PLD2 silencing did not significantly affect basal POS phagocytosis by ABC cells. Our results demonstrate the expression of classical PLD isoforms in a new RPE cell line and their role in the modulation of the mTOR/S6K pathway. Further experiments are needed to fully elucidate the role of PLD1 and 2 in the phagocytic process of RPE cells exposed to inflammatory conditions. Our findings contribute to the knowledge of the molecular bases of retinal inflammatory and degenerative diseases.

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REGULATION OF PROTEIN KINASE A SUBUNITS EXPRESSION DURING THERMOTOLERANCE IN *SACCHAROMYCES CEREVISIAE*

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Organisms are constantly exposed to environmental changes. Variations in external conditions directly affect cellular homeostasis so that organisms have developed different strategies to overcome those situations. During stress a reprogramming of gene expression occurs, which involves not only the global inhibition of translation initiation but also the large-scale induction of stress-responsive mRNAs through both transcriptional and translational regulation. Several mechanisms allow organisms to be prepared for recurring stressors. One of them is the anticipatory response or cellular memory, through which a current environment acts as a signal or input resulting in adaptation to future challenges. This response is known as "acquired stress resistance". *S. cerevisiae* PKA is composed of two catalytic subunits encoded by *TPK1*, *TPK2* and *TPK3* genes and two regulatory subunits encoded by *BCY1* gene. The specificity of the PKA pathway depends on several factors as substrates specificity and interaction with anchoring proteins (AKAPs). Moreover, transcriptional regulation and PKA subunits expression level are also events involved in maintaining specificity. We have previously demonstrated that all PKA subunits share a negative autoregulatory mechanism mediated by PKA activity. *TPK1* is the only PKA subunit that is transcriptionally upregulated during heat shock. To further understand the molecular process involved in regulating PKA subunits expression, the existence of a memory mechanism was evaluated. To this aim, cells were exposed to a scheme of two consecutive heat shocks: a 30-minute heat treatment at 37°C followed by a second 10-minute heat treatment at 45°C. *TPKs* and *BCY1* promoter activities, mRNA and protein levels were assessed in mild log cells exposed to the thermotolerance scheme. We demonstrated that only *TPK1* expression increases during thermotolerance. In yeast cells the final protein output of a genetic program is determined not only by transcription control and mRNA translation, but also by regulating mRNA localization and turnover rates. As part of this dynamic process, it has been proposed that components of the mRNA decay machinery can directly regulate transcription. The 5'-3' exonuclease Xrn1 is known as a key regulator of general mRNA decay pathways which also participates in transcription activation. We have previously demonstrated that Xrn1 affect *TPK1* mRNA half-life. In order to evaluate the role of Xrn1 in *TPK1* expression during thermotolerance we assessed *TPK1* promoter activity and measured mRNA and protein levels in a mutant *Δxrn1* strain. Our results showed that *TPK1* expression is regulated during thermotolerance and that Xrn1 has an important role in this regulation.

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14-3-3γ SILENCING IMPAIRS OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE DERIVED-MESENCHYMAL STEM CELLS

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14-3-3 proteins constitute a family of regulatory molecules that participate in a plethora of cellular processes mainly through protein-protein interactions. Even though 14-3-3 protein family members show some functional redundancy, there is growing evidence that indicates evolutionary and biochemical diversity. Consistent with the literature, previous research from our laboratory showed that expression levels of 14-3-3 paralogs are independently regulated during the adipogenesis and osteogenesis of human adipose derived-mesenchymal stem cells (hASCs). In the current work, we used a validated approach to isolate hASCs and studied the implication of 14-3-3γ on the osteogenic commitment of these cells. To address this purpose, we delivered a 14-3-3γ shRNA construct into hASCs by pAd-BLOCKiT, an adenoviral vector containing a human U6 promoter, and examined the effect on the differentiation potential into osteoblasts. The latter was evaluated by: i) measuring alkaline phosphatase (ALP) activity, an early-stage osteoblast differentiation biomarker, and ii) detecting Runt-related transcription factor 2 (Runx2, master regulator of bone formation) protein levels. Cells were either maintained for 14 days with standard growth media (control, low glucose DMEM; 5% FBS) or induced with an osteogenic differentiation medium (ODM; an optimized drug cocktail that includes dexamethasone, β-glycerophosphate, and 2-phospho-L-ascorbic acid). Our results clearly showed a decrease in both Runx2 protein levels and ALP activity in 14-3-3γ depleted hASCs. This also accords with our earlier observations, which showed that reduced expression of 14-3-3γ had a negative impact on the osteoblastic transdifferentiation of NIH3T3-L1 cells. Taken together, these findings suggest a regulatory role for 14-3-3γ in hASC differentiation to the osteogenic lineage.

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