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Effects of two phosphatases, HePTP and VHR, on mast cell activation

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Mast cells play an important role in allergic and inflammatory reactions. Stimulation of mast cells results in the release of secretory granules, the activation of mitogen-activated protein (MAP) kinase cascade, and the generation of cytokines. Here we have studied the effects of two phosphatases, HePTP (a tyrosine phosphatase) and VHR (a dual-specific phosphatase), on mast cell activation after the ligation of IgE receptors. When we measured the distribution of HePTP and VHR in mast (RBL-2H3) cells, HePTP was localized in the cytoplasm while VHR existed in both the cytoplasm and the nucleus. In RBL-2H3 cells stably overexpressing either HePTP or VHR, antigen-induced intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) was increased in similar extent to wild type cells. However, the subsequent degranulation and MAP kinase (ERK2) phosphorylation were inhibited in HePTP- and VHR-overexpressing cells. In wild type RBL cells, the nuclear import of ERK2 reached the maximum at 5 - 7 min after antigen stimulation, and thereafter the imported ERK2 was exported to the cytoplasm. In HePTP-overexpressing cells, the amount of ERK2 accumulated in the nucleus was much less than that in wild type cells though the nuclear import of ERK2 reached the maximum in the similar time-course. The production of TNF- α , a proinflammatory cytokine, was also inhibited in HePTP- and VHR-overexpressing cells. These results suggested that HePTP and VHR diminished the signaling in downstream of $[\text{Ca}^{2+}]_i$ increase in mast cell activation.

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An attempt at imaging of single functioning synaptosomes

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Synaptosome is a saclike structure (particle size 500~700nm) formed by nerve ending at a synapse that remains intact after homogenization of nerve tissue. Synaptosome contains a lot of synaptic vesicles and organelle and maintains nerve terminal functions including accumulation in synaptic vesicle and release of neurotransmitter. For these reasons, synaptosome has been examined a lot to reveal mitochondrial function in nerve terminal and regulation mechanism of accumulation and/or release of neurotransmitters. So far, when the activity of synaptosome was measured, synaptosomal suspensions were conventionally used. Therefore, the behavior of synaptosomes reported were based on an average of signal which occurred in synaptosomes. However, the internal environment of synaptosome is different in each, and it is thought that the a half of total synaptosomes contains mitochondria in particular. The energy state in nerve terminal is very important in a neurotransmission process, so differences of internal environment of synaptosome (especially presence or absence of mitochondria) must be considered when phenomena in synaptosome are studied. Therefore we attempt imaging of single synaptosomal behaviors. To distinguish several types of synaptosomes, synaptosomes were adsorbed on a dish using an adsorbent, and observed with fluorescence microscopy. The details will be discussed.

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Gravity-induced calcium transient devoid of amyoplast contribution may be involved in an early phase of gravitropism in Arabidopsis

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Gravity field affects growth and morphogenesis of most plants. Such phenomena are called gravitropism, in which gravity sensing is the earliest critical event. Starch-statolith hypothesis has been a most popular mechanism for gravity sensing in plants, insisting that sedimentation of high dense starch-filled plastids (amyoplasts) triggers gravity sensing. Although amyoplast sedimentation has been hypothesized to induce intracellular Ca^{2+} increases, exact mechanism is completely unknown. We reported previously that cytoplasmic free calcium concentration ($[\text{Ca}^{2+}]_c$) transiently increased in response to gravistimulation in Arabidopsis hypocotyls and petioles. In order to clarify the relationship between amyoplasts and gravi-induced $[\text{Ca}^{2+}]_c$ transients, we examined changes in the $[\text{Ca}^{2+}]_c$ in amyoplast-less mutant (*eal1*). Unexpectedly, the gravi-induced $[\text{Ca}^{2+}]_c$ transient in *eal1* was normal, indicating that intact amyoplasts are not required for the $[\text{Ca}^{2+}]_c$ transient. The hypocotyls of *eal1* showed normal gravitropism in 5 h of gravistimulation, whereas it slowed down after this period. On the other hand, gravitropism in wild type hypocotyls within 5 h after gravistimulation was attenuated by an extracellularly applied Ca^{2+} chelator (BAPTA), which completely inhibited the $[\text{Ca}^{2+}]_c$ transient. These observations suggest that the gravi-induced $[\text{Ca}^{2+}]_c$ transient is involved partially in an early phase of gravitropism and intact amyoplasts are not involved in the $[\text{Ca}^{2+}]_c$ transient and the early phase of gravitropism in Arabidopsis hypocotyls.

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Reactive oxygen species modulate CDK4/cyclin D1 in differentiation of PLB-985 cells: a crucial role of NADPH oxidase

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We have studied the effect of reactive oxygen species (ROS) on the progress of cell cycle in the differentiation of myelomonoblastic PLB-985 cells. In previous study we have reported that ROS generated by NADPH oxidase have mediated the expression of adhesion molecule, Mac-1 α /CD11b, during monocytic differentiation. In this research, we aimed to elucidate both the role of ROS in cell cycle. To analyze the cell cycle during differentiation, PLB-985 wild type cells and CGD-mutant cells, NADPH oxidase gp91^{phox} negative mutant, were incubated with 50 nM phorbol 12-myristate 13-acetate (PMA) for 3-5 days. Cell cycle was monitored with flow cytometer. ROS from activated NADPH oxidase were assayed with the method combining ESR and spin-trapping. NADPH oxidase of wild-type PLB-985 cells was activated immediately after 50 nM PMA treatment. Cell cycle analysis of wild type PLB-985 cells revealed G1 arrest after PMA treatment for 5 days. The rate of CGD-mutant cells in G1 phase after PMA treatment was lower than that of wild type cells. Co-incubation with CDK4/cyclin D1 inhibitor, Foscarnin, during PMA-induced differentiation showed the lower rate of cells in G1 phase than that of no inhibitor. Foscarnin also reduced the adherency of PMA-treated wild-type PLB-985 cells. These results indicate that CDK4/cyclin D1 process is essential to G1 arrest during PMA-induced monocytic differentiation in PLB-985 cells. ROS generated from NADPH oxidase should be trigger of activation of CDK4/cyclin D1 in this process.