論文内容の要旨

論文題目　"Detection and Analysis of Subcellular Calcium Dynamics"
「細胞内局所カルシウムダイナミクスの観測と解析」

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Ca^{2+} signals are involved in many essential processes including cell differentiation, apoptosis, development, and neural plasticity in various living organisms, During Ca^{2+} signals, Ca^{2+} is imported into the cytosol via two major pathways; Ca^{2+} influx from extracellular space and and Ca^{2+} release from intracellular Ca^{2+} stores.Inositol-1,4,5-trisphosphate receptor (IP3R) is one of the two major receptor channels involved in Ca^{2+} release from endoplasmin reticulum (ER). IP3R channel activity is known to be regulated by inositol-1,4,5-trisphosphate (IP3) and Ca^{2+} itself. The smallest unit. Or elementary Ca^{2+} event through IP3R is thought to be local and transient Ca^{2+} increase in the cytosol, which is referred to as "Ca^{2+} puff". Ca^{2+} puu is believed to be correspondent to the co-operative opening of a cluster of IP3R channels.
Several groups have identified and extensively studied Ca^{2+} puffs in several celltypes such as *Xenopus* oocyte and HeLa cells. However, the criteria for Ca^{2+} puff detection or the definition of the event is not often clearly described. This arbitrariness in the definition of Ca^{2+} puffs can have an artifactual effects on the result. The consequent misunderstanding of Ca^{2+} puffs as elementary Ca^{2+} signals can easily lead to the misunderstandubg of Ca^{2+} signal in general.
Therefore, I tried to establish and automatic detection algorithm for Ca^{2+} puffs by analyzing the data acquired in histamine-applied HeLa cells. To visualize Ca^{2+} puffs, I used total internal reflection microscope (TIRFM) . or evanescent wave microscope. In many studies on Ca^{2+} puffs, line-scan confocal microscope was used to obtain the dadtaacquisition rate fast enough to visulalize Ca^{2+} puffs, shich is typically ~100msec in duration. These studies necessarily ignored 2-dimensional spatial information . TIRFM can obtain the acquisition rate of =50Hz without losing spatial information.
Under TIRFM, HeLa cells exhibited spontaneous loca and transient Ca^{2+} increase without any extracellular ligands when radiated with strong laser.. I termed this

phenomenon as "light-induced Ca^{2+} puff", and succeeded in inhibiting this background event by lowering the laser intensity by mutral density filters.

I started with developing a simple automatic detection method for the data without any significant global Ca^{2+} signals, and succeeded in qualitatively reproduce the properties of Ca^{2+} puffs reported so far. However, the use of ratio value and smoothing processeswas required for this detection method, and this method could not be applied to the data with global Ca^{2+} signals. I went further to improve the algorithm to conquer these problems.

The improved version of the automatic detection method were used to investigate the ligand(histamine) concentration dependency of both temporal and spatial size of Ca puffs. The dependency of Ca^{2+} puffs on ligand concentration has not been systematically studied so far.

Considering that higher ligand concentration cause higher cytosolic IP3 and Ca^{2+} concentration, I had expected that there must be a shift in the duration and area (spatial size) of Ca^{2+} puffs corresponding to the change in IP3R activity. However, statistical analysis of duration and area of Ca^{2+} puffs showed no significant change in either the shape of the histogram or the average value of these properties.

It seemed that this implied that IP3R activities underlying Ca^{2+} puffs are not affected by Ca^{2+} or IP3, but closer look at the correlation between both duration and area of Ca puffs and global Ca^{2+} concentration revealed that the opening of IP3Rs are positively regulated by the intermideate concentration of Ca $^{2+}$, which goes well with thewell-known "bell-shaped" Ca^{2+} regulation of IP3R activity.

In this study, I have succeeded in establishing the automatic detection algorithm for local and transient Ca^{2+} puffs. I used the method to show Ca^{2+} regulation of IP3R channels in vivo.