FLIPPER: a new genetically encoded probe for correlated light and electron microscopy

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Abstract

Fluorescent proteins (FPs) have allowed major insight into biological processes as they occur in living cells. Despite recent advances in fluorescence imaging, electron microscopy (EM) is still superior to resolve structures ranged within the wavelengths of visible light. Genetically encoded probes that can be visualized at the EM level allow correlating live-cell imaging and ultrastructural examination. This family of probes allows stringent fixation, while avoiding harsh permeabilization conditions affecting the cellular ultrastructure and protein localization (1). However, there is a lack of genetically encoded probes to take advantage of both worlds.

Here, we designed and applied a new genetically-encoded probe enabling visualization of proteins in live cells and by EM, named FLIPPER (Fluorescent Indicator and Peroxidase EM Resolution). FLIPPER is based on Golgi apparatus and ER-targeted FPs of choice coupled to horse radish peroxidase. Linking the typical EM structure of these organelles to their in vivo behavior has proved difficult. Only recently, the behavior of the Golgi during mitosis was characterized using correlated microscopy, showing a completely novel behavior of these well-studied organelles (2). We apply FLIPPER to study the dynamics and ultrastructure of the Golgi system, allowing multi-color visualization of Golgi and ER dynamics in living cells, followed by analysis at EM resolution by conversion of diaminobenzidine (DAB). We find that FLIPPER can be used for visualizing the dynamics of Golgi twins during mitosis, validating this probe in vivo. Furthermore, we apply FLIPPER to study EpCAM, a plasma membrane protein involved in cancer and intestinal disease. Using fluorescence microscopy, we find that the congenital tufting enteropathy-causing EpCAM disease mutant is retained in the ER. At EM level, FLIPPER allows to explore the effect of this disease mutation on ER morphology.

In all, here we present FLIPPER, a new genetically encoded probe which allows visualization of organelles at correlated light and electron microscopic level non-invasively. Furthermore, we demonstrate that FLIPPER can be used to study the effect of disease-causing mutations and to discover novel biological mechanisms.

References