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Beyond the Cell Cycle: A New Role for Cdk6 in Differentiation

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Abstract Over 10 years ago, cdk6 was identified as a new member in a family of vertebrate cdc-2 related kinases. This novel kinase was found to partner with the D-type cyclins and to possess pRb kinase activity in vitro and has since been understood to function solely as a pRb kinase in the regulation of the G_1 phase of the cell cycle. In the past 2 years, several independent studies in multiple cell types have indicated a novel role for cdk6 in differentiation. For example, cdk6 expression must be reduced to allow proper osteoblast and osteoclast differentiation, forced cdk6 expression blocked differentiation of mouse erythroid leukemia cells and cdk6 expression in primary astrocytes favors the expression of progenitor cell markers. Since exit from the cell cycle is a necessary step in terminal differentiation, down-regulation of a mitogenic factor may be expected in this process, however it is surprising that this association has not been previously uncovered and that it is apparently not shared with cdk4, long understood to be a functional homolog of cdk6. The mechanism of cdk6 function in differentiation is not understood, but it may extend beyond the established role of cdk6 as a pRb kinase. As this story unfolds it will be important to discover if the function of cdk6 in differentiation is pRb-dependent or pRb-independent, since pRb has long been established as a key factor in initiating and maintaining cell cycle exit during differentiation. J. Cell. Biochem. 97: 485–493, 2006.

Key words: cdk6; differentiation; G_1 phase; pRb; cdk4; cyclin D

The cell cycle is divided into four main phases: G_1, S, G_2, and M phase. G_1 and G_2 phase were originally named gap phases since the early microscopists saw these as breaks between the visible activities of S-phase-where DNA replication occurred and M-phase-where cytokinesis and mitosis occurred. We now understand that these gaps contain crucial regulatory events that prepare the cell for the events of DNA replication and mitosis.

In G_1 phase and early S-phase, positive and negative regulatory proteins control the onset of DNA replication. The tightly regulated entry into S-phase ensures that damaged DNA is not copied and passed on to new cells and that the DNA replication necessary for cell division only occurs under the appropriate conditions. To regulate the onset of DNA replication, cyclin dependent kinases (cdk) partner with cyclins to phosphorylate the retinoblastoma (pRb) protein. When sufficiently phosphorylated, pRb releases its binding partner E2F. E2F is a transcriptional activator that, once freed from pRb, is able to activate the transcription of genes necessary to replicate DNA. Working against the mitogenic activity of the cdks are cell cycle inhibitor proteins. Two families of proteins-the INK family (containing $p15^{INK4b}$, $p16^{INK4a}$, $p18^{INK4c}$, and $p19^{INK4d}$) and the CIP/KIP family (containing $p21^{Cip1/Waf1}$, $p27^{Kip1}$, and $p57^{Kip2}$) have been shown to regulate the capacity of the cyclin/cdk complex for pRb phosphorylation. The relative abundance of inhibitors may determine the ability of the cyclin/cdk complex to phosphorylate pRb. For instance, if the levels of p16 protein are high, cdk4 will more likely partner with p16 than with cyclin D, thereby inactivating the kinase and disallowing phosphorylation of pRB. Kinases

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cdk4 and cdk6 partner with D-type cyclins in early G_1 phase while cdk2 binds to both cyclin E and cyclin A in later G_1 and early S phase. Three proteins make up the cyclin D family, cyclin D1, D2, and D3. Cyclin D proteins and associated kinases are uniquely positioned to regulate the cell cycle because they link mitogenic signals to the cell cycle machinery (for review see [Ekholm and Reed, 2000]).

Cdk4 and cdk6, share 71% amino acid identity and both partner with all three D-type cyclins to phosphorylate pRb. Both cdk4 and cdk6 are expressed ubiquitously [Meyerson et al., 1992; Meyerson and Harlow, 1994] and historically have been understood to function redundantly in G_1 phase of the cell cycle. Both cdk4 and cdk6 phosphorylate pRb but poorly phosphorylate Histone H1, the preferred in vitro substrate of cdk2 [Matsushime et al., 1992, 1994; Ewen et al., 1993; Kato et al., 1993]. Initial experiments identifying cdk6 activity were conducted in T-cells and it was suggested that cell-type specific expression might explain the need for two D-cyclin kinases [Lucas et al., 1995b]. However, more recent data indicate that both kinases are expressed in most cell types (P. Sicinski, personal communication), suggesting that these kinases have discrete, nonoverlapping functions.

Although there has been unwavering belief in the field of a common function of cdk4 and cdk6, several descriptions of differences between cdk4 and cdk6 have been published. A decade ago, Lucas and coworkers found that activation of cdk6 preceded cdk4 activation by several hours in T-cells [Lucas et al., 1995a,b]. More recently, these kinases were shown to have distinct responses to the p21 inhibitor in human leukemia cells. In this study, p21 was shown to bind to cdk2, cdk4, and cdk6 and to inhibit the activity of cdk2 and cdk4, but not cdk6 activity [Munoz-Alonso et al., 2005]. Residue selectivity of the kinases has also now been demonstrated. Cdk4 preferentially phosphorylates the threonine residue at amino acid 826 on the retinoblastoma protein while cdk6 preferentially phosphorylates threonine 821, indicating differences in the in vitro substrate recognition of these two kinases [Takaki et al., 2005].

Studies of tumors have also demonstrated differences between cdk4 and cdk6. Certain types of tumors selectively amplify either cdk4 or cdk6. For instance, cdk4 is specifically mutated in human melanoma [Wolfel et al., 1995; Zuo et al., 1996], while cdk6 activity has been found to be elevated in squamous cell carcinomas [Timmermann et al., 1997] and neuroblastomas [Easton et al., 1998], without alteration of cdk4 activity. Because random chance would predict that each kinase would be equally likely to be disrupted or amplified, a preference for amplification of kinase activity in tumors of certain tissues or cell types suggests that these D-cyclin kinases indeed have non-overlapping functions in these cells.

Cdk4 and cdk6 also demonstrate distinct patterns of sub-cellular localization. In U2OS cells, cdk6 was predominately localized to the cytoplasm [Grossel et al., 1999]. In mouse astroctyes, cdk6 was also localized predominately in the cytoplasm with cdk4 localized almost entirely to the nucleus [Ericson et al., 2003]. In T-cells, cdk6 was detected in both the nucleus and cytoplasm with only the nuclear fraction active as an pRb kinase [Mahony et al., 1998]. Cdk6 has even been localized to the ruffling edge of spreading fibroblasts, suggesting a function for cdk6 in controlling cell spreading [Fahraeus and Lane, 1999].

These observed differences in subcellular localization could indicate a mechanism for regulating kinase acivity, or may be indicative of the presence of a cytoplasmic role or substrate(s) of cdk6. While pRb has historically been the only known substrate of the D-cyclin kinases, a new substrate for cdk2 and cdk4 was recently revealed. While cdk6 was not studied in this report, the identification of a novel substrate for these cdk may prove pivotal to the field. Smad3 was found to be a substrate of both cdk2 and cdk4 and phosphorylation of Smad3 by these kinases inhibited transcriptional activity of Smad3 [Matsuura et al., 2004]. Thus, Smad3 may prove to be the first of several novel substrates of cdks that function to regulate DNA replication. Thus, in the past decade several studies have shown differences in timing and activity, of localization, tumor selectivity and possibly even substrates of the D-cyclin kinases.

A NEW FUNCTION FOR CDK6

The historical understanding that cdk4 and cdk6 are functionally redundant is currently being reconsidered. In the past 2 years, findings have demonstrated a new role for cdk6 in the differentiation of a variety of cell types. This function is apparently not shared with cdk4.

One of the earliest reports of a role for cdk6 in differentiation was published in 2000 [Matushansky et al., 2000]. The entry of murine erythroid leukemia (MEL) cells into terminal differentiation was accompanied by a decline in the activity of cdk2 followed by a decline in cdk6 activity and inhibitors that blocked the kinase activity of cdk2 and cdk6 triggered differentiation while inhibition of cdk2 and cdk4 did not. Later studies expanded this work to clearly show that MEL cells expressing an inhibitor-resistant form of cdk6 (cdk6R31C), but not an inhibitor-resistant form of cdk4 (cdk4R24C), failed to differentiate [Matushansky et al., 2003]. At the same time, expression of cdk6 in mouse astrocytes was found to be associated with the expression of progenitor cell markers [Ericson et al., 2003]. In astrocytes, expression of cdk6, but not cdk4, resulted in a drastic morphology change and in expression of markers consistent with less-differentiated glial precursor cells [Ericson et al., 2003]. More recently, studies have shown that BMP-2-stimulated osteoblast differentiation was inhibited by over-expression of cdk6 but not by cdk4 over-expression [Ogasawara et al., 2004b]. A related study showed that cdk6 protein levels, but not cdk4 protein levels, were down-regulated by RANKL-induced osteoclast differentiation of murine monocytic RAW cells [Ogasawara et al., 2004a].

These data are compelling in both their number and in the variety of cell types studied. However, the cdk6 knockout mouse has recently been published and indicates that mice lacking cdk6 are viable and develop relatively normally. Consistent with a prescribed role for cdk6 in blood cell differentiation, these mice contained defects in hematopoesis including decreased cellularity of the thymus, and of red blood cells and lymphocytes [Malumbres et al., 2004]. Female mice were reduced in size and one-third of females were sterile [Malumbres et al., 2004]. The mild phenotype of this knockout mouse suggests that functions of cdk6 may be compensated for by cdk4, or possibly by cdk2, which has been observed in association with D-type cyclins in cdk4/cdk6 double knockout mice [Malumbres et al., 2004]. The double knockout of cdk2 and cdk6 did not exacerbate developmental defects, but mice lacking both cdk4 and cdk6 die in embryonic development due to severe anemia. These data from the double knockout mice indeed support a hypothesis of functional compensation between cdk4 and cdk6 [Malumbres et al., 2004]. Functional compensation between cell cycle regulators is not a new paradigm. The pocket proteins p130, p107, and pRb are commonly believed to have distinct functions, but in mouse cells with compromised pRB function, p130 and p107 function as important cell cycle regulatory factors [Sage et al., 2000]. In the past 2 years, several studies in a variety of model systems have indicated that cdk6 and cdk4 have discrete functions and that cdk6 blocks differentiation.

CELL CYCLE REGULATORS IN DIFFERENTIATION

The CIP/KIP family of proteins, including p21, p27, and p57, are proteins that regulate the cell cycle through regulation of kinase activity. This family of proteins has also been studied as regulators of differentiation. Because the CIP/KIP proteins are important regulators of cdk6 activity, it is important to consider the role of these proteins in the process of differentiation as we build models of cdk6 function. In 1995 several papers were published simultaneously that showed that the cell cycle inhibitor, p21, halted cell proliferation during differentiation of muscle cells (see [Marx, 1995]). Since then, both p27 and p57 have also been implicated in changes in proliferation and differentiation. The idea that CIP/KIP inhibitors might be the link that would couple differentiation with cell cycle withdrawal was attractive, but studies of knockout mice have revealed that this model is too simplistic. Mice that lack p21 undergo normal development and have no gross alterations in their organs [Brugarolas et al., 1995; Deng et al., 1995] and mice nullizygous for p27 did not demonstrate dramatic defects in tissue differentiation [Fero et al., 1996; Kiyokawa et al., 1996; Nakayama et al., 1996]. Interestingly, mice lacking p27 showed increased levels of p21 protein, suggesting that the remaining inhibitors can functionally compensate for the deleted inhibitor. These knockout mice complicate the simple model that one or more of the CIP/KIP proteins act as the link between cell cycle withdrawal and differentiation. Nevertheless, the p21 and p27 proteins have proven to be important components of the differentiation process in cell-based studies. For instance, studies in oligodendrocyte precursor cells indicate that p27 may be a part of an intrinsic timer

of cell division that arrests the cell cycle and induces differentiation at the appropriate time [Durand et al., 1998]. In addition to arresting cell division, the inhibitor proteins have also been shown to function in morphology changes and enhanced motility that can be associated with differentiation. For instance, the p57 protein was shown to modulate the subcellular localization of LIMK, a serine/threonine kinase that is involved in the regulation of actin filaments [Tanaka et al., 2002; Yokoo et al., 2003]. Also, expression of p27 induced the migration of cultured hepatocytes and p27-deficient MEFs failed to migrate in cell culture migration assays [McAllister et al., 2003]. The role of p27 and p57 in these functions appears to be distinct from the role of these proteins as inhibitors of cell division. For instance, regions of p57 not involved in cdk-binding (termed QT domains) may be involved in LIMK localization [Zhang et al., 1997], and the ability to mediate cell cycle arrest is not a requirement for p27 function in cell migration [McAllister et al., 2003]. Thus, the CIP/KIP family of proteins, long understood to be potent inhibitors of cell division, are now understood to regulate diverse aspects of differentiation including proliferation control, regulation of actin dynamics, and cell migration.

DOES CDK6 REQUIRE pRB FOR ITS FUNCTION IN DIFFERENTIATION?

Another cell cycle regulatory protein that has been implicated in processes related to differentiation is the retinoblastoma protein. The retinoblastoma protein has been shown to promote terminal differentiation of several cell types including myocytes, adipocytes, neurons, and chondroctyes [Cobrinik et al., 1996]. Indeed, pRb knockout mice exhibit pronounced defects in erythroid, neuronal, and lens development. In mice lacking pRB, these lineages were able to initiate differentiation but did not fully differentiate, suggesting that pRb might maintain cell cycle withdrawal that precedes expression of tissue-specific genes [Lipinski and Jacks, 1999]. However, recent data suggest that pRb may have a role in cell proliferation that follows differentiation [Sage et al., 2005].

One role that the retinoblastoma protein plays in differentiation is the expression of tissue-specific genes required for terminal differentiation. For example, pRb may activate the MyoD family of transcription factors [Yee et al., 1998] and may act as a transcriptional coactivator with the osteoblast transcription factor, Runx2 in osteogenic differentiation [Thomas et al., 2001]. In addition, some differentiation-specific transcription factors act in opposition to the function of retinoblastoma. For example, the transcription factor Id-2 can bind and inactivate pRb and the loss of Id-2 rescued differentiation defects in the nervous systems of Rb-null embryos [Iavarone and Lasorella, 2004]. The transcription factor PU.1 has also been shown to bind pRb through its activation domain [Hagemeier et al., 1993]. Increasingly, data support interplay of pRb and the tissue-specific transcription factors that control differentiation.

It will be of great interest to understand if the role of cdk6 in differentiation is pRb-dependent or pRb-independent. One clue suggests that pRb is unlikely to be the only mediator of cdk6-driven inhibition of differentiation. A recent study found no correlation between the binding of pRb to the osteocalcin promoter and the cdk6-induced block to differentiation in these cells [Ogasawara et al., 2004b]. The retinoblastoma protein and cdk6 may together provide a link between the complex cellular processes of differentiation and proliferation and in this way maintain the delicate balance between cellular division and differentiation.

MECHANISMS OF CDK6 FUNCTION

The mechanism by which cdk6 expression exerts its block on differentiation is not understood. One possibility is that cdk6 exerts its role in differentiation through the regulation of cell cycle. While cdk6 has long been understood as a mitogenic factor, some studies have indicated that cdk6 can inhibit cell proliferation. For example, NIH3T3 cells overexpressing cdk6 showed a reduced proliferation rate as compared to parental NIH 3T3 cells [Nagasawa et al., 2001]. Also, breast tumor cell lines that were transfected with cdk6 showed a reduced rate of proliferation compared with parental tumor cell lines and normal mammary epithelial cells had a high level of cdk6 protein but breast tumor-derived cell lines had much lower levels of cdk6 [Lucas et al., 2004]. These findings are consistent with a role for cdk6 in halting the cell cycle prior to differentiation. Other studies seem to suggest a role that is independent of cell cycle regulation. In osteoblast differentiation,

the inhibition of differentiation by cdk6 expression was declared independent of its role in cell cycle regulation since cell cycle changes were not observed in this study [Ogasawara et al., 2004b]. While it is simple to the segregate the processes of differentiation and proliferation, it is important to consider that they are not mutually exclusive events; cells in higher organisms only cease dividing at the end of the process of differentiation. That is, proliferation is a necessary part of the extended process that results in terminal differentiation [Coffman, 2003].

Examination of upstream and downstream factors involved in the cdk6 inhibition of differentiation is underway. For instance, the transcription factor PU.1 has been shown to regulate the synthesis of cdk6 mRNA in MEL differentiation [Matushansky et al., 2003]. Interestingly, this factor blocks erythroid differentiation via repression of GATA-mediated transcriptional activation. GATA-1 is a zinc finger transcription factor that is required for erythroid differentiation, thus PU.1 both blocks GATA transactivation and regulates cdk6 synthesis to allow erythroid differentiation. Likewise, the signaling molecule Bone Morphogenic Protein 2 (BMP-2) may regulate the synthesis of cdk6 message in osteoblasts. BMP-2 is a signaling molecule that stimulates osteoblast differentiation through Smad mediator proteins. In BMP-2-induced osteoblast differentiation, cdk6 was down-regulated by BMP-2/Smad mediated transcriptional repression and this down-regulation of cdk6 was required for differentiation [Ogasawara et al., 2004b]. In a related study, cdk6 overexpression prevented the transcription factor, Runx2, from loading on the osteocalcin promoter, but, perhaps showing specificity, it did not exclude pRb from the same promoter [Ogasawara et al., 2004a]. Together, these data provide a foundation to begin to predict a mechanism of cdk6 function.

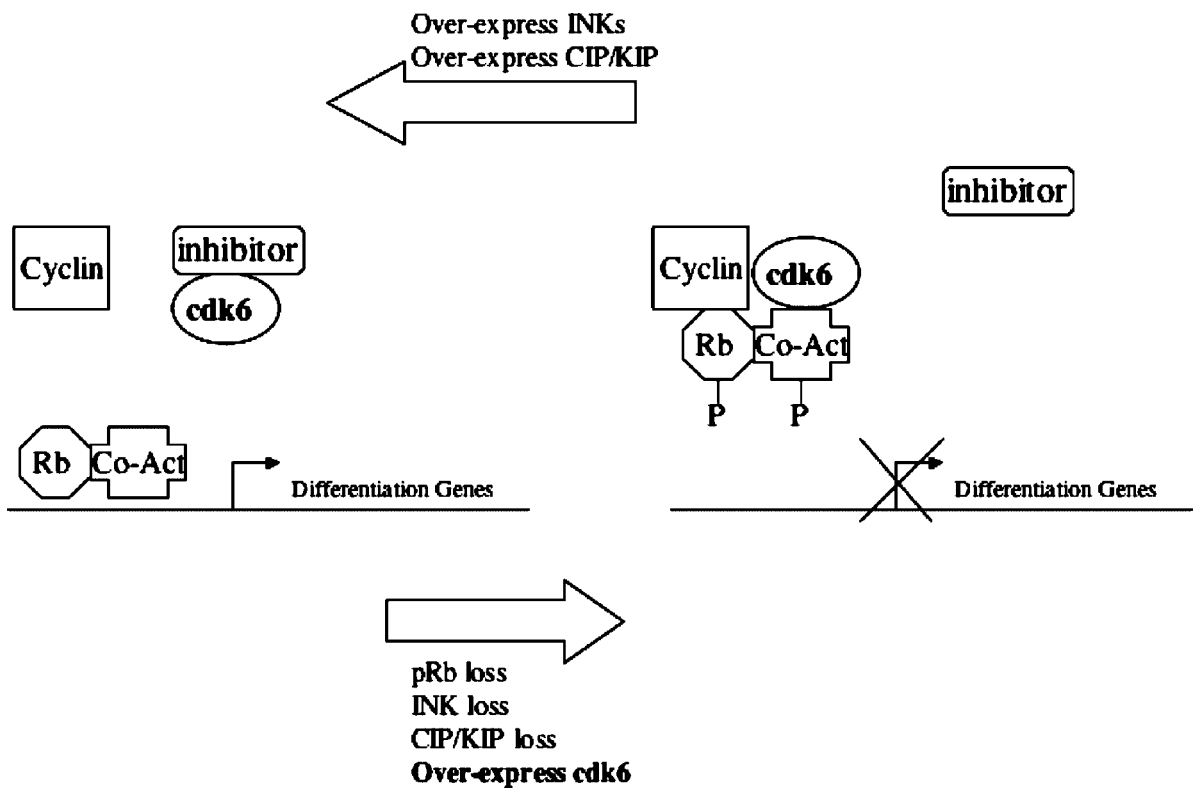

Fig. 1. A model for cdk6-regulated control of differentiation. Based on a model proposed for phosphate regulation in yeast [Cross, 1995], cdk6 regulates the activity of transcription factors that control expression of differentiation-specific genes. In conditions where inhibitor protein(s) exceed cdk6 concentration, Rb and tissue-specific co-activators function at the promoters of differentiation-specific genes. Under conditions where cdk6 exceeds the concentration of inhibitor protein(s), cdk6 functions to phosphorylate and remove transcription factors from the promoter.

MODELS OF CDK6 FUNCTION

As we begin to identify molecules that function with cdk6 in blocking differentiation, we can build models of cdk6 function. Perhaps cdk6 functions via a mechanism similar to that proposed by Fred Cross to model the regulation of phosphate metabolism in yeast [Cross, 1995]. In this model, genes involved in phosphate metabolism are controlled through transcriptional regulation that is itself regulated by cyclin-dependent-kinase phosphorylation of transcription factors. This model, proposed in Figure 1, predicts that cdk6 would phosphorylate differentiation-specific transcription factors to activate or repress expression of differentiation-specific genes. In this transcriptional regulatory role, cdk6 would phosphorylate transcription factors on the appropriate promoters to block differentiation. If this system mimicked that of yeast, cdk6 could partner with a novel cyclin that would function with cdk6 in transcriptional regulation but not in cell cycle control. Once phosphorylated, transcription factors (activators and repressors) are inactivated and removed from the promoter.

Data from systems presented here suggest a transcriptional mechanism of cdk6 function in the process of differentiation. In MEL cells, the PU.1 transcription factor regulates cdk6 expression. If cdk6 regulated PU.1 activity, this could create a feedback loop to control cdk6 levels during differentiation. In the astrocyte model, elevated levels of Id4 protein coincided with a change in differentiation status of cdk6-expressing cultured mouse astroyctes [Ericson et al., 2003]. Id4 is a member of the Id family of proteins (for inhibitor of differentiation) that set the timing of cell differentiation in several neural cell types. Id proteins sequester basic helix-loop-helix transcription factors to block differentiation and sustain cell cycle activation [Iavarone and Lasorella, 2004]. Overexpression of Id genes can inhibit the differentiation of B lymphocytes, muscle cells, mammary epithelial cells, erythroid cells, and oligodendrocyte precursor cells [Kondo and Raff, 2000]. As shown in Figure 2, Id could influence the differentiation of Glia.

Perhaps the most compelling data in support of the model proposed in Figure 1, is the finding that cdk6 expression resulted in the loss of Runx from the osteocalcin promoter. Runx is a family of transcriptional regulators that function in tissue-specific differentiation and also possibly function in proliferation during differentiation that is not tissue-specific. Runx proteins have been reported to regulate p21 protein levels,

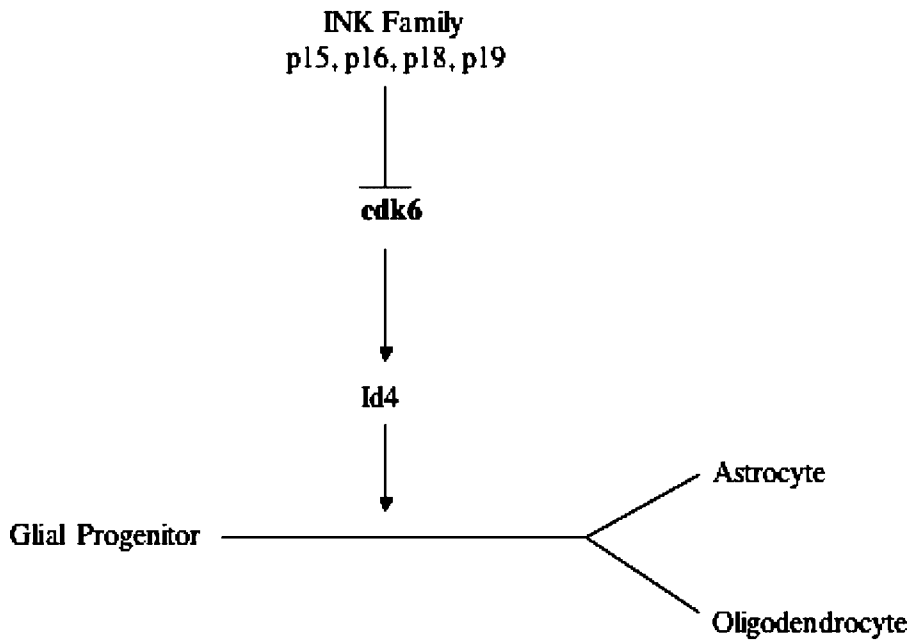

Fig. 2. A model for cdk6-regulated differentiation of glia. Overexpression of cdk6 may overcome inhibition by INK family members and result in increased levels of Id4 protein. Id4 inhibits differentiation through a known mechanism that involves the formation of inactive heterodimers with some members of the helix-loop-helix family of transcription factors.

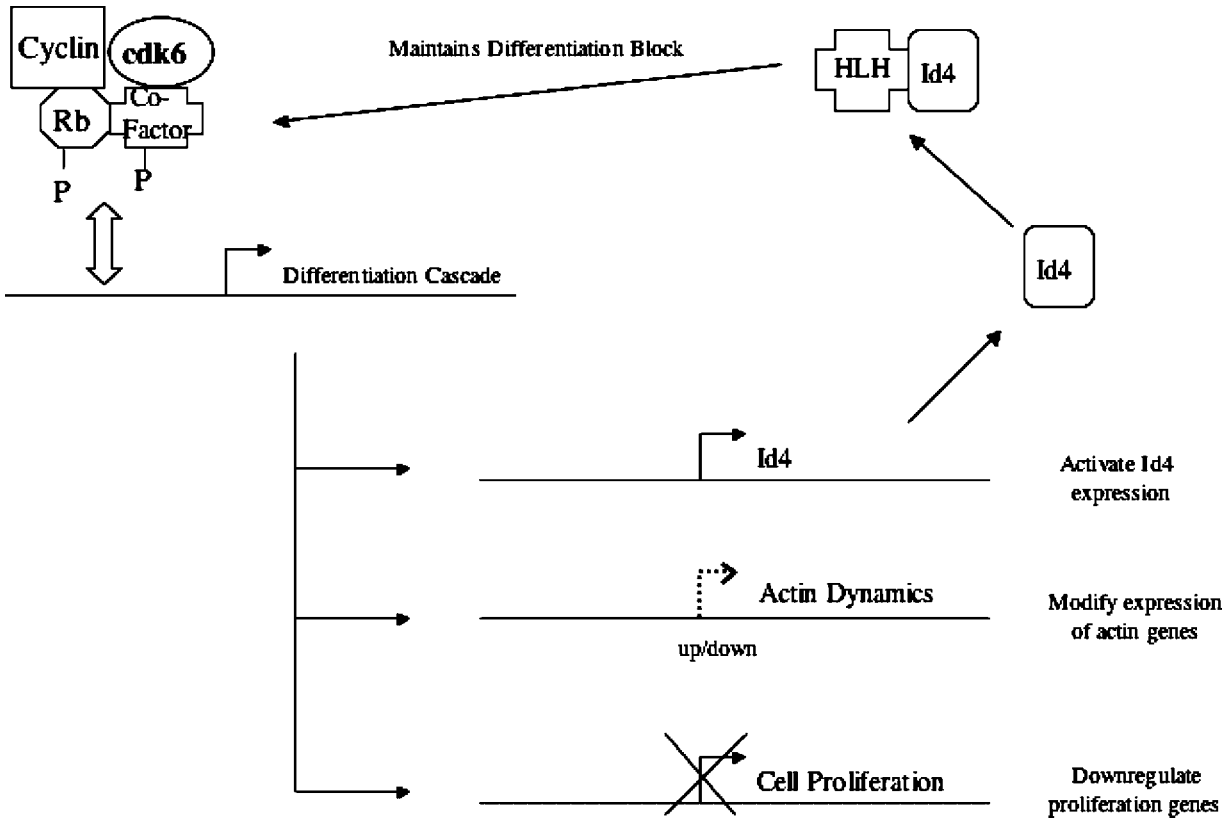

Fig. 3. Differentiation likely involves a cascade of transcriptional regulation. The process of differentiation likely involves the regulation of many classes of genes. Depicted in this graphic are (1) cdk6-activation of Id4 expression. Overexpression of cdk6 results in increased levels of Id4, depicted here through transcriptional regulation. Cdk6 could regulate this process by phosphorylating and removing a repressor protein from the Id4 promoter. (2) Cdk6-regulation of genes that control actin dynamics. Cdk6 may regulate these genes through the phosphorylation of activator and/or repressor proteins. Changes in actin filament formation are necessary for morphological changes associated with differentiation and increased migration of cells that can accompany differentiation. (3) Cdk6-down-regulation of genes that promote cell proliferation. Studies have shown that cdk6 expression can result in reduced proliferation in some cell types. Tight regulation of cell proliferation is an important part of the process of differentiation.

have been reported to partner with PU.1 in blood differentiation and even to function with Smad proteins in this role [Coffman, 2003; Stein et al., 2004]. Could then Runx and other transcriptional regulators be the target of cdk6 phosphorylation? Might pRb deliver these regulators to cdk6 to be phosphorylated? Importantly, pRb has been shown to associate with Runx2 to the osteocalcin promoter [Thomas et al., 2001] and PU.1 has been shown to bind pRb.

How then, can cdk6 be affecting the multitude of diverse processes required for differentiation? The model in Figure 3 shows a cascade of events, all regulated by cdk6-regulated transcription. Cdk6 could regulate transcription of a variety of activators and repressors that influence expression of several genes, all of which contribute to different aspects of differentation. For instance, based on data summarized in this work, cdk6 overexpression may result in the activation of Id4 gene expression. An increase in Id4 protein levels would result in the formation of inactive heterodimers with transcriptional activators and the inhibition of differentiation. Another result of cdk6-regulated gene expression may be to modify the expression of genes involved in actin dynamics. The Runx proteins may influence actin dynamics to control cellular morphology changes and motility during differentiation. These gene products may result in changes in actin filament formation necessary for morphological changes associated with differentiation and in the increased migration of cells that is often necessary in differentiation, especially of the nervous system. The regulation of actin dynamics may occur at several promoters through the regulation of both positive and negative regulatory factors. Runx proteins are capable of either

activating or repressing transcription. Finally, the cdk6-driven inhibition of cell division observed in some systems may also be regulated by transcription. As part of the cascade of events controlled by cdk6 expression, cdk6 could directly phosphorylate activators of cell proliferation, resulting in turning off genes that induce cell proliferation.

This model then predicts that cdk6 is positioned to regulate a cascade of events that control differentiation, and by simply phosphorylating transcriptional regulators, cdk6 is positioned to regulate all aspects of differentiation that have been discussed in this review. It will be of great interest to test these models and to understand the role of pRb as a possible co-factor in these processes.

CONCLUSIONS

Recent evidence seems to indicate that differentiation and cell cycle regulation may be independently regulated, simultaneous events. These events may involve cdk6, pRb and cell cycle inhibitors, in dual roles of cell cycle withdrawal and transcriptional regulation. Retinoblastoma may have a direct role in activating differentiation-specific transcription and this transcriptional role of pRb and its associated co-factors could be regulated by cdk6 phosphorylation. The distinction between cdk6 and cdk4 in this regard could be the recently uncovered site-specific phosphorylation of pRb by these two kinases. It will be interesting to learn if cdk6 mimics known mechanisms of differentiation such as cell cycle withdrawal and altering actin dynamics through transcriptional regulation, or if it will reveal an entirely new function to regulate the process of differentiation.

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REFERENCES

Brugarolas J, Chandrasekaran C, Gordon JI, Beach D, Jacks T, Hannon GJ. 1995. Radiation-induced cell cycle arrest compromised by p21 deficiency. Nature 377:552–557.

Cobrinik D, Lee MH, Hannon G, Mulligan G, Bronson RT, Dyson N, Harlow E, Beach D, Weinberg RA, Jacks T. 1996. Shared role of the pRB-related p130 and p107 proteins in limb development. Genes Dev 10:1633–1644.

Coffman JA. 2003. Runx transcription factors and the developmental balance between cell proliferation and differentiation. Cell Biol Int 27:315–324.

Cross F. 1995. Transcriptional regulation by a cyclin-cdk. Trends Genet 11:209–211.

Deng C, Zhang P, Harper JW, Elledge SJ, Leder P. 1995. Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. Cell 82: 675–684.

Durand B, Fero ML, Roberts JM, Raff MC. 1998. p27Kip1 alters the response of cells to mitogen and is part of a cell-intrinsic timer that arrests the cell cycle and initiates differentiation. Curr Biol 8:431–440.

Easton J, Wei T, Lahti J, Kidd V. 1998. Disruption of the cyclin D/cyclin-dependent kinase/INK4/retinoblastoma protein regulatory pathway in human neuroblastoma. Cancer Res 58:2624–2632.

Ekholm SV, Reed SI. 2000. Regulation of G(1) cyclin-dependent kinases in the mammalian cell cycle. Curr Opin Cell Biol 12:676–684.

Ericson KK, Krull D, Slomiany P, Grossel MJ. 2003. Expression of cyclin-dependent kinase 6, but not cyclin-dependent kinase 4, alters morphology of cultured mouse astrocytes. Mol Cancer Res 1:654–664.

Ewen ME, Sluss HK, Whitehouse LL, Livingston DM. 1993. TGF beta inhibition of Cdk4 synthesis is linked to cell cycle arrest. Cell 74:1009–1020.

Fahraeus R, Lane DP. 1999. The p16(INK4a) tumour suppressor protein inhibits alphavbeta3 integrin-mediated cell spreading on vitronectin by blocking PKC-dependent localization of alphavbeta3 to focal contacts. EMBO J 18:2106–2118.

Fero ML, Rivkin M, Tasch M, Porter P, Carow CE, Firpo E, Polyak K, Tsai LH, Broudy V, Perlmutter RM, Kaushansky K, Roberts JM. 1996. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. Cell 85:733–744.

Grossel MJ, Baker GL, Hinds PW. 1999. cdk6 can shorten G(1) phase dependent upon the N-terminal INK4 interaction domain. J Biol Chem 274:29960–29967.

Hagemeier C, Bannister AJ, Cook A, Kouzarides T. 1993. The activation domain of transcription factor PU.1 binds the retinoblastoma (RB) protein and the transcription factor TFIID in vitro: RB shows sequence similarity to TFIID and TFIIB. Proc Natl Acad Sci USA 90:1580–1584.

Iavarone A, Lasorella A. 2004. Id proteins in neural cancer. Cancer Lett 204:189–196.

Kato J, Matsushime H, Hiebert SW, Ewen ME, Sherr CJ. 1993. Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. Genes Dev 7:331–342.

Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, Khanam D, Hayday AC, Frohman LA, Koff A. 1996. Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). Cell 85:721–732.

Kondo T, Raff M. 2000. The Id4 HLH protein and the timing of oligodendrocyte differentiation. EMBO J 19:1998–2007.

Lipinski MM, Jacks T. 1999. The retinoblastoma gene family in differentiation and development. Oncogene 18: 7873–7882.

Lucas JJ, Szepesi A, Domenico J, Tordai A, Terada N, Gelfand EW. 1995a. Differential regulation of the synthesis and activity of the major cyclin-dependent kinases, p34cdc2, p33cdk2, and p34cdk4, during cell cycle entry and progression in normal human T lymphocytes. J Cell Physiol 165:406–416.

Lucas JJ, Szepesi A, Modiano JF, Domenico J, Gelfand EW. 1995b. Regulation of synthesis and activity of the PLSTIRE protein (cyclin-dependent kinase 6 (cdk6)), a major cyclin D-associated cdk4 homologue in normal human T lymphocytes. J Immunol 154:6275–6284.

Lucas JJ, Domenico J, Gelfand EW. 2004. Cyclin-dependent kinase 6 inhibits proliferation of human mammary epithelial cells. Mol Cancer Res 2:105–114.

Mahony D, Parry K, Lees E. 1998. Active cdk6 complexes are predominatly nuclear and represent only a minority of the cdk6 in T cells. Oncogene 16:603–611.

Malumbres M, Sotillo R, Santamaria D, Galan J, Cerezo A, Ortega S, Dubus P, Barbacid M. 2004. Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. Cell 118:493–504.

Marx J. 1995. Cell biology. Cell cycle inhibitors may help brake growth as cells develop. Science 267:963–964.

Matsushime H, Ewen M, Strom D, Kato J, Hanks S, Foussel M, Sherr C. 1992. Identification and properties of an atypical catalytic subunit (p34PSK-J3/cdk4) for mammalian D type G1 cyclins. Cell 71:323–334.

Matsushime H, Quelle DE, Shurtleff SA, Shibuya M, Sherr CJ, Kato J. 1994. D-type cyclin-dependent kinase activity in mammalian cells. Mol Cell Biol 14:2066–2076.

Matsuura I, Denissova NG, Wang G, He D, Long J, Liu F. 2004. Cyclin-dependent kinases regulate the antiproliferative function of Smads. Nature 430:226–231.

Matushansky I, Radparvar F, Skoultchi AI. 2000. Reprogramming leukemic cells to terminal differentiation by inhibiting specific cyclin-dependent kinases in G1. Proc Natl Acad Sci USA 97:14317–14322.

Matushansky I, Radparvar F, Skoultchi AI. 2003. CDK6 blocks differentiation: Coupling cell proliferation to the block to differentiation in leukemic cells. Oncogene 22: 4143–4149.

McAllister SS, Becker-Hapak M, Pintucci G, Pagano M, Dowdy SF. 2003. Novel p27(kip1) C-terminal scatter domain mediates Rac-dependent cell migration independent of cell cycle arrest functions. Mol Cell Biol 23:216–228.

Meyerson M, Harlow E. 1994. Identification of G1 kinase activity for cdk6, a novel cyclin D partner. Mol Cell Biol 14:2077–2086.

Meyerson M, Enders GH, Wu C, Su L, Gorka C, Nelson C, Harlow E, Tsai L. 1992. A family of human cdc2-related protein kinases. EMBO 11:2909–2917.

Munoz-Alonso MJ, Acosta JC, Richard C, Delgado MD, Sedivy J, Leon J. 2005. p21Cip1 and p27Kip1 induce distinct cell cycle effects and differentiation programs in myeloid leukemia cells. J Biol Chem 280:18120–18129.

Nagasawa M, Gelfand EW, Lucas JJ. 2001. Accumulation of high levels of the p53 and p130 growth-suppressing proteins in cell lines stably over-expressing cyclin-dependent kinase 6 (cdk6). Oncogene 20:2889–2899.

Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, Horii I, Loh DY, Nakayama K. 1996. Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell 85:707–720.

Ogasawara T, Katagiri M, Yamamoto A, Hoshi K, Takato T, Nakamura K, Tanaka S, Okayama H, Kawaguchi H. 2004a. Osteoclast differentiation by RANKL requires NF-kappaB-mediated downregulation of cyclin-dependent kinase 6 (Cdk6). J Bone Miner Res 19:1128–1136.

Ogasawara T, Kawaguchi H, Jinno S, Hoshi K, Itaka K, Takato T, Nakamura K, Okayama H. 2004b. Bone morphogenetic protein 2-induced osteoblast differentiation requires Smad-mediated down-regulation of Cdk6. Mol Cell Biol 24:6560–6568.

Sage J, Mulligan GJ, Attardi LD, Miller A, Chen S, Williams B, Theodorou E, Jacks T. 2000. Targeted dis ruption of the three Rb-related genes leads to loss of G(1) control and immortalization. Genes Dev 14:3037–3050.

Sage C, Huang M, Karimi K, Gutierrez G, Vollrath MA, Zhang DS, Garcia-Anoveros J, Hinds PW, Corwin JT, Corey DP, Chen ZY. 2005. Proliferation of functional hair cells in vivo in the absence of the retinoblastoma protein. Science 307:1114–1118.

Stein GS, Lian JB, van Wijnen AJ, Stein JL, Montecino M, Javed A, Zaidi SK, Young DW, Choi JY, Pockwinse SM. 2004. Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression. Oncogene 23:4315–4329.

Takaki T, Fukasawa K, Suzuki-Takahashi I, Semba K, Kitagawa M, Taya Y, Hirai H. 2005. Preferences for phosphorylation sites in the retinoblastoma protein of D-type cyclin-dependent kinases, Cdk4 and Cdk6, in vitro. J Biochem (Tokyo) 137:381–386.

Tanaka H, Homma K, Iwane AH, Katayama E, Ikebe R, Saito J, Yanagida T, Ikebe M. 2002. The motor domain determines the large step of myosin-V. Nature 415:192–195.

Thomas DM, Carty SA, Piscopo DM, Lee JS, Wang WF, Forrester WC, Hinds PW. 2001. The retinoblastoma protein acts as a transcriptional coactivator required for osteogenic differentiation. Mol Cell 8:303–316.

Timmermann S, Hinds PW, Munger K. 1997. Elevated activity of cyclin-dependent kinase 6 in human squamous cell carcinoma lines. Cell Growth Differ 8:361–370.

Wolfel J, Hauer M, Schneider J, Serrano M, Wolfel C, Klehmann-Hieb E, De Plaen E, Hankelen T, Meyer zum Buschenfelde K, Beach D. 1995. A p16 INK4a-insensitive CDK4 mutant targeted by cytolitic T lymphocytes in a human melanoma. Science 269:1281–1284.

Yee AS, Shih HH, Tevosian SG. 1998. New perspectives on retinoblastoma family functions in differentiation. Front Biosci 3:D532–D547.

Yokoo T, Toyoshima H, Miura M, Wang Y, Iida KT, Suzuki H, Sone H, Shimano H, Gotoda T, Nishimori S, Tanaka K, Yamada N. 2003. p57Kip2 regulates actin dynamics by binding and translocating LIM-kinase 1 to the nucleus. J Biol Chem 278:52919–52923.

Zhang P, Liegeois NJ, Wong C, Finegold M, Hou H, Thompson JC, Silverman A, Harper JW, DePinho RA, Elledge SJ. 1997. Altered cell differentiation and proliferation in mice lacking p57KIP2 indicates a role in Beckwith–Wiedemann syndrome. Nature 387:151–158.

Zuo L, Weger J, Yang Q, Goldstein A, Tucker M, Walker G, Hayward N, Dracopoli N. 1996. Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. Nat Genet 12:97–99.