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ABSTRACTS

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100 A "CAAT" Box Element supports Cyclin B1 Promoter Basal Activity in Proliferating C2C12 Cells and mediates its Downregulation during Differentiation in vivo.

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The expression of cyclin B1 gene is restricted to proliferating cells and is negatively regulated by arrest of proliferation. We investigate the expression and the transcriptional regulation of cyclin B1 gene during induction of differentiation of C2C12 cells. First, we demonstrate that cyclin B1 mRNA is downregulated during differentiation of these cells. Using promoter fragments fused to a CAT reporter gene stable transfected, we demonstrate that also cyclin B1 promoter activity is downregulated in C2C12 differentiated cells. In vivo footprinting of the promoter region sufficient for the downregulation occurring in differentiated cells, reveals a protection of a "CAAT" box element located from nucleotide -10 to -15 relative to the start site. Electromobility shift assays, performed with nuclear extracts from both proliferating and differentiated cells, show that nuclear proteins bind this sequence only in proliferating cells. NFY-specific antibodies interfere with the binding activity of the complexes to this probe. Consistent with the loss of binding activity, we show that mRNAs of NFY subunits (A,B,C), are downregulated in C2C12 differentiated cells. In conclusion, our data demonstrate that cyclin B1 expression is regulated at transcriptional level in C2C12 differentiated cells and indicate that the downregulation of cyclin B1 promoter occurring in differentiated cells depend on a loss of a functional NFY protein complex.

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101 **ROLE OF 4-HYDROXYNONENAL, A PRODUCT OF LIPID PEROXYDATION, ON CYCLIN EXPRESSION IN HL-60 CELLS.**

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4-Hydroxynonenal (HNE), a product of lipid peroxidation, is able to affect many biological parameters, like for example inhibition of cell growth in several cell lines. In the HL-60 cell line HNE, at concentrations founded in normal cells, is able to block cell proliferation and induces a granulocytic-like differentiating program. Moreover this aldehyde induces an accumulation of cells in the G0/G1 phase of the cell cycle. This specificity let us to suppose that the cyclin/cyclin-dependent protein kinase (cdk) system, controlling the cell cycle progression, could be involved. We have analysed the expression of cyclins A, B, D1, D2, E, as well as the expression of cdk2 and cdk4 in HL-60 cells after HNE treatments. DMSO-treated cells were used as a positive control. We found that HNE is able to affect specific set of genes of the cyclin/cdk system and, in particular, we demonstrated a down-regulation of cyclins D1, D2 and A, while cyclins B, E and protein kinase cdk2 and cdk4 are not affected. These results confirm a possible role of lipid peroxidation products in the control of cell progression through the cell cycle phases.

102 Flow cytometric analysis of cell size changes during nutritional shifts in budding yeast.

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The analysis of protein distributions obtained by flow cytometry is a powerful tool to analyse the coordination between cell growth and cell cycle progression in budding yeast (1). Further information can be obtained by a biparametric staining procedure in which proteins are stained with TRITC, while a cell wall specific staining is given by ConcanavalinA conjugated to FITC. Using this double tag it is possible to follow the cell growth of individual cells at different set-points of the cell cycle (2-4).
In this report the previously described two-tags technique has been applied to the analysis of the average cell size, measured as total protein content, of the newborn daughter cells (Po), of the cells at the entrance in the S phase (Ps) and at cell division (Pd) during growth transitions. In a shift-up, while Pd increases from the beginning, Ps remains initially constant, then it increases over a short period of time. Similar data, but showing opposite behaviours, have been obtained during a nutritional shift-down. In budding yeast cAMP is involved in the response to nutrients. Following the addition of cAMP to permeable cultures, Po, Ps and Pd increase; however, in this case, Ps increases from the beginning, while Pd remains initially constant.
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