Intravital imaging at subcellular resolution reveals multiple actin filament populations involved in exocytosis of secretory granules

A. Masedunskas[1]*, M. Appaduray[2], E. Hardeman[2] and P. Gunning[1]
[1]Oncology Research Unit & [2]Neuromuscular and Regenerative Medicine Unit, School of Medical Sciences, UNSW Australia, Sydney, Australia a.masedunskas@unsw.edu.au
* Corresponding Author

Keywords: *exocytosis, tropomyosin, myosin, formins, Arp2/3, intravital.*

Abstract

We established an intravital microscopy-based system to study actin dynamics in secretory granules (SGs) during exocytosis in salivary glands of living mice and rats. Using transgenic mouse models that express selected fluorescently labeled molecules, we discovered several key aspects of exocytosis that were not seen in *ex vivo* models. In the current study we sought to explore how tropomyosins (Tms), which form co-polymers along the length of actin filaments, may be modulating the actomyosin scaffold, and hence controlling exocytosis. There are about 40 different Tm isoforms and they are known to regulate the actin cytoskeleton by specifying the functional characteristics of actin filaments in time and space either via either a direct effect on filament stability or by regulating recruitment of actin binding proteins and myosin motors. We found that the Tm4 and Tm5NM1 isoforms line the apical canaliculi of the acinar structures. Interestingly, the distribution of these Tms did not overlap completely with the phalloidin signal suggesting that these isoforms associate with a subset(s) of actin filaments. Upon stimulation with isoproteranol Tm5NM1 and Tm4 were recruited onto the fused SGs together with F-actin, but with different kinetics to that of bulk filaments. Intravital microscopy of transfected rat salivary glands revealed that Tm5NM1 and Tm4 were recruited after fusion of the SGs with the apical plasma membrane and coincided with F-actin recruitment to the granules. Genetic ablation of Tm5NM1 or exposure to an anti-Tm compound altered the kinetics of granule exocytosis but did not prevent the completion of granule exocytosis. We conclude that Tm isoforms are present at the apical membranes and, in the case of Tm5NM1 and Tm4, directly participate in actin scaffolding and gradual delivery of granule contents post-fusion.