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An Echinoderm Model to Study the Ribbon Organization of the Golgi Apparatus

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Abstract

The Golgi apparatus is central to the physiology of eukaryotic cells and its main functions are the post-translational modifications of proteins and the sorting of cargoes. The common consensus among cell biologists is that the Golgi subunits of the Golgi apparatus -the mini-stacks- are scattered in the cytoplasm; but these subunits are centralized into a superstructure called “ribbon” in vertebrates. Structural alterations of the ribbon, collectively known as Golgi “fragmentation”, are linked to pathologies such as neurodegenerative diseases and cancer. To date, the ribbon’s biological roles remain unclear, precluding insight into how Golgi structural changes may lead to pathological outcomes. Investigation into ribbon functions has been hindered by the complexity of the mammalian molecular machinery involved in its formation.

In the sea urchin embryo, electron microscopy data from our lab and literature surveys suggest that the Golgi switches from the typical architecture with separated mini-stacks to a mammalian-like ribbon from the pre-hatching blastula until the pluteus larva, suggesting that this structural change plays fundamental roles in development. As sea urchins are Echinoderms, a sister phylum of Chordates, this observation has an important evolutionary implication: the Golgi ribbon is not exclusive to vertebrates, as generally believed, but a feature of the phyla with which they share common ancestry (Deuterostomes). Importantly, in contrast to vertebrates, sea urchins have non-duplicated genomes, making the molecular machinery involved in the formation of their Golgi ribbon likely less redundant than that found in Mammals.

Given these observations, this thesis' objective is to test the Mediterranean sea urchin *Paracentrotus lividus* embryo as a new suitable experimental system to study the role of the Golgi ribbon organization in the context of development.

During the first two years of this thesis, using a bioinformatics approach I looked into the core components of the mammalian ribbon machinery in order to define which factors could explain the formation of the ribbon structure by combination of mini-stacks: This gave also the possibility to establish which components of the ribbon machinery are conserved in metazonans and, consequently sea urchin, in order to select them as actor for the functional experiments that I conducted. The results of this survey indicate that the evolutionary innovation represented by the mini-stack tethering into the ribbon-like Golgi cannot be reduced to the appearance of novel tethers belonging to the Golgin class.

Since this subcomponent of the ribbon machinery is conserved between mammals and sea urchin, I tested for the first time strategies targeting factors and processes known to affect ribbon formation in mammalian cells. These strategies were designed to induce precocious formation of the ribbon during sea urchin early stages of development through an overexpression pf a recombinant Pl-GRASP and to interfere with ribbon formation during development using the overexpression of two dominant negative mutants (i.e. Pl-GRASP(YY/AA) and the head domain of Pl-Golgin-160), the overexpression of the chimera protein Hsa-GEF-GRIP and the pharmacological treatment with the novel drug GRASPIN. This approach were based on evidence from the literature in order to interfere with the tethering function of GRASP, the dissociation from microtubule components through the head domain of Pl-Golgin-160, the induction of actin hyper-polymerization through the chimera protein Hsa-GEF-GRIP and the interference of the binding site of GRASP55 and Golgin-45 with the use

of GRASPIN. Experimentally, all of these events leads to the disruption of the ribbon in mammalian cells. While these experiments did not succeed in interfering with ribbon assembly, since we observed the formation of the ribbon structure and a normal development in treated animals, they provided useful indications to refine technical approaches and explore new strategies of manipulation of the ribbon dynamics in the sea urchin embryo.

In conclusion, this thesis work represented a first informative attempt at using sea urchin in functional experiments to interfere with ribbon formation in a whole organism.