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Review

Crosstalk between cell cycle regulators and the myogenic factor MyoD in skeletal myoblasts

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Abstract. During the early process of skeletal muscle differentiation, myogenic factors are not only involved in muscle-specific gene induction but also in regulating the transition from the proliferative stage, when MyoD and Myf5 are already expressed, to the orderly exit from the cell division cycle. This key step in skeletal muscle differentiation involves the down-regulation of cell cycle activators such as cyclins and cdks, and up-regulation of cell cycle inhibitors such as Rb, p21, p27, and p57. In particular, Rb and p21 have been shown to play an important role in the growth arrest of differentiating myoblasts. Their level and/or activity, while being negatively controlled by growth factors, appear to be positively linked with the myogenic factor MyoD, which plays a cooperative role in the induction of growth arrest. MyoD can block proliferation independently of its transcriptional activity. Therefore, the interplay between G1 cyclins and cdk inhibitors, on the one hand, and MyoD and its co-factors, on the other, plays a critical role in myoblast cell cycle withdrawal. Accurate synchronization of dividing myoblasts revealed that MyoD and Myf5 are themselves subject to specific cell cycle-dependent regulation, with MyoD at its highest level in early G1 and its lowest level at the G1 to S phase transition. The time-window when cells exit their cycle into differentiation is in G1, when MyoD is maximal and Myf5 is down. In contrast, quiescent non-differentiating myoblasts (i.e., in G0) present an opposite pattern for the two factors: high Myf5 and no MyoD. Several recent studies have focused on MyoD phosphorylation and its potential role in ubiquitination-mediated degradation of the protein. Linking this phosphorylation to the cell cycle-dependent drop in MyoD protein before S phase leads, to a mechanism implying cdk2-cyclin E and its inhibitors (p57kip and p21cip) in the tight control of MyoD levels and subsequent myoblast cell cycle progression or exit into differentiation.

Key words. MyoD; cell cycle; skeletal myoblast; myogenesis; transition-proliferation-differentiation.

Introduction

The commitment of myogenic cells into skeletal muscle differentiation requires prior irreversible cell cycle withdrawal. The decision to progress through a new division cycle appears primarily regulated before the G1 to S phase transition. At the molecular level, several positive and negative cell cycle regulators have been identified. Among positive regulators are the cyclin-dependent kinases (cdks) and their cyclins [1, 2], whereas the negatively acting regulators comprise the cdk inhibitors (CKIs) [3] and pocket protein family: the product of the retinoblastoma susceptibility gene (Rb protein) and the two related Rb family proteins p107 and p130 [4, 5].

The biological activity of cell cycle phase-specific cyclin/cdk complexes allows progression into successive phases of the cell cycle. Cdk1, the first characterized cdk, forms complexes with cyclins A and B, which are crucial

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for the cell to progress from G2 into M phase. Subsequent passage through G1 into S phase is controlled by cdks that are sequentially regulated by cyclins D (cdk4 and cdk6), cyclin E, and cyclin A (cdk2). One of the most extensively studied targets for cdks during the G1/S transition is Rb. In its hypophosphorylated form, Rb is a tumor suppressor gene which exerts its function at the transcriptional level by interacting with and modulating the activities of a variety of transcription factors [6, 7], thus repressing the transcription of genes essential for entry into S phase. Three transcription factors of the E2F family (E2F1, E2F2, and E2F3) are the most extensively described targets for Rb binding during the G1 phase [8, 9]. Sequential phosphorylation of Rb by G1 cyclin-cdk complexes (cdk4-cyclinD and cdk2-cyclinE) promotes its dissociation from E2F, thus allowing entry of cells into S phase [10]. Therefore, the control of cell cycle progression during the G1 phase is largely dependent on Rb/E2F-mediated gene regulation.

Similar mechanisms control the cell cycle of myogenic cells, which undergo a phase of active proliferation before cell cycle arrest and fusion into myotubes. The specification, differentiation, and fusion of myoblasts require myogenic factors [11, 12]. Four myogenic factors have been identified: MyoD [13], myogenic [14], Myf-5 [15], and MFR4/Myf-6/herculin [16]. These muscle-specific bHLH transcription factors are each capable of activating the complete program of skeletal muscle differentiation when expressed ectopically in a variety of non-muscle cell types [15, 17, 18; reviewed in ref. 11]. In addition to these four myogenic factors, two paired-box transcription factors, Pax-3 and Pax-7, are involved in the determination/commitment of muscle cells. Pax-3 is an upstream regulator of MyoD during development and plays an essential role in committing multi-potent somite cells into differentiating muscle [19, 20], whereas Pax-7 is required for the specification of adult satellite cells [21]. The four myogenic factors activate transcription of muscle-specific genes by binding, upon heterodimerization with ubiquitous E proteins [22], the E-box consensus sequence (CANNTG) in muscle gene promoters and enhancers [23]. They are expressed in a defined sequence in myogenic cells: Myf-5 and MyoD are typically expressed the earliest, followed by myogenin and lastly MRF4 [24, 25]. This sequential expression of myogenic factors identifies early (Myf-5/MyoD) and late (MRF4/myogenin) stages in the life of a muscle cell. It is also related to their respective roles in muscle development as determined by knock-out experiments in mice: MyoD and Myf-5 play a role in specifying muscle lineage (determination), myogenin controls the differentiation process, and MRF4 is thought to be involved in the maturation of myotubes [reviewed in refs. 11, 12].

There is clear evidence that deregulation of the cell cycle leading to uncontrolled proliferation antagonizes the function of myogenic factors, resulting in a general absence of differentiation-specific gene expression in dividing cells. Conversely, the myogenic factor MyoD seems to cooperate with cell cycle inhibitors (CKI, Rb) in the pathway leading to cell cycle arrest in G1 and commitment to differentiation. Both the hypophosphorylated form of Rb and CKIs play important roles in maintaining a permanent cell cycle withdrawal of differentiated myotubes [26–30].

This review will address the molecular events underlying the balance between proliferation and differentiation of skeletal myoblasts, by shedding light on the crosstalk mechanisms taking place between cell cycle regulators (cdks, CKI, E2F, Rb) and myogenic factors. We will focus in particular on MyoD because it is the myogenic factor shown to be critically required in the process of muscle regeneration [31]. This process, which is mimicked in myogenic cell lines, requires a phase of cell proliferation before cell cycle exit and fusion of post-mitotic myoblasts.

Growth factors and antagonism between myoblast proliferation and differentiation

Differentiation of skeletal myoblasts in culture is under negative control by serum which prevents entry into differentiation until its concentration is reduced below a critical threshold. The immediate early gene products c-myc, c-fos, and c-jun are induced upon serum stimulation of quiescent cells and these genes are inhibitory to myogenesis [32–34], suggesting that signaling pathways induced by growth factors will facilitate myoblast proliferation and inhibit myogenesis. Indeed, two of the most potent inhibitors of myoblast differentiation in culture are fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β) [35–37] and both are expressed at appropriate times and places during embryogenesis, playing a role in the regulation of myogenesis. TGF-β inhibits the activity of myogenin and MyoD without affecting their ability to bind DNA [30]. Since ongoing protein synthesis is required for growth factor-dependent repression of differentiation [36], growth factor-induced early genes must be essential mediators of this inhibition. c-jun, for example, has been reported to interact directly with the bHLH region of MyoD and inhibit its activity [38]. Protein kinase C (PKC) was proposed as one of the downstream effectors of growth factor inhibition on myogenesis. It directly phosphorylates myogenin at a conserved Thr 115 in the basic domain, resulting in the inhibition of DNA binding activity [39]. However this model was contradicted by further studies showing that inhibition of myogenic regulatory factor (MRF)4 by FGF occurs independently of its phosphorylation on the conserved Thr 115 [40]. Apart from the fact that myogenin is only present in myoblasts

when they enter the post-mitotic stage (i.e., low-growth factors), and neither MyoD nor Myf5 have been shown to be phosphorylated by PKC in vivo, the role shown so far for phosphorylation of myogenin by PKC has been in down regulating the expression of specific acetylcholine receptor (AceR) isoforms (containing an E-box in their promoters) in response to Ca^{2+} influx at the neuromuscular junction [41]. Thus PKC role(s) in myogenesis more likely relate to specific regulation in muscle fibers, i.e., after commitment of myoblasts into differentiation.

In addition to immediate early genes, two growth factor-induced genes have been shown to inhibit MRFs: Id and cyclin D1. Id family members (Id1, Id2, Id3, and Id4) are HLH proteins which lack a basic region and inhibit the activity of other HLH proteins by forming biologically inactive hetero-oligomers [42]. Id expression is induced by serum stimulation and is down-regulated upon serum withdrawal and during myogenesis [43]. Id oligomerizes preferentially with E proteins the major cofactors of MRFs, thereby competing for the formation of transcriptionally active MRF/E heterodimers. However, tethering E47 to MyoD showed that these chimeras still displayed higher activity in growth-arrested differentiating cells [44]. This indicates the existence of growth factor-induced inhibitory pathways other than those mediated by Id. Expression of cyclin D1 is induced by growth factors such as FGF and TGF-β and is maximal during the G1 phase [2]. Cyclin Dl overexpression accelerates G1 progression [45] and inhibits muscle-specific gene transactivation in part via the Rb/E2F pathway [46–48]. In addition to its role in cell cycle arrest, Rb was shown to be required for muscle differentiation in cooperation with MyoD [49] and by promoting the transcriptional activity of MEF2 [50]. Recent reports from Paterson's group have postulated that cyclin D1-mediated inhibition of MyoD activity would be the result of a direct interaction between cdk4 and MyoD which could only take place when cyclin D1 is present to shuttle cdk4 in the nucleus [51]. Reciprocally, MyoD binding to cdk4 would inhibit cdk4 activity and thereby cell cycle progression [52]. The problem with these conclusions is that they rely almost completely on binding studies between heterologous systems, i.e., chicken MyoD versus mouse cdk4. This is not trivia considering that the protein domain mapped on chicken MyoD as binding to mouse cdk4 differs by 6 out of 16 amino acids from the corresponding human or mouse MyoD sequence [52]. Similarly the rationale for ruling out a previously reported [49] interaction between pRb and MyoD in this study was based on two-hybrid experiments using chicken MyoD versus human pRb [52]. In addition, the binding of cdk4 to MyoD should be effective in driving cdk4 in the nucleus at any time, including in differentiated myotubes: it is supposed to involve a region of MyoD C-terminal to the bHLH domain [52], and therefore would not mask and impair the two very efficient nuclear localization signals (NLP) identified in MyoD [53]. For example, when trying to neutralize MyoD protein by microinjection of specific anti-MyoD antibodies in the cytoplasm, we have observed that these antibodies are imported into the nucleus very rapidly and efficiently (M. Vandromme, N. Lamb, A. Fernandez, unpublished observation).

Expression and activity of cell cycle regulators during myogenesis

To date, all existing data establish clearly that the presence of serum, above a critical threshold, stimulates progression of cultured myoblasts through S phase and antagonizes their commitment into differentiation. Conversely, permanent withdrawal of myotubes from the cell cycle requires that major positive cell cycle regulators are and remain inhibited, and this involves the up-regulation of cdk inhibitors and active Rb family members [27–30, 47].

Indeed, the expression and activity of positive cell cycle regulators are down-regulated during myogenesis. If the expression of cdk4 and cdk2 remains unchanged during skeletal differentiation, those of cdk1, cyclin A, and cyclin D1 decline [54]. Exit of myoblasts from the cell cycle is associated with down-regulation of cdk activity, and the inability of the skeletal muscle malignancy rhabdomyosarcoma (RD) cells to exit from the cell cycle is related to a high level of both cyclin E and cyclin A [55]. As for cyclin D1, cdk2/cyclin A or cdk2/cyclin E overexpression in proliferative myoblasts leads to inhibition of myogenin and MyoD myogenic activity and consequent inhibition of skeletal differentiation [48]. Inhibition of myogenic transcriptional activity is a general feature of Cdk activity which appears to be mediated by an Rb/E2F-dependent pathway [48]. Rb and E2F expression and activity change during myogenesis: Rb accumulates during embryonic development and cell differentiation and participates in the terminal differentiation of various cell lineages [56, 57]. During myogenic differentiation of C2 cells, Rb gene expression is enhanced by MyoD by a mechanism that is distinct from its myogenic function [58]. Skeletal myogenesis involves interaction of Rb, in its active hypophosphorylated form, with myogenic factors of the MyoD family [49] and Rb cooperates with MyoD to promote MEF2 transcriptional activity in differentiating myoblasts [50]. Although mice genetically deficient in Rb have histologically normal skeletal musculature [59], in contrast to wild-type muscle, terminal cell cycle arrest is not maintained in Rb–/– differentiated skeletal muscle. Myotubes from Rb–/– cells could synthesize DNA after restimulation with serum growth factor-rich medium [26, 27].

Changes in E2F function and regulation also occur upon muscle differentiation [60]. E2F is a family of proteins

comprising six members [E2F1–6) and different E2F-containing nucleoprotein complexes are detected in proliferative myoblasts compared with post-mitotic myotubes [60]. E2F1 is the most extensively E2F studied in myogenesis. Its expression is down-regulated during myogenesis, and myocytes overexpressing E2F1 fail to exit the cell cycle under differentiation conditions [61]. E2F1 inhibits the activation of gene transcription by MyoD and myogenin, this repression being dependent on the Rb/E2F pathway [62]. Recent data have implicated E2F4 and E2F5 in G1 arrest in response to the INK family of cdk inhibitors [63]. These two E2F members appear to be involved in the post-mitotic state and differentiation through complexes implicated in transcriptional repression rather than activation [64]. For example, E2F4-p130 complexes are known to be associated with the G0 quiescence stage [65]. Recent studies in differentiated muscle cells have shown that changes in sub-cellular localization of E2F members (likely related to their phosphorylation and association with different co-factors), are involved in maintaining the post-mitotic stage in terminally differentiated myotubes [66]. The expression of cdk inhibitors, in particular p21 [28–30, 47], but also p27 [67] and p18 [68], is up-regulated during myogenesis in developing mouse embryos and in myogenic cell lines, thus explaining how cdks activities are down-regulated. There are two CKI families: the CIP/KIP family with p21, p27, and p57, and the INK family, which includes p15, p16, p18, and p19. The INK family of inhibitors is specific for cdk4 and cdk6 complexes and inhibit kinase activity by binding to the cdk subunit thereby preventing their binding to cyclin D family members. The CIP/KIP class of inhibitors are more promiscuous in that they can inhibit all cdk complexes, but are less potent inhibitors because they only bind the cyclin-cdk complex and need to bind as a homodimer to be inhibitory [69–72]. As mentioned, MyoD induces p21 expression during myogenesis [28–30]. Forced p21 expression in myoblasts is sufficient for cell cycle arrest in mitogenic-rich medium [28]. The mechanisms of induction of p18, p27, and p57 expression during skeletal differentiation are not yet known. However, expression of either p57 or p21 is required for muscle differentiation as shown by double knock out of p57 and p21 which gave the same muscle-deficient phenotype as observed with myogenin(–/–) mice [73]. In addition to p21 [28–30] and Rb [58], MyoD also activates expression of cyclin D3, by a mechanism independent of protein synthesis and which requires the MyoD coactivator p300 [74]. Cyclin D3 is the only G1 cyclin (a family of cyclins normally associated with proliferation) surprisingly found to be up regulated upon myogenic differentiation [75, 76]. It contributes critically to the irreversible cell cycle exit of myoblasts and is found entirely associated with cdk4 and unphosphorylated Rb in myotubes [74].

Expression and activity of the myogenic factor MyoD during the myoblast cell cycle

As differentiating myoblasts permanently withdraw from the cell cycle, there is direct cooperation between MyoD and the blockade to cell cycle progression, as shown by the link between MyoD and induction of p21, Rb, and cyclin D3. When expressed ectopically in a number of cell types, MyoD inhibits the cell cycle before the S phase independently of its DNA binding and the induction of myogenic differentiation [77, 78].

Early investigations noted that dividing myoblasts displayed heterogeneous levels of MyoD [17], but in the absence of an efficient means of synchronizing myoblasts, no link to the cell cycle could be made. Synchronization of growing myoblasts was confronted with the difficulty that when they are moved to low growth factor-containing media, to arrest their growth, a great proportion of myoblasts leaves the cell cycle into differentiation instead of arresting in G0 quiescence, like non-myogenic cells. The use of medium depleted of an essential amino acid, methionine, has overcome this problem and yields accurately synchronized myoblasts [79]. In such synchronously growing myoblasts, expression of the two myogenic factors, Myf5 and MyoD, shows opposite patterns between G0 and S phase entry, with the MyoD protein level peaking in early G1 and dropping to a minimum just before S phase [79] (fig. 1). Confirming these data from artificial synchronization, MyoD and Myf5 also show opposite patterns of expression in myoblasts (and in cultured primary satellite cells) induced to differentiate. Myf5 is absent from all differentiating cells (G1 exit) which express high levels of MyoD and myogenin, whereas it is present in myoblasts which do not differentiate but instead exit their cell cycle into quiescence (G0) and contain neither MyoD nor myogenin [79, 80]. This sub-population of circa 10% undifferentiated quiescent myoblasts, which always arises upon induction of differentiation, shows expression of Myf5 and no MyoD as in synchronized G0 quiescent cells [79]. It is proposed to represent a pool of ‘reserve’ cells [81]. Indeed, when stimulated to grow, these cells will multiply and, when reaching the appropriate density, differentiate and fuse into myotubes, again leaving a subset of myoblasts in a quiescent undifferentiated stage [79, 81]. This sub-population of reserve cells shows a specific up-regulation of the pocket protein p130 (and not of Rb or p107) [82]. In addition, forced expression of p130 in myoblasts not only inhibits their proliferation, as also seen with other Rb family members, but also inhibits their myogenic differentiation, unlike pRb and p107. This inhibition appears to involve a direct effect of p130 on MyoD expression and activity [82] and suggests a role for p130 in establishing the myoblast quiescence stage in ‘reserve’ cells.

In addition to being regulated at the level of its expression along the cell cycle, MyoD is phosphorylated in growing

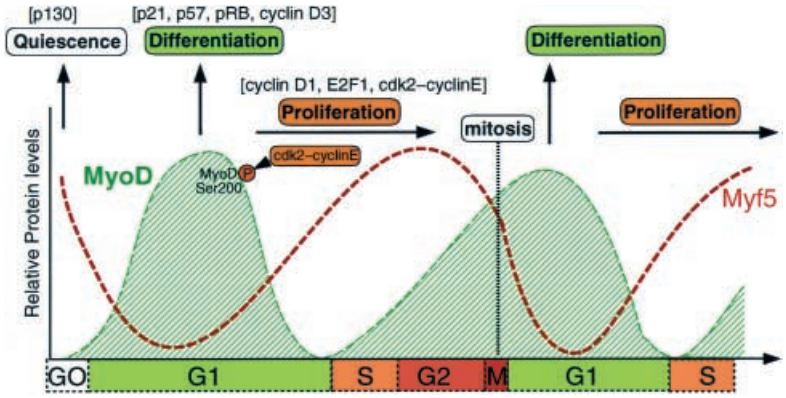

Figure 1. Protein expression profiles for Myf5 and MyoD in the course of the myoblast cell cycle with key stages and factors involved in the choice between continued proliferation, cell cycle exit into differentiation, or quiescence. Shown is a complete myoblast division cycle from G0 quiescence to mitosis, and the following cycle from mitosis to G1/S. The time scale is represented proportionally on the x-axis and takes 20 h from G0 to mitosis. Major factors shown to interfere with MyoD and implicated in sustained proliferation or exit into differentiation or quiescence are indicated between brackets. Shown also is the role of cdk2-cyclin E in phosphorylation of MyoD Ser 200 which triggers ubiquitination-dependent degradation of MyoD at the G1/S transition.

myoblasts, and this phosphorylation diminishes when myoblasts differentiate into myotubes [83]. MyoD was found to be phosphorylated on Ser 200 in growing myoblasts, a phosphorylation which can be carried out by either cdk1 or cdk2 immunoprecipitated from myoblasts, whereas neither cdk4 nor cdk5 are able to phosphorylate MyoD [83]. This phosphorylation of MyoD Ser 200 is involved in regulating MyoD turn-over since the non-phosphorylatable MyoD ala 200 mutant shows a three-to four-fold extended half-life [83, 84]. In addition, the Ala 200 mutant displays clearly increased myogenic activity as measured by myogenic conversion of fibroblasts [83]. In myoblasts, the CIP/KIP class of inhibitors (p57, p21, and p27), and not the INK family p16, were able to specifically enhance transcriptional transactivation promoted by MyoD [85]. This effect could be directly related to inhibition of cdk2-cylin E activity by p57 family inhibitors [86].

Considering the rapid decrease in MyoD protein levels observed in synchronized myoblasts at the transition from G1 to S phase and the known implication of cdk-dependent phosphorylation in ubiquitin-mediated degradation [87–89], these results support a model whereby cdk2-dependent phosphorylation of MyoD is involved in ubiquitin-dependent degradation of MyoD during G1–S. This point was recently proven using roscovitine, a specific inhibitor of cdk2 (which does rot inhibit cdk4) on synchronized myoblasts. Roscovitine, by inhibiting the phosphorylation of MyoD, prevented its degradation normally seen in synchronized myoblasts at the end of G1, and the same effect was also observed using an inhibitor of the proteasome [90]. Because cyclin Dl is essential for the induction of cyclin E [1, 2] the role of cdk2-cyclin E in MyoD phosphorylation for ubiquitination and degradation provides a link to the inhibitory role of cyclin D1 in myogenesis (in addition to its role in Rb phosphorylation). A recapitulation of these regulations interrelating cell cycle progression and the level and activity of the myogenic factor MyoD is represented in figure 1.

Differentiation signaling pathways and MyoD cofactors

Although not the main focus of the present review, it is important to keep in mind that a number of signaling pathway components and cotranscriptional regulators are critically involved in myoblast differentiation, in many cases through stimulation/inhibition of MyoD activity. For example, in contrast to the ability of FGF and TGF-β to inhibit muscle gene expression, insulin-like growth factor (IGF)-I and IGF-II are potent activators of myoblast differentiation. IGF effects are known to pass via the Pi3K to Akt/PKB and to calcineurin-NFAT-mediated pathways but the final targets of this activation are not yet identified. MyoD itself seems to be a potential target for the IGF differentiation signaling pathway, since there is a positive feedback loop between IGF-II and MyoD [91]. Such an IGF-induced pathway may be related to the induction of MyoD expression upon exit from the quiescent state, which is the state of satellite cells in vivo before their activation for regeneration.

In addition, MyoD has been shown to interact with cofactors which positively regulate its activity, such as the MEF2 family or the acetyl transferases p300/CBP and PCAF. While MEF2s and PCAF are more involved in cooperative induction of myogenic differentiation after cell cycle withdrawal, p300 is also implicated in the initiation of cell cycle arrest by MyoD [92]. However, p300/CBP acetyl transferase activity appears to be cell cycle enhanced through cdk2-cyclin A-mediated phosphorylation and therefore positively linked to proliferation [93]. Indeed, the role of p300 in myogenic differen-

tiation is independent of its acetyl transferase activity, and involves targeting PCAF for interaction with MyoD [94] and subsequent MyoD acetylation, recently shown to play an important role in MyoD activity [95].

These examples emphasize a complementary effect on MyoD possibly played by molecules that are not directly cell cycle related, but that may contribute to the fine regulation of MyoD in the course of myoblast cell cycle progression or withdrawal.

Concluding remarks

Gene knock-out studies of MyoD and Myf5 have provided useful information on the implication of these two MRFs during embryogenesis, and show that they can compensate for each other in the specification of muscle lineage [96]. In contrast, the specific role of MyoD at adult stages was less easily identified simply from knock-out studies and took 4 more years [31]. This illustrates some of the limitations of knock-out technology and the need to complement such an approach with 'ex vivo' studies. With respect to embryogenic myogenesis, MyoD can compensate for the absence of Myf5, whereas in the post-natal stage, the unique role of MyoD in the process of muscle regeneration cannot be substituted by Myf5 [31]. These features indicate that MyoD expression and activity must be under different regulatory pathway control during development and the adult stage. This point should be kept in mind when considering MyoD expression and activity in relation to the cell cycle; the studies and regulatory processes described here relate mostly to myogenic cell lines and primary cultures from adult muscle. Indeed, the existence of distinct regulatory pathways seems to be reflected in the promoter of MyoD where the 'core enhancer' (528 bp at –20 kb) appears involved in MyoD expression during embryogenic myogenesis [97, 98], whereas the distal regulatory region (DRR; –5 kb) together with the proximal region (PRR) [99] could be more implicated in the post-natal expression of MyoD. The specific role and regulation of MyoD in adult muscle cells can only be clarified in light of all the 'in vitro' studies performed or satellite cells and derived cell lines, such as described in this review. Recent papers have described defects in differentiation and at the proliferation-differentiation transition of satellite cells derived from adult MyoD –/– mice [100, 101]. Linking such studies to further investigation of the promoter regions of MyoD will be useful.

Several mechanisms can account for the clear anti-proliferative effect of MyoD and the requirement for tight regulation of MyoD levels to proceed through the cell cycle. First, MyoD was shown to bind the family of CIP/KIP cdk inhibitors: p21, p27, and p57 [85]. Such binding may cooperate in their homodimerization, resulting in their increased level and subsequent inhibition of cdks. Second, through its interaction with Rb, [49] and/or cdk4 (if proven relevant between members from the same or homologous species) [51, 52], MyoD may interfere with Rb phosphorylation and E2F1 induction and thereby counteract the NF-κB pathway which was recently shown to induce an increase in cyclin D1 expression [102]. Indeed MyoD–/– myoblasts cultured from knock-out mice display clearly increased cyclin D1, D2, and E levels, and these levels remain high after mitogen withdrawal [M. Parker and M. Rudnicki, Ottawa Hospital Research Institute, personal communication]. Given the possibility of accurate synchronization of proliferative myoblasts, it will be of interest to compare in wild-type and MyoD–/– myoblasts, the cell cycle profile of key participants in myoblast cell cycle exit (p21, p57, cyclin D3, Rb), as well as MyoD inhibitors such as cyclin D1, or the nuclear repressor N-CoR1 [103].

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