The cell cycle is formed by a number of different complex biochemical pathways ensuring that the start of a particular event depends upon the successful and right end of previous steps in the pathway (De Luca et al., 2003). During the cell-division cycle, an important role is played by cyclins, a family of proteins named for their cyclical expression and degradation. These proteins are synthesized immediately before being used, and their levels fall abruptly after their action because of degradation through ubiquitination (King et al., 1996). Cyclins act as regulatory subunits of complexes together with a family of related protein kinases called cyclin-dependent kinases (CDKs) that function as catalytic subunits. Interaction between the cyclins and the CDKs occurs at specific stages of the cell cycle, and the progression through the cell cycle requires their activities. Unlike the cyclins, the protein levels of the CDKs do not oscillate throughout the cell cycle, suggesting the cyclins are an important regulative element of the complexes. In fact CDKs are often present in an inactive form until the synthesis of the cyclins (De Falco and Giordano 1998). Intriguingly, CDK/cyclin complexes not only regulate cell division by phosphorylating different substrates, but are able also to control several cellular pathways such as signal transduction, differentiation and apoptosis (De Luca et al., 2003). Among these, some CDKs/cyclin couples, such as CDK7/cyclin H, CDK8/cyclin C, and CDK9/cyclin T, seem to direct their activity in a cell cycle independent manner and appear to be involved in transcription during the initiation or the elongation steps (Dynlacht 1997). In particular, the Cdk9-cyclin T complex is known as P-TEFb and was originally identified as a positive transcription elongation factor in Drosophila transcription extracts (Marshall and Price 1995;
Herrmann and Mancini 2001). Peng et al., (1998) and Wei et al., (1998) showed that human P-TEF-b contains multiple cyclin subunits: cyclin T1 and T2. Cyclin T contains an amino terminal box motif that is highly conserved from Drosophila to human (De Luca et al., 2003). The “cyclin homology box”, formed by 290 amino acids, is the most conserved region among different members of the cyclin-family and serves to bind CDK9. More precisely, the region of cyclinT1 from amino acid 1 to 188 is necessary and sufficient to interact with CDK9 in vivo. Cyclin T2 has two forms termed T2a and T2b, that likely arise by an alternative splicing of the primary transcript, a process commonly used to produce variety in other cyclins. Cyclins T2a and T2b share the first 642 amino acids but have different carboxyl termini. All three cyclins T are expressed in a wide variety of human tissues and are found complexed with CDK9 in HeLa nuclear extracts. Cyclin T1 is not a typical cell cycle regulatory since its levels do not oscillate at any phase during the cell cycle (De Luca et al., 2003). Immunohistochemical analysis of cyclin T2a expression in a large human tissue bank shows that cyclin T2a is widely expressed in all cell types, even if higher levels are found in some terminally differentiated tissues such as muscle, blood, lymphoid tissue, and connective cells (De Luca et al., 2001a). This expression pattern is very similar to that described above for cyclin T1 (De Luca et al., 2001b) and in agreement with mRNA distribution for cyclin T2a (Peng et al., 1998). Differences between CDK9/cyclin T1-T2 complexes and the other CDKs/cyclin complexes involved in regulation of the cell cycle may reflect a different function during terminal differentiation in muscle cells. The induction of myocyte differentiation on partial serum withdrawal, as occurs with C2C7 cells, is a good in vitro system in which to look at the CDK9/cyclin T1-T2 functions during terminal differentiation since it has been investigated the cdk9 activity in these cells (Bagella et al., 1998). Moreover, cyclin T2a protein level is high in human adult skeletal muscle cells (De Luca et al., 2001b). The high immunohistochemical expression level of cyclin T2a in adult muscle cells (De Luca et al., 2001b), as well as the peak of expression reached during in vitro muscle differentiation, suggests that CDK9/cyclin T2a complex might promote myogenic differentiation eliciting the expression of muscle-specific genes (Simone et al., 2002a). Human cyclin T2a gene maps on chromosome locus 2q21 (De Luca et al., 2001b), a locus involved in different forms of autosomal dominant myopathies (Nicolao et al., 1999; Pelin et al., 1999; Xiang et al., 1999). Interestingly, the immunohistochemical expression of cyclin T2 is undetectable in the skeletal muscle cells from two patients with centronuclear myopathy, in contrast with its very high expression in normal skeletal muscle tissue. These findings could indicate an involvement of the CDK9/cyclin T2a complex in the centronuclear myopathy (De Luca et al., 2001b). Skeletal muscle formation during development is a multistep process that involves the determination of multipotential, mesodermal cells to give rise to myoblasts, withdrawal of the myoblasts from the cell cycle, and differentiation into muscle fibers (Stockdale 1992; Miller et al., 1999; Shen et al., 2003). These processes are controlled by muscle-specific transcriptional regulators that determine cell fate and differentiation and by external signals that couple myogenesis to development and growth of the organism (Miller et al., 1999; Bailey et al., 2001; McKinsey et al., 2001). At the molecular level, myogenic determination and muscle-specific gene expression involve myogenic regulatory factors (MRFs) as well as muscle-specific helix-loop-helix factors (MyoD family) and the MEF2 family of MADS-box myocyte enhancer-binding factors (Lassar et al., 1994; Molkentin and Olson 1996). MRFs include MyoD, Myf5, myogenin, and MRF4. Expression of MRFs in several nonmyogenic cell lines is sufficient to induce myogenic differentiation (Davis et al., 1987; Wright et al., 1989; Braun et al. 1989). In last years, it has been hypothesized that myogenic factors can be subdivided into primary and secondary MRFs (Ishibashi et al., 2005) and it has been demonstrated that in the course of muscle differentiation in vivo, MRFs are expressed in a defined sequence (Figure 1): MyoD and Myf-5 are expressed in proliferating, undifferentiated cells, whereas myogenin expression is only induced upon muscle differentiation (Smith et al., 1993, 1994). Particularly, both MyoD and Myf 5 are active transcription factors in proliferating myoblasts (Wyzkowski et al., 2002), but whereas whereas MyoD levels peak at the differentiation checkpoint in G1 of the cell cycle, Myf5 levels are high in S/G2 and G0 in association with
proliferation and a failure to differentiate (Kitzmann et al., 1998). These data strongly reinforce the concept of a role for Myf5 in myoblast proliferation versus MyoD instigating myogenic differentiation (Ishibashi et al., 2005). MRF4 is unique since it is expressed both in early stages of myogenesis, and later during muscle development and in adult muscle tissue (Hinterberger et al., 1991; Shen et al., 2003). MRFs operate by heterodimerizing with the E2A gene products (E12 and E47) via the HLH domain and by binding to certain recognition sites in the regulatory regions of muscle-specific genes (Murre et al., 1989; Lassar et al., 1991; Weintraub et al., 1991). Full-length cyclin T2a and N-terminal region of CDK9, interacting with bHLH domain of MyoD, allow the formation of a complex able to stimulate the transcription of specific muscle genes (Simone et al., 2002a). In this complex, cyclin T2a interacts physically with MyoD that is phosphorylated by CDK9. CDK9/cyclin T2a-MyoD complex has several positive and negative regulating signals (Simone and Giordano 2001). Therefore, CK9/cyclin T2a binds and phosphorylates the C-terminus of pRb (Simone et al., 2002b), an essential cofactor during muscle differentiation. At this stage, pRb is present in the active hypophosphorylated form, especially due to downregulation of cyclins A, E and D1 and the upregulation of cdk inhibitors (Puri and Sartorelli 2000) (Figure 2). It is possible that CDK9/CycT2 kinase activity is involved in the basal phosphorylation of the retinoblastoma protein and that pRb and CDK9/CycT2 cooperate to support MyoD-mediated myogenic transcription (Simone and Giordano 2001). In the last years several studies seem to confirm that whereas cyclin T1 has CDK9 as the most important intra-cellular partner, cyclin T2a could have one or more “Cdk9-like” partners (Cottone et al., 2005). In order to demonstrate this hypothesis, we performed a two-hybrid screening in yeast using the full-length human cyclin T2a as bait, and a human heart cDNA library as a prey source (Cottone et al., 2005). Upon several interesting genes selected for interaction, our attention has been focused on the one coding for PKNα, a fatty acid- and Rho-activated serine/threonine protein kinase, having a catalytic domain homologous to protein kinase C family (Mukai 2003). Several functions of this kinase are thought to be involved in cytoskeleton rearrangement, because many PKNα targets are structural or regulative components of the microfilaments-microtubules-intermediate filaments network; however some published experimental data also suggest a nuclear role for this kinase. In fact, it has been shown that PKNα is translocated from cytoplasm to nucleus of in vitro cultured cells under stress conditions such as heat shock or serum starvation (Mukai et al., 1996), and that this kinase can interact with a neuron-specific basic Helix-Loop-Helix transcription factor (NDRF/NeuroD2) (Shibata et al., 1999). Moreover, PKNα is able to regulate ANF gene transcription in cardiomyocytes through a serum response element (Morissette et al., 2000). In addition, it has been demonstrated that PKN is able to interact with skeletal muscle α-actinin (Mukai et al., 1997), which is the major component of the Z-band in myofibrils. PKN phosphorylates α-actinin to maintain the integrity of sarcomere structure in skeletal muscle. Mutations in the PKN protein could therefore disturb the dynamics of sarcomeric proteins in skeletal muscle (Bartsch et al., 1998). In particular, we have shown that the Ser/Thr kinase domain sub-region

Figure 1. Expression of myogenic regulatory factors (MRFs) during cell growth and differentiation.

Figure 2. Differential expression of some cell cycle-related proteins during cell growth and differentiation.
of PKN, spanning from aa 782 to 873 was sufficient, but not necessary, for a weak interaction with cyclin T2a in vitro (Cottone et al., 2005). This suggests that different portions of the full-length PKN protein may be somehow involved in the binding to cyclin T2a, or more probably that the whole serine/threonine kinase domain is required for the interaction in vivo. Moreover, by luciferase assays performed using a MyoD-responsive promoter, we pointed out that PKNα alone enhanced MyoD-mediated transcriptional activity, and that cyclin T2a co-overexpression caused a further increase in luciferase-reporter expression. Finally, we have shown that in C2C12 cells the overexpression of both cyclin T2a and PKNα increased and anticipated the expression of myogenic differentiation markers like myogenin and MHC during starvation-induced differentiation. In confirmation of our studies, it has been demonstrated that PKNα is able to regulate the gene transcription of the atrial natriuretic factor (ANF) gene, one of the few currently studied hypertrophic marker (Morisette et al., 2000; Cottone et al., 2005). These results suggest that cyclin T2a could strengthen MyoD-dependent transcription and stimulate myogenic differentiation by kinase partner either Cdk9 or PKNα that could act in synergy or antagonism (Cottone et al., 2005).

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References


Molkentin JD, Olson EN. Combinatorial control of muscle development by basic helix-loop-helix and MADSbox transcription factors. Proc Natl Acad Sci U S A 1996; 93:9366-73.


Mukai H. The structure and function of PKN, a protein kinase Having a catalytic homologous to that of PKC. J Biochem 2003; 133:17-27.


Simone C, Giordano A. New insight in CDK9 function: from Tat to MyoD. Front Biosci 2001; 6:d1073-82.

Wright WE, Sassoon DA, Lin VK. Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD. Cell 1989; 56:607–17.