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Rab32 and Rab38 genes in chordate pigmentation: an evolutionary perspective

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Abstract

Background: The regulation of cellular membrane trafficking in all eukaryotes is a very complex mechanism, mostly regulated by the Rab family proteins. Among all membrane-enclosed organelles, melanosomes are the cellular site for synthesis, storage and transport of melanin granules, making them an excellent model for studies on organelle biogenesis and motility. Specific Rab proteins, as Rab32 and Rab38, have been shown to play a key role in melanosome biogenesis. We analysed the Rab32 and Rab38 genes in the teleost zebrafish and in the cephalochordate amphioxus, gaining insight on their evolutionary history following gene and genome duplications.

Results: We studied the molecular evolution of Rab supergroup III in deuterostomes by phylogenetic reconstruction, intron and synteny conservation. We discovered a novel amino acid stretch, named FALK, shared by three related classes belonging to Rab supergroup III: Rab7L1, Rab32LO and Rab32/Rab38. Among these, we demonstrated that the Rab32LO class, already present in the last common eukaryotic ancestor, was lost in urochordates and vertebrates. Synteny shows that one zebrafish gene, *Rab38a*, which is expressed in pigmented cells, retained the linkage with tyrosinase, a protein essential for pigmentation. Moreover, the chromosomal linkage of *Rab32* or *Rab38* with a member of the glutamate receptor metabotropic (Grm) family has been retained in all analysed gnathostomes, suggesting a conserved microsynteny in the vertebrate ancestor. Expression patterns of *Rab32* and *Rab38* genes in zebrafish, and *Rab32/38* in amphioxus, indicate their involvement in development of pigmented cells and notochord.

Conclusions: Phylogenetic, intron conservation and synteny analyses point towards an evolutionary scenario based on a duplication of a single invertebrate *Rab32/38* gene giving rise to vertebrate *Rab32* and *Rab38*. The expression patterns of *Rab38* paralogues highlight sub-functionalization event. Finally, the discovery of a chromosomal linkage between the *Rab32* or *Rab38* gene with a *Grm* opens new perspectives on possible conserved bystander gene regulation across the vertebrate evolution.

Keywords: Pigmentation, Amphioxus, Zebrafish, Synteny, Phylogeny, Intron Conservation, Gene Duplication, Genome Duplication

Background

Intracellular compartmentalization, via membrane-delimited organelles, is a fundamental feature of the eukaryotic cell and membrane trafficking between organelles became vital for these cells.

These mechanisms are typically regulated by Rab proteins, which form by far the biggest family among the small GTPases, with more than 60 members in humans [1]. These proteins play a crucial role in the regulation of cellular membrane trafficking in all eukaryotes [2]. This role is orchestrated mainly by the switching between the GTP/GDP-bound states of these proteins, controlled by the guanine nucleotide exchange factors (GEF) and GTPase activating proteins (GAP). Most Rab GTPases consist of 220 amino acids and are roughly 24 kDa [3]. Rab proteins possess some conserved domains: the P-loop, a well-known nucleotide binding motif, fundamental for the switching between GTP/GDP-bound states; Switch I and Switch II that are necessary for the binding of guanine nucleotides [4]. Each Rab has a distinct subcellular localization and regulates a specific transport step. Evolutionary studies suggest that twenty Rab proteins, divided into six supergroups,

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