

## Analysis of Aldosterone-ENaC Pathway in Lungfish: Evolutionary Insight to the Conquest of Land of Vertebrates

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### Summary

In tetrapod, adrenal cortical hormone, aldosterone, plays a major role in the renal Na homeostasis by regulating expression and activity of an epithelial sodium channel (ENaC). It is thought that the aldosterone-ENaC system evolved with the emergence of the tetrapods, because the presence of both aldosterone and ENaC has not thus far been demonstrated in fishes. Lungfish, an archaic group of fish belonging to the class of lobe-finned fish (Sarcopterygii), are recognized as the closest living relatives of terrestrial tetrapods among fishes. They overcome the dry season by estivating in subterranean mud cocoons for several months without water. In this study, we explored genes of ENaC and mineralocorticoid receptor in African lungfish, *Protopterus annectens*, and investigated effects of estivation on renal ENaC expression and plasma aldosterone level. We successfully cloned ENaC and MR from the lungfish. Phylogenetic analysis showed that cloned three subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) of ENaC belong to each subunit group of tetrapods. Gene expression of ENaC  $\alpha$ -subunit was detected in the gill, kidney and rectum, which are known as osmoregulatory organs. Functional plasma aldosterone concentration was detected in the lungfish kept in freshwater (FW), but not in gold fish. Unexpectedly, artificial estivation (EST) for 8 weeks decreased plasma aldosterone and renal ENaC expression.

Our results indicate that lungfish already possess the Na regulating mechanism mediated by aldosterone and ENaC in the kidney, but the system is likely functional in aquatic environment than in the terrestrial estivating condition. This suggests that lungfish conducts a different Na regulation from teleost fish of ray-finned fish lineage.