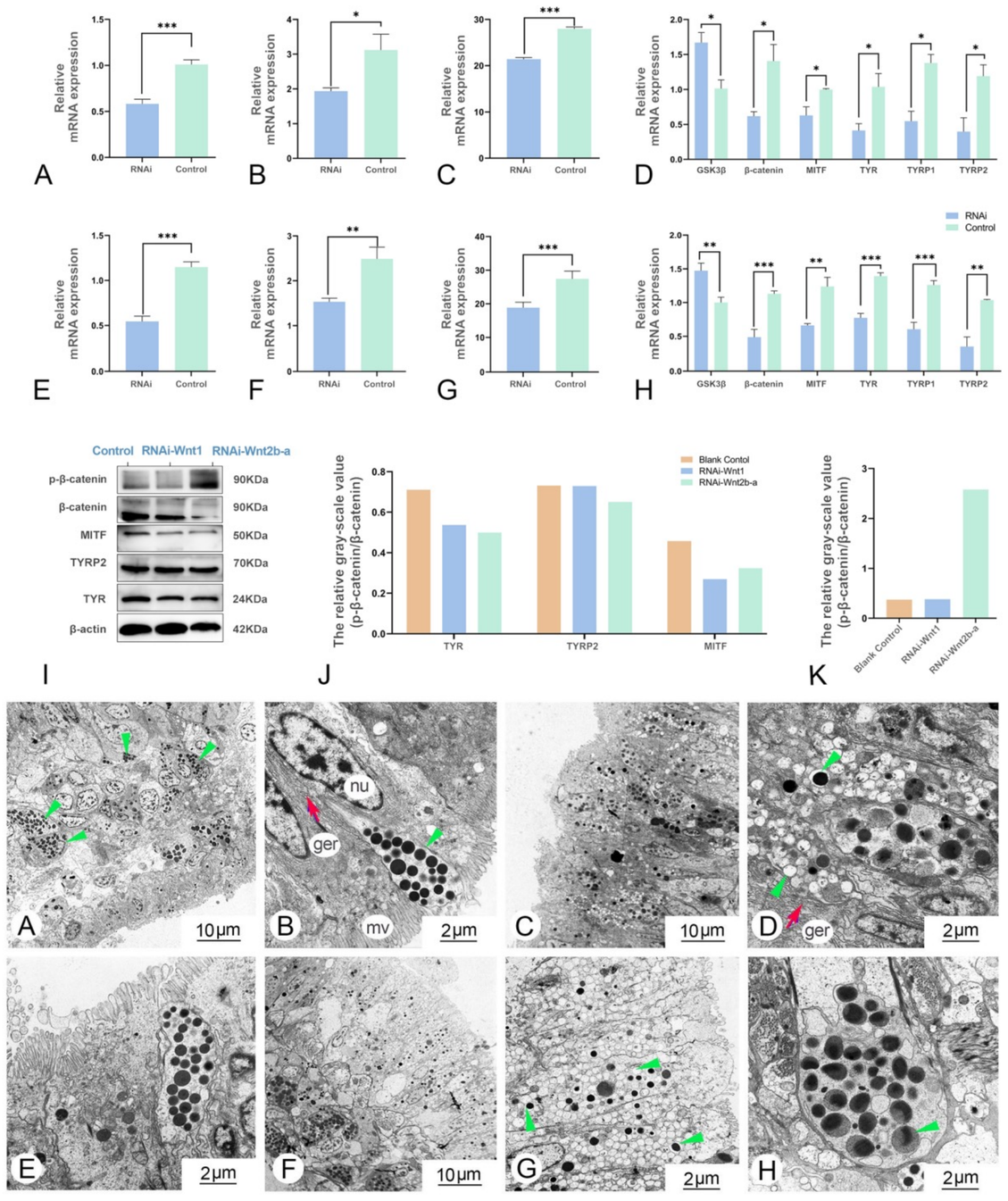
Result 2: CgWnt1 and CgWnt2b-a showed significantly higher expression in the edge of black-shelled oysters than in white-shelled oysters.



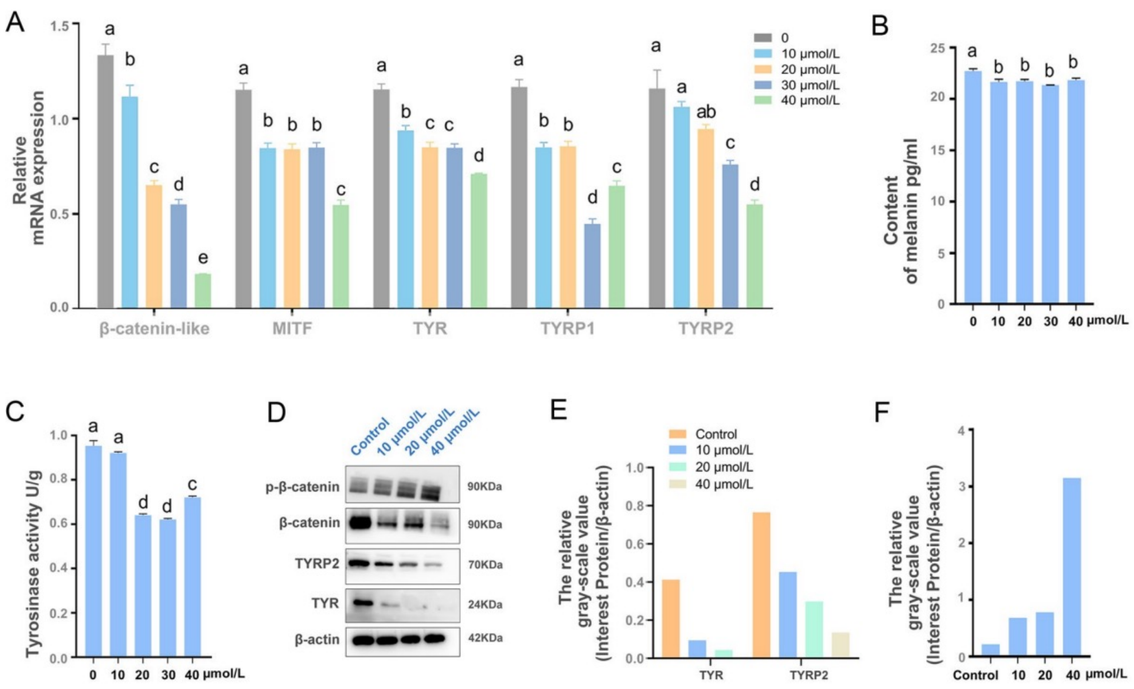
Results 4: CgWIF-1 significantly inhibited Wnt signals, with downstream genes downregulated.



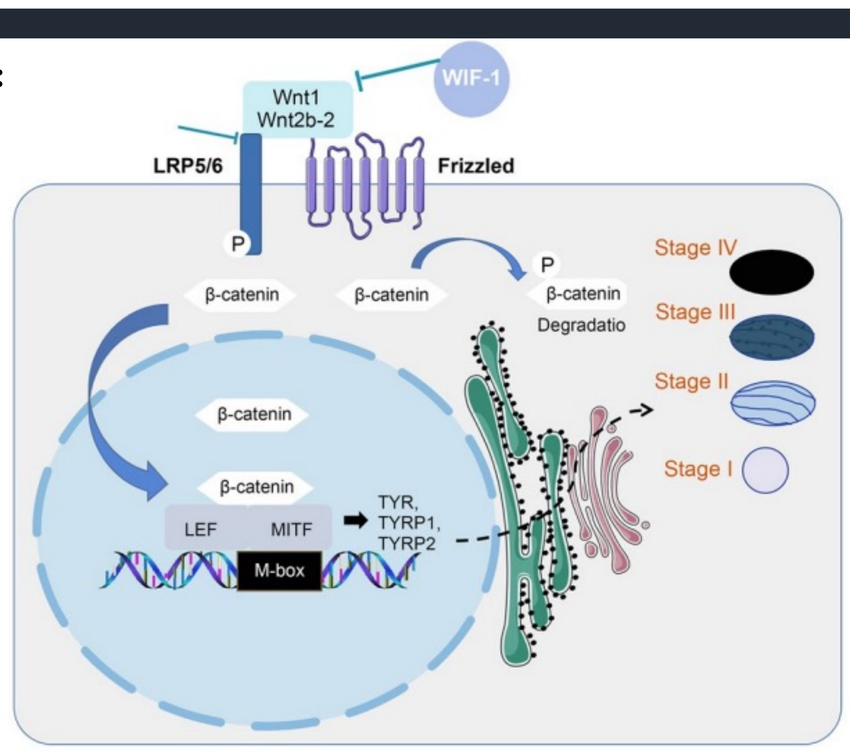
Result 3: Knockdown of CgWnt1 and CgWnt2b-a blocked melanogenesis



Result 5:Salinomycin treatment with mantle tissues significantly downregulated melanogenesis-related genes expression.



Conclusions:



Our results demonstrated that the inhibition of the Wnt/β-catenin pathway effectively blocks melanogenesis in *C. gigas*. The reduction in melanin production may be attributed to the downregulation of both MITF and its downstream target gene expression