

Real-time imaging of Golgi derived vesicle exocytosis during the formation of growth cone lamellipodium after axotomy of cultured *Aplysia* neurons

Micha E. Spira and Guy Malkinson

Life Sciences Institute, The Hebrew University of Jerusalem.

The transformation of a stable axonal segment into a motile growth cone is a critical step in the regeneration of amputated axons. Axotomy of cultured *Aplysia* neurons is followed by rapid extension of a growth cones (GC) lamellipodium. Calculations revealed that to account for this rate of growth, 80-180 vesicles/sec fuse with the plasmalemma.

Here we began to explore where, when and how Golgi derived vesicles fuse with the plasma membrane in support of the growth process. To that end, we expressed the pH-sensitive GFP fused to the luminal domain of VAMP - synapto-pHluorin (a gift from J. E. Rothman, Memorial Sloan-Kettering Cancer Center, NY) in cultured neurons. Detection of vesicles distribution before, during and after axotomy as well as throughout the process of GC extension was done by confocal imaging using excitation wavelength of 488 nm to image the probe during or after fusion of the labeled vesicles, and by imaging of the total population of synapto-pHluorin labeled vesicles with excitation wavelength of 405nm. Calcium was imaged by intracellular injection of Fluo-4 or Rhod -2. A fraction of these indicators is taken up by the vesicles and reveals relatively high intravesicular calcium levels.

We found that axotomy leads to: (a) Fusion of anterogradly transported vesicles with the plasma membrane at the tip of the axon where the $[Ca^{2+}]_i$ is elevated to $> 1\text{mM}$. (b) After the recovery of the $[Ca^{2+}]_i$ to control, Synapto-pHluorin labeled vesicles accumulate at the forming GC's center. (c) These vesicles fuse with the GC's plasma membrane in the vicinity of the GC center. The fusion process proceeds in the absence of any detectable fluctuations in the $[Ca^{2+}]_i$.

In conclusion, the addition of new membrane to axotomyzed axon is exclusively localized to the GC center. We propose that the preferential fusion of vesicles at the GC center is facilitated by release of calcium from the vesicles themselves and their local concentration. Supported by: The ISF and the BSF