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ABSTRACTS

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52 Identifiaction of proteins which interact with YRB1.

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The mammalian GTP binding protein, Ran/TC4, has been implicated in a wide variety of cellular activities including the regulation of the nuclear localisation of transcription factors and control of cell cycle progression (1). Several regulators of Ran/TC4 activity have been identified in mammals and also in yeast. We (and other laboratories) have cloned a yeast homologue of the Ran binding protein RanBP1, now called YRB1 (2). To further address the function of YRB1, we have used the Brent interaction trap method to identify novel proteins which interact with YRB1. A screen of a *Saccharomyces cerevisiae* library has led to the identification of a number of potentially interesting clones, including the *ARGR3* transcription factor and *PRG1*, which encodes a yeast proteasome-related gene. *YRB1* acts as a high-copy-number supressor of the thermosensitive phenoype of a number of *prg1* mutations, suggesting the interaction is physiologically relevant. Mutants in *prg1* are defective in cell division, and may be associated with degradation of CLB2 (3). We are investigating the possibility that *YRB1* involvement in cell cycle regulation acts through *PRG1*.

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53 Inhibition of retinoblastoma protein phosphorylation in terminally differentiated muscle cells.

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Terminal differentiation of murine C2 skeletal muscle cells is associated with augmented expression of the retinoblastoma gene and complete de-phosphorylation of its gene product (pRb). We have demonstrated that upon differentiation the levels of the cyclin-dependent kinases cdk2 and cdk4 did not change significantly, while that of the p21 cdk-inhibitor was rapidly induced. Concerning cyclins, cyclin E expression remained constant, cyclin A and cyclin D1 were downregulated, while the expression levels of cyclin D3 strongly increased. Imunoprecipitation-coupled immunoblot analyses demonstrated that in growing myoblasts cdk4 was associated with cyclin D1 and low amounts of p21, while following differentiation, cdk4 interacted with cyclin D3 and augmented amounts of p21. According with the enhanced interaction of p21 with cdk4, the Rb kinase activity associated with cdk4 was greatly reduced in differentiated cells. The cdk4 complexes, immunoprecipitated from either undifferentiated or differentiated C2 cells, also contained proliferating cell nuclear antigen (PCNA), the auxiliary protein of DNA polymerases δ and ε, required for DNA replication and repair. Since DNA replication does not occur in terminally differentiated cells that have permanently withdraw from the cell cycle, we hypothesize that PCNA could be stored in inactive complexes in these cells, until its function would be required during DNA repair. Interestingly, we found that after DNA damage induced by the anti-cancer agent adriamycin, myotubes normally negative for PCNA staining after methanol fixation, displayed the typical nuclear punctuated staining pattern observed during DNA repair synthesis.

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54 Modeling cell cycle organization

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The coordination between growth and cell cycle progression is described by a two-thresholds cell cycle model that holds that a critical protein level (Ps) has to be achieved to activate DNA replication as well as that a second threshold (Pm) is operative over the entrance into mitosis. The events from the beginning of DNA replication to the exit of mitosis are given temporal order by the sequential activation of various cyclin dependent kinases (Cdk), different at least for their cyclin counterparts.

In this report a mathematical model is presented to analyze the dynamics of the cyclin/Cdk cascade.

The dynamical behavior associated to the proposed representation as well as the sensitivity to the model parameters are investigated by simulation.

To have an ordered progression of events in the cell cycle the backbone structure of the cyclins/Cdk cascade has to be synchronized and its end has to be related to the beginning of the next cascade, that, as previously told, requires the attainment of the critical threshold Ps. A threshold, in which a growth-dependent cyclin and Cdk titrate an inhibitor of cyclin-dependent kinase able to inhibit DNA replication, is proposed as molecular mechanism of Ps. Simulations have tested the self-consistency of the proposed cell cycle organization in the transition from quiescence to proliferation in mammalian cells.

The results are discussed to illustrate how a dynamical model may provide a useful tool to interpeter observations made on molecular determinants of the cell cycle control