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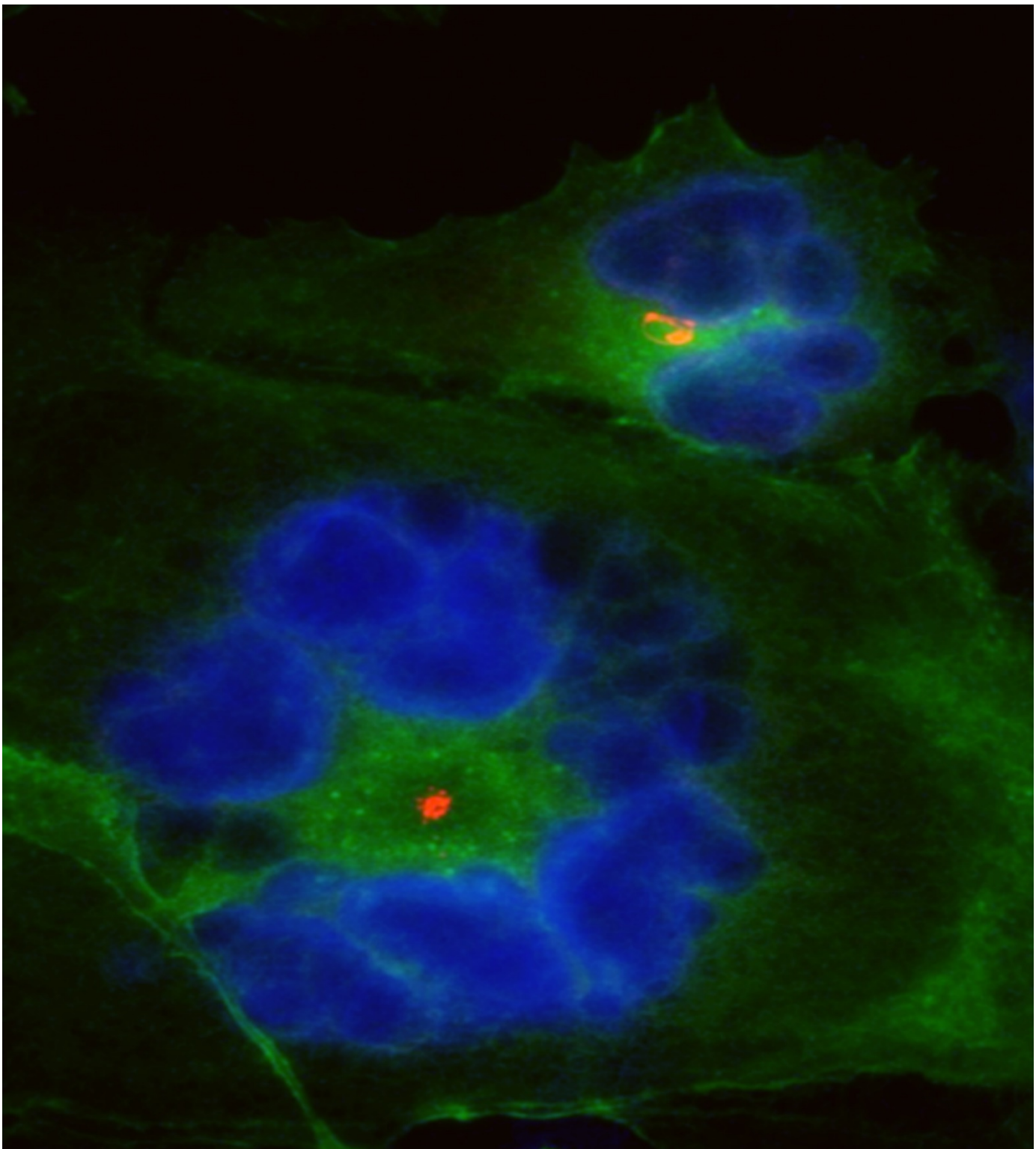

CB-P47
THE INITIAL PICTURE OF PREADIPOCYTE RESPONSE TO A NITRO-OXIDATIVE ENVIRONMENT

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Cumulative evidence indicates that obesity-related metabolic dysfunction is more associated with the existence of hypertrophic dysfunctional adipocytes rather than to numerous small adipocytes. For this reason, the study of adipocyte development as a major cause of obesity has become a relevant area of research. We have previously shown that menadione-induced oxidative stress inhibits adipogenesis and increases triglyceride content in the mature adipocyte, with the PI3K/Akt pathway playing a crucial role. Here, we tested the effect of nitro-oxidative stress (NS) induced by sodium nitroprusside (SNP) on 3T3-L1 preadipocytes. For this purpose, we exposed 3T3-L1 preadipocytes to 0.0–1.0 mM SNP for 24 h, and we characterized the level of cellular injury triggered by NS. Cell mortality was close to 100% after 24-h incubation in the presence of concentrations of SNP over 0.2 mM. Low SNP concentrations (0.1–0.2 mM) altered neither mitochondrial functionality (MF) nor increased reactive oxygen species (ROS). However, lipid peroxides were found to be increased, and the permeability of the cell membrane (measured as LDH leakage) was significantly altered. These findings indicate that SNP triggers the generation of ROS that are early scavenged, and only the remaining lipid peroxidation products and damage to cell membrane are detected after 24 h. We also evaluated the short-term effect of the exposure to SNP by incubating 3T3-L1 cells with 0.0–10.0 mM SNP for 2 h. It was found that MF was not affected by 0.5 mM SNP, but significantly diminished by concentrations of SNP over that (20%, 40%, and 60% decrease by 1 mM, 5 mM, and 10 mM, respectively). Moreover, 0.5 mM tripled ROS levels, doubled the levels of lipid peroxides and significantly altered membrane permeability (4-fold increased LDH leakage). We then evaluated the phosphorylation levels of Akt, ERK1/2, and the stress-activated protein kinase/Jun-amino-terminal kinase (SAPK/JNK), four well known kinases involved in cellular redox signaling, after the incubation with 0.5 mM SNP (increased markers of NS without affecting MF) and 5 mM SNP (increased markers of NS with decreased MF). The four kinases were found to increase their phosphorylation level, thus, to be activated by both concentrations of SNP. Immunocytochemistry analysis revealed a different subcellular distribution of the kinases upon SNP treatment: the active form of Akt showed a preferential nuclear localization, regardless of the concentration of SNP; p-ERK1/2 increased their nuclear localization only in the condition where MF was not affected; p-SAPK/JNK increased all over the cell. Together, our findings show the response-time distribution that arises from the SNP treatment of preadipocytes. Our next goal is to evaluate the differentiation capacity of these preadipocytes acutely exposed to SNP.

ENZYMOLOGY

EN-P01
A CO-PURIFYING CONTAMINANT LOWERS THE PHOSPHOENZYME LEVEL OF THE SPF1

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P5-ATPases are eukaryotic proteins believed to actively transport a yet not identified substrate. They are necessary for cellular functions associated with the ER and the endo-lysosomal membranes, and in humans, mutations in the P5-ATPases genes are associated with neurological disorders. At the molecular level, the best-characterized P5-ATPase is the Spf1 from *Saccharomyces cerevisiae*. We have previously shown that purified micellar preparations of recombinant Spf1 can hydrolyze ATP and produce the phosphoenzyme intermediate (EP) characteristic of the transport reaction cycle of P-ATPases. Moreover, Spf1 was proposed to be modulated by Ca^{2+} by decreasing the level of EP. Here we present results suggesting that at least part of the effect of Ca^{2+} is mediated by traces of contaminant proteins that co-purify with Spf1. When the reaction media contained EGTA, the preparation exhibited a low ATPase activity that was either increased or inhibited by the addition of $CaCl_2$ depending on each particular preparation. The addition of 1 mM of the phosphatase inhibitor ammonium molybdate abolished the stimulation of the ATPase. The catalytic death mutant Spf1-D487N showed a marginal ATPase activity in EGTA but was highly stimulated by Ca^{2+}. These results suggest that the increase of ATPase did not result from a direct effect of Ca^{2+} on Spf1. Moreover, the stimulation of the ATPase activity was also produced by other metals like Mn and Zn, suggesting that the increase in ATPase was not a specific effect of Ca^{2+} but the result of decreasing the free EGTA. The level of EP formed by Spf1 was higher in the presence of EGTA and decreased with the increase in the Ca^{2+} of the media. The magnitude of this Ca^{2+} effect on the EP level showed a positive correlation with the stimulation of the ATPase activity in each preparation of Spf1 tested. Altogether these results suggest that the catalytic function of Spf1 may be regulated by a protein phosphatase. Analysis by mass spectrometry of the Spf1 preparation after SDS-PAGE detected the presence of several kinases and phosphatases proteins.