2P204 脂肪分化及び脂質分解における脂肪細胞の非同期的な挙動
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Asynchronous behavior of adipocytes during differentiation and lipolysis
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Adipocytes store excess energy as triacylglycerol in lipid droplets (LDs) and release the energy as free fatty acid and glycerol via lipolysis in response to physiological demands. To date, many molecular processes involved in adipocyte proliferation, differentiation and function have rapidly been identified using biochemical and molecular biological techniques. However, adipocytes behavior at cellular level (~ several 10 μ m, several days) has not been fully characterized yet.

In the present study, we performed time-lapse observations of (i) adipocyte differentiation from primary stromal-vascular cells (SVCs) and (ii) adipocyte lipolysis induced by stimulation with norepinephrine. According to the time-lapse observations, individual SVCs accumulated numerous small LDs in the perinuclear region at 76.5 ± 13.4 h (mean $\pm$ SD) after inoculation; subsequently, several LDs grew to more than 10 μ m in diameter and occupied the cytoplasm. We also found that 25% of the SVCs that started accumulating LDs are capable of division. At 8 days after inoculation, more than 80% of the cells expressed the adipocyte phenotype. When such adipocytes with large LDs were stimulated with norepinephrine, the LDs shrunk rapidly and finally disappeared in several hours. However, not all of adipocytes shrunk LDs in their cytoplasm. In summary, our time-lapse observations have provided a detailed account of asynchronous behavior of adipocytes during differentiation and lipolysis.

2P206 セミインタクト細胞における転写因子 ATF4 翻訳過程の再構成系の確立
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Reconstitution of stress-induced translation of ATF4 transcription factor in semi-intact cells
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Under various cellular stress, eIF-2 α , a translation initiation factor, is phosphorylated and the formation of translational preinitiation complex is reduced, which leads to inhibition of most cellular protein synthesis. However, translation of the ATF4 transcription factor is specifically induced under stress. ATF4 translocates from cytosol to nucleus, resulting in the activation of stress-induced genes. In this study, we reconstituted the translation of ATF4 in semi-intact cells by incubation with cytosol, which was prepared from cells under endoplasmic reticulum (ER) stress. Translation of ATF4 was quantified by measuring the fluorescence intensity of ATF4-d1EGFP at the nucleus. The accumulation of ATF4 at the nucleus was induced by ER stress cytosol, not by normal cytosol, and was inhibited by cycloheximide, an inhibitor of translation. In addition, we examined the localization of mRNA under stress conditions. mRNA was visualized by coexpression of mRNA containing MS2-tag sequence and GFP (green fluorescence protein) labeled MS2 coat protein, which could bind to MS2 tag sequence. Interestingly, we found the presence of ATF4 mRNA at processing bodies that contain proteins involved in translation repression and mRNA degradation, and stress granules, cytoplasmic foci where translation-stalled mRNA-protein complexes assemble. We will discuss the mechanisms of stress-induced translation of ATF4, and also present a short introduction of a device for preparing semi-intact cell chips automatically.

2P205 細胞ストレス下における ATF4-GADD34 feedback loop の kinetics 解析
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Kinetic analysis of ATF4-GADD34 feedback loop under different cellular stress
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Cells have various mechanisms to respond to different stress such as heat shock, UV irradiation, oxidative stress, and ER stress. However, there is a common response module driven by a transcriptional factor ATF4 involving apoptosis, protein folding, and metabolism control. ATF4 transcriptionally activates GADD34, which in turn down-regulates ATF4 translation. The ATF4-GADD34 feedback loop is known to determine the working period of this stress response module.

In this study, we attempted to find the dynamic properties of the ATF4-GADD34 system by analyzing the kinetics of ATF4 and GADD34 expression under multiple stimuli. We have established CHO cell lines that stably express ATF4-EGFP and measured pulsive expression of ATF4-EGFP under oxidative and ER stress at single-cell level. We also measured the time-course of expression of endogenous ATF4 and GADD34 by Western Blotting. From these experiments, two interesting features of this system were derived: (1) The dynamics of this system changes considerably depending on the type and intensity of the stimuli. (2) Although peak level of ATF4-EGFP differs widely, the difference of peak time and pulse width between individual cells is relatively small under the same stimulus. In addition, we examined the dependence of the dynamics on the kinetic parameters by using simple mathematical models. These results indicate that this ATF4-GADD34 system exhibits robustness to changes of some parameters, but also sensitiveness to the type and intensity of the stimuli.

2P207 ラント培養心筋細胞の収縮、カルシウムイオン濃度振動に対する BTS の作用
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Effect of BTS on the auto-oscillatory properties of contraction and Ca²⁺ concentration in cultured cardiac muscle cells
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A cardiac muscle cell beats in response to the oscillation of [Ca²⁺], which is regulated by sarcoplasmic reticulum (SR). At the same time, the intracellular Ca²⁺ concentration also controls the switching between the three states of the actomyosin contractile system, which are the contraction, relaxation, and spontaneous oscillatory contraction (SPOC).

We attempted to clarify the properties of the cross-talk between the SR system and the actomyosin contractile system. We loaded cultured rat cardiac muscle cells with fluo4-AM, which is a Ca²⁺-sensitive fluorescent dye, and found that BTS (N-benzyl-p-toluene sulphonamide), which is a myosin II-ATPase inhibitor, disturbed and weakened contraction and oscillation of [Ca²⁺] and sometimes completely terminated them in cultured cells. Based on these results, we suggest the existence of a feedback loop between the contractile system and the SR system. To confirm it, there is a need to rule out the possibility that BTS directly affects the SR system, or to quantify the relationship between the SR system and the contractile system in the presence of BTS. We plan to characterize the effect of BTS on isolated SR.