EVOLUTIONARY AND DEVELOPMENTAL SURVEY OF GENES INVOLVED IN CHORDATE PIGMENTATION

Ugo Coppola

Sponsoring Establishment:

STAZIONE ZOOLOGICA ANTON DOHRN

NAPOLI, ITALY

February, 2018

EVOLUTIONARY AND DEVELOPMENTAL SURVEY OF GENES INVOLVED IN CHORDATE PIGMENTATION

A thesis submitted to the Open University of London for the degree

of

DOCTOR OF PHILOSOPHY

By

Ugo Coppola

Director of Studies:

Dr. Filomena Ristoratore

External Supervisor:

Dr. Prof. Sebastian Shimeld

Sponsoring Establishment:

STAZIONE ZOOLOGICA ANTON DOHRN

NAPOLI, ITALY

February, 2018

DEDICATION AND ACKNOWLEDGEMENTS

This thesis is dedicated to my father, my mother and Miriana: they are simply the most important people of my life.

First of all, I acknowledge Stazione Zoologica Anton Dohrn and OU University for the opportunity of studying in one of leading institutions in marine biology. During my time spent in Stazione Zoologica, I have met a lot of fantastic people. In particular, I have to mention my favourite lab technician Mara and my favourite technologists Giovanna. Among several colleagues, Claudia, Filomena, Ivan and Maria Concetta will be not comparable with the people of all the laboratories that I will visit during my future career. During these years, has been crucial for me the help of Marta, Miriam, Alessandra and Francesca: they are talented students.

A further, fantastic person is the researcher Antonietta Spagnuolo. I think that my external supervisor, prof. Sebastian Shimeld (Oxford), is a fantastic person and a great scientist that improved more and more my PhD; moreover, I have to thank my favourite collaborator, prof. Ricard Albalat Rodriguez (Barcelona University), which gave me enormous insights in evolutionary biology.

It is important to mention and thank Salvatore D'Aniello: from my first day in Stazione Zoologica he rendered me a member of his family and laboratory, opening my mind to the evolution, day by day. In these years, I met many people interested in embryo development and I attended conferences and seminars regarding embryology but, nevertheless, I consider my PI, Filomena Ristoratore, a great developmental biologist and a fantastic person. I think that "Mena" and the institute should understand this, because she is a resource for SZN. I hope that Stazione Zoologica will represent for future students what has been for me, i.e. a legendary place for studying animal evolution and development.

ABSTRACT

The pigmentation represents one of the most interesting topics in animal life because it is a decisive factor for the evolutionary success and the conquest of new ecological niches. Through the combination of developmental biology and comparative genomics, some aspects of pigmentary dynamics have been studied.

Given Rabs regulate a plethora of trafficking steps, the members belonging to this family are central to the transport of molecules involved in pigmentation. Moreover, comprehending the evolution of Rab family is relevant to understand the establishment of eukaryotic cellular organization and for its implication in many human pathologies. For the first time, I reconstructed the evolutionary scenario of Rab family in eleven species of metazoans, spanning from cnidarians to human. Phylogeny, intron code and synteny conservation prompted me to depict Rab evolution, with a special focus on chordates that exhibit a highly dynamic evolutionary pattern.

I clarified the evolution of Rab32/38 subfamily, fundamental in regulation of trafficking related to melanogenesis. It has been clarified the evolutionary history of Rab32/38 genes in deuterostomes and the expression pattern in key species as zebrafish and amphioxus, demonstrating how events as whole-genome duplications have influenced their role during embryogenesis.

In order to find new genes involved in pigmentation, I analyzed a Kelch-like member in ascidian *Ciona robusta* (*Cr-Klhl21*). My results point at this gene as a marker of pigmented cells, with a dynamic expression profile during embryogenesis: from middle tailbud stage, it is expressed specifically in the otolith. Moreover, *Cr-Klhl21* shows an intricate regulatory scenario with the possible intervention of a transcription factors combination (*Cr-Mitf*, *Cr-msxb*, *Cr-Dmrt*). This work contains first data about a Kelch-like member in ascidians, providing new insights in pigmentation or pigment cell specification. This encourages further analyses on its gene regulatory network and possible function.