The cyclin-dependent kinase inhibitor p27Kip1 is stabilized in G0 by Mirk/dyrk1B kinase.

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Elevated levels of the cyclin-dependent kinase (CDK) inhibitor p27 block the cell in G0/G1 until mitogenic signals activate G1 cyclins and initiate proliferation. Post-translational regulation of p27 by different phosphorylation events is critical in allowing cells to proceed through the cell cycle. We now demonstrate that the arginine-directed kinase, Mirk/dyrk1B, is maximally active in G0 in NIH3T3 cells, when it stabilizes p27 by phosphorylating it at Ser-10. The phospho-mimetic mutant p27-S10D was more stable, and the non-phosphorylatable mutant p27-S10A was less stable than wild-type when expressed in G0-arrested cells. Following phosphorylation by Mirk, p27 remains a functional CDK inhibitor, capable of binding to CDK2. Mirk did not induce the translocation of p27 from the nucleus in G0, but instead co-localized with nuclear p27. Depletion of Mirk by RNA interference decreased the phosphorylation of p27 at Ser-10 and the stability of endogenous p27. RNAi to Mirk increased cell entry from G0 into G1, as shown by increased expression of proliferating cell nuclear antigen and decreased expression of p27. These data suggest a model in which Mirk increases the amount of nuclear p27 by stabilizing it during G0 when Mirk is most abundant. Mitogen stimulation then causes cells to enter G1, reduces Mirk levels (Deng, X., Ewton, D., Pawlikowski, B., Maimone, M., and Friedman, E. (2003) J. Biol. Chem. 278, 41347–41354), and initiates the translocation of p27 to the cytoplasm. In addition, depletion of Mirk by RNAi, in postmitotic C2C12 myoblasts decreased protein but not mRNA levels of p27, suggesting that stabilization of p27 by Mirk also occurs during differentiation.

The cyclin-dependent kinase inhibitor p27 (1, 2) plays a critical role in regulating progression through the cell cycle. Rising levels of p27 block the cell in G0/G1 until mitogenic signals activate G1 cyclins and initiate proliferation. Post-translational regulation of p27 by different phosphorylation events is critical in allowing cells to proceed through the cell cycle. CDK2/cyclin E phosphorylates p27 at Thr-187 (3, 4) and promotes its degradation by the ubiquitin-proteasome pathway at the G1/S checkpoint (5). Mitogenic signals also induce the kinase KIS, which mediates phosphorylation of p27 at Ser-10 (6), facilitating the translocation of p27 to the cytoplasm at later times in G1 (7, 8). However, p27 has been shown to be already phosphorylated at Ser-10 in G0/early G1 before KIS induction (7, 8). Furthermore, p27 is solely nuclear in G0 and does not even bind to its exportin CRM1 until cells enter G1, following mitogen stimulation (9), implying that phosphorylation at Ser-10 may fulfill another function. In this study we identify the arginine-directed serine/threonine kinase Mirk/dyrk1B as a kinase active in G0, which phosphorylates p27 at Ser-10 without inducing its translocation from the nucleus.

Mirk/dyrk1B is a member of the Dyrk/minibrain family of arginine-directed serine/threonine kinases. Mirk is transcriptionally up-regulated by Rho family members (10) and itself functions as a transcriptional activator (11) under control of the MKK3/p38 MAPK signaling system (11, 12). Mirk is expressed at elevated levels in some tumor cells and in normal skeletal muscle (13) where it is active in muscle differentiation (10). However, Mirk is widely expressed at low levels, suggesting it performs some general role. In the current study we demonstrate a novel function for Mirk, stabilization of the CDK inhibitor p27 kip1 during the G0 phase of the cell cycle.

EXPERIMENTAL PROCEDURES

Materials—Antibody to p27(Ser10) was from Transduction Laboratories; antibodies to PCNA,1 Cdk2, β-tubulin, and cyclin A were from Santa Cruz Biotechnology, and antibody to the FLAG-epitope was from Sigma. Antibody to the Ser-10-phosphorylated form of p27 was purchased from Zymed Laboratories Inc.. Affinity-purified rabbit polyclonal antibody to a unique sequence at the C terminus of Mirk was raised as described (13). In some experiments affinity-purified rabbit polyclonal antibody to the unique N terminus of Mirk was used (13). GST-ERK2 was purchased from Upstate Biotechnology, Inc. Polyvinylidene difluoride transfer paper Immobilon-P was purchased from Millipore. PLUS reagent and LipofectAMINE were from Invitrogen. All radioactive materials were purchased from PerkinElmer Life Sciences, ECL reagents from Amersham Biosciences, and tissue culture reagents from Mediatech (Fisher). All other reagents were from Sigma.

Cell Culture—C2C12 mouse myoblasts and NIH3T3 cells were obtained from the ATCC. NIH3T3 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. C2C12 cells were maintained in growth medium: Dulbecco’s modified Eagle’s medium containing 20% fetal calf serum, and switched to differentiation medium/Dulbecco’s modified Eagle’s medium containing 2% horse serum. NIH3T3 and C2C12 cells were transfected with LipofectAMINE/PLUS according to the supplier’s manual.

Plasmids—pcDNA-Mirk, pcDNA-MirkYF, and pcDNA-MirkKR (kinase-inactive Mirk) had been generated previously (13), pGEX2T p27 wild-type, pGEX2T p27-NADA, pGEX2T p27-VPKK, and pGEX2T p27-NADA/VPKK were the kind gift of Dr. B. Amati (14). Wild-type p27(133) (pGEX2T-p27) was mutated by site-directed mutagenesis (Gene-Editor system from Promega) and subcloned into pCMV-tag2 (Stratagene) to make FLAG epitope-tagged constructs. All mutant p27 constructs were sequenced to confirm the mutated sequence. RNA sequences to murine Mirk were s1, GACCTACAGAGCATGATT, and s13, CAGAGCTACCGTACAG, whereas another sequence mutant at two positions to

1 The abbreviations used are: PCNA, proliferating cell nuclear antigen; EGFP, enhanced green fluorescent protein; DTT, dithiothreitol; PBS, phosphate-buffered saline; GST, glutathione S-transferase; CDK, cyclin-dependent kinase; HNF1α, hepatocyte nuclear factor 1α.

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murine Mirk, si2, GCTCTCTGGACCTCA, served as the mutant control. Each was inserted into the pSilencer plasmid (Ambion).

Phosphatase Treatment of Phosphorylated p27—FLAG-tagged p27 was immunoprecipitated from total cell lysates, and the immunocomplex was treated with 1 unit of alkaline phosphatase (Promega) in the presence of protease inhibitors (15).

In Vitro Kinase Assay—The immunoprecipitates were washed five times with lysis buffer and then three times with kinase buffer (50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 0.5 mM DTT), incubated for 15 min at 30 °C with 20 μl of kinase buffer containing 50 μM cold ATP plus 5 μCi of [γ-32P]ATP and 1 μg of purified recombinant p27 protein as substrate, and then analyzed by PAGE and autoradiography. TN T products were produced as detailed previously (13).

FIG. 1. Mirk is a G0 kinase that phosphorylates the CDK inhibitor p27. A, cell cycle-dependent expression of Mirk. NIH3T3 cells were maintained at confluent density for 3 days and then replated at 1/3 density in fresh medium. Western blotting (WB) for cyclin A and Mirk was performed on parallel cultures at the times indicated. B, phosphorylation of p27 by Mirk at various points in the cell cycle. NIH3T3 cells were synchronized in G0/G1 (14), a p27 construct mutant at its conserved C-terminal CDK substrate, and then analyzed by PAGE and autoradiography (AR). Western blotting for Mirk and p27 was performed on the immunoprecipitates, and for cyclin A on the total cell lysates (lower panel). IVK, in vitro kinase; CB, Coomassie Blue.

The secrete/threonine kinase Mirk/dyrk1B is widely expressed at low levels in normal tissues (13), suggesting that Mirk may play a general role in either cell physiology or cell growth. In addition, Mirk protein levels are known to vary widely in response to treatment of cells both with mitogens and with anti-proliferative stimuli (13), so we determined whether Mirk exhibited any regulation within the cell cycle. NIH3T3 cells arrest in G0/G1 when grown to confluence and move into S phase in a synchronous wave 10–14 h after release (17). Protein levels of cyclin A were used to monitor cell cycle progression through late G1 to S. Mirk protein levels were elevated in density-arrested NIH3T3 cells, and these levels fell sharply when cells transited late G1 and entered S phase (Fig. 1A). Thus Mirk levels were elevated in G0/G1, and were reduced in cycling cells. Levels of the CDK inhibitor p27 are elevated when cells reach confluence and arrest in G0 (18, 19), the cell cycle phase in which Mirk activity was greatest, so we tested the hypothesis that Mirk phosphorylated p27 in G0. Mirk was immunoprecipitated from NIH3T3 growth-arrested cells and from cells at various times after release, and then its capacity to phosphorylate exogenous p27 was determined by an in vitro kinase assay. The greatest phosphorylation of p27 by Mirk was seen in cells in G0/G1 concordant with the highest Mirk levels 0–6 h post-release (Fig. 1B). At later time points, cells had undergone the G1/S transition as shown by the increase in cyclin A, and the levels and the capacity of Mirk to phosphorylate p27 decreased.

Mirk Phosphorylates p27 in Vivo and in Vivo at Ser-10—We next determined the specific site at which Mirk phosphorylated p27. Recombinant Mirk is a constitutively active kinase (13), so its capacity to phosphorylate p27 was assayed using in vitro kinase assays. Mirk phosphorylated the CDK inhibitor p27, with kinase-inactive YF-Mirk serving as the negative control (Fig. 2). In contrast, recombinant p38α and JNK1 had little kinase activity on p27 (data not shown). CDK2 phosphorylation at Thr-187 initiated the ubiquitination and rapid degradation of p27 by the proteasome (3, 4), so we tested whether mutation of the CDK-binding site, the Thr-187 phosphorylation site, or the Ser-10 site, which facilitates the translocation of p27 to the cytoplasm (8, 20), altered Mirk activity. The double mutant p27-F62A/F64A, a construct deficient in interaction with CDKs (14), a p27 construct mutant at its conserved C-terminal CDK target site (T187V), and a triple mutant (F62A/F64A/T187V)
were each phosphorylated by Mirk to a similar extent as wild-type p27 (Fig. 2), indicating these sites were irrelevant to Mirk action in vitro. Moreover, Mirk is a highly selective p27-S10 kinase. Mirk was unable to phosphorylate p27-S10A, whereas it phosphorylated wild-type p27, p27-S178A, and the p27 CDK phosphorylation site mutant T187V to a similar extent in in vitro kinase reactions (Fig. 3). In contrast, ERK2 phosphorylated each of the mutant p27 constructs to an equal extent, somewhat less than wild-type p27. These results show that Mirk has a very defined substrate requirement for the Ser-10 residue in p27 and suggest that Mirk is at least one of the kinases responsible for phosphorylation at Ser-10 in vivo.

Ser-10 is the major phosphorylation site for p27 in vivo, accounting for about 70% of the total amount of p27 phosphorylation as determined by metabolic labeling with [32P]orthophosphate (7). Phosphorylation of p27 at Ser-10 has been shown, by use of a p27-S10D phospho-mimetic mutant, to facilitate p27 translocation to the cytoplasm in response to mitogens, whereas mutation of Ser-10 to alanine prevents export to the cytoplasm (8, 20). In G0, p27 is solely nuclear and does not even bind to its exportin CRM1 until cells enter G1 following mitogen stimulation (9). These studies indicate that phosphorylation of p27 at Ser-10 is a major control point in the cellular mitogenic response. The mitogen-induced kinase KIS has been identified as one kinase that mediates phosphorylation of Ser-10 in vivo (6). However, p27 has been shown to be phosphorylated already at Ser-10 to some extent in G0/early G1 before KIS induction (7, 8). Mirk activity is highest in density-arrested NIH3T3 cells in G0 (Fig. 1), and it is rapidly down-regulated by serum mitogens (13). These properties suggest that Mirk could phosphorylate p27 at Ser-10 during G0, before mitogen induction of KIS.

To confirm that Mirk phosphorylates p27 in vivo, either wild-type or kinase-inactive KR-Mirk was co-transfected with either wild-type or p27-S10A FLAG epitope-tagged expression constructs into NIH3T3 cells. Following expression, the constructs were labeled for 16 h with 32P, and then the 32P-labeled p27 was immunoprecipitated with anti-FLAG antibody and analyzed by PAGE and autoradiography. The amount of 32P incorporated into wild-type p27 was severalfold higher when wild-type Mirk was co-expressed (Fig. 4, 3rd lane), whereas little incorporation was seen when wild-type p27 was co-expressed with kinase-inactive Mirk or vector control. In contrast, co-expression of wild-type Mirk did not increase the low basal level of 32P incorporated into mutant p27-S10A (Fig. 4, last 3 lanes). Western blotting for transfected p27 using anti-FLAG antibody demonstrated equal expression of all constructs (Fig. 4, lower lanes). These metabolic labeling data confirm that Mirk phosphorylates p27 in vivo at Ser-10.

Phosphorylation can impede protein mobility on SDS-PAGE. Mirk phosphorylation of p27 in vivo reduced its electrophoretic mobility. Constructs for wild-type p27 and either wild-type Mirk, kinase-inactive YF-Mirk, or the pcDNA(HisA) vector were co-transfected into NIH3T3 cells and allowed to express, and the mobility of p27 was determined by Western blotting (Fig. 5A, 1st 3 lanes). Only the p27 co-transfected with wild-type Mirk exhibited a decrease in mobility. When wild-type Mirk was co-expressed with the mutant p27 constructs S10A, T170A, S178A, or T187V, all of the mutants except S10A exhibited the mobility shift. The p27-T187V construct is itself a little longer than wild-type p27 so its shift was confirmed by co-transfection with the HisA epitope-tagged vector (Fig. 5, last 3 lanes on right).

Phosphatase treatment demonstrated that the p27 mobility shift was caused by phosphorylation (Fig. 5B). Immunoprecipitated FLAG epitope-tagged p27 constructs (wild-type or S10A) were incubated with calf intestinal alkaline phosphatase before Western blotting. Treatment with the phosphatase eliminated the p27 mobility shift induced by co-transfected wild-type Mirk, thus demonstrating definitively that Mirk phosphorylates p27 in vivo solely at Ser-10.

p27 Phosphorylated at Ser-10 by Mirk Binds to CDK2—We next determined whether p27 phosphorylated at Ser-10 could function as a CDK inhibitor. FLAG-p27 and Mirk were co-expressed in NIH3T3 cells. Following phosphorylation by Mirk in vivo, p27 was still capable of binding to CDK2 (Fig. 6A). The FLAG-p27 bound to immunoprecipitated CDK2 exhibited decreased mobility on SDS-PAGE following co-expression with Mirk (Fig. 6A, arrows), indicating that it had been phosphorylated. The equal amount of immunoglobulin heavy chain (H-chain) indicates equal amounts of antibody were found in the immunoprecipitates. These data demonstrate that p27 phosphorylated by Mirk in G0 was still capable of functioning as a CDK inhibitor.

Mirk Phosphorylation of p27 Blocked by RNAi to Mirk—The capacity of endogenous Mirk to phosphorylate p27 at Ser-10
was further evaluated by expression of small interfering RNA to Mirk using the pSilencer vector. Two sequences within the Mirk coding region were targeted to murine Mirk sequences and termed si1 and si3. For the RNAi control, a mutant sequence si2 was used. NIH3T3 cells were co-transfected with wild-type pSilencer expressing si1 or si3, and lysates were immunoprecipitated with anti-FLAG antibody and collected with protein G-agarose. The immunoprecipitates were analyzed by SDS-PAGE and autoradiography and then Western-blotted (WB) for the FLAG epitope.

**Fig. 5. In vivo phosphorylation of co-transfected p27 by Mirk decreases p27 mobility.** A, NIH3T3 cells were co-transfected with wild-type (WT) FLAG-p27 or mutant FLAG-p27—S10A into NIH3T3 cells. The p27-T187V construct was slightly longer in the C terminus than wild type, as shown by DNA sequencing, and displayed shorter arrow mobility, and the shorter arrow indicates expression in complete medium. B, Western blotting of WB) to the FLAG epitope. The long arrow indicates p27 with normal mobility, and the shorter arrow indicates p27 with reduced mobility due to phosphorylation. (C), indicates expression in complete medium. B, phosphatase treatment. NIH3T3 cells were co-transfected with wild-type pEGFP construct and then analyzed by Western blotting. The amount of antibody used in the immunoprecipitates was identical as shown by the abundance of heavy chains in each lane.

**Phosphorylation of p27 at Ser-10 by co-transfected Mirk.** Mirk, kinase-inactive KR-Mirk, or HisA pcDNA vector were co-transfected with either wild-type (WT) FLAG-p27 or mutant FLAG-p27—S10A into NIH3T3 cells. Cells were labeled for 16 h with [32P]orthophosphate, and then lysates were immunoprecipitated with anti-FLAG antibody and collected with protein G-agarose. The immunoprecipitates were analyzed by SDS-PAGE and autoradiography and then Western-blotted (WB) for the FLAG epitope.
FIG. 6. Phosphorylation of p27 at Ser-10 by Mirk stabilizes p27, allows binding to CDK2, and is blocked by RNAi to Mirk, which enhances mitogen-free cell cycling. A, p27 phosphorylated by Mirk in vivo remains capable of binding to CDK2. NIH3T3 cells were co-transfected with wild-type FLAG-p27 together with wild-type Mirk or vector control, immunoprecipitated (IP) with antibody to CDK2, and the amount of FLAG-p27 associated with CDK2 assayed by Western blotting (WB) the immunoprecipitates. The amount of immunoglobulin heavy chain (H-chain) by cross-reactivity in the immunoprecipitates is shown as an internal control. The lowest panel shows the input amount of FLAG-p27, as determined by Western blotting the lysates for the FLAG epitope. B, depletion of endogenous Mirk by two different RNAi sequences decreases phosphorylation of p27 at Ser-10. NIH3T3 cells were co-transfected for 16 h in serum-free medium with the pSilencer vector encoding RNAi sequences si1 or si3, or mutant control si2, pcDNA-EGFP (10:1 ratio), and FLAG-p27. After an additional 24 h, lysates were examined by Western blotting for Mirk, p27, and tubulin as a blotting control, and the phosphorylated form of p27-S10. C, depletion of endogenous Mirk by RNAi enhances cell cycling. NIH3T3 cells were transfected for 24 h in serum-free medium with the pSilencer vector encoding either si1 or control, and then medium was changed to serum-free and culture continued for 48 h. Lysates were examined by Western blotting for Mirk, p27, PCNA, or β-actin. After normalization to β-actin, the percent of the proteins compared with control values were calculated (mean ± S.E., n = 3). Transfection efficiency, as monitored by co-transfection with EGFPP, was equivalent in si1-treated and control cultures and averaged 30%. D, the amount of Mirk normalized to tubulin and the amount of p27 phosphorylated at Ser-10 to total p27 from the experiment shown in B and a duplicate experiment are shown (±S.D. if >5%). E, exogenous p27 is stabilized by phosphorylation at Ser-10. NIH3T3 cells were co-transfected for 16 h with wild-type (WT) FLAG-p27, FLAG-p27-S10A, or FLAG-p27-S10D and then cultured for 2 days in serum-free medium with cycloheximide added at 50 μg/ml for the last 0–12 h, as noted. The abundance of the FLAG epitope normalized to tubulin is shown for two experiments. The mean ± S.D. for the values shown is 4%. F, endogenous p27 is stabilized by phosphorylation at Ser-10. NIH3T3 cells were co-transfected for 16 h in serum-free medium with the pSilencer vector encoding the RNAi, sequence si1 and pcDNA-EGFP (10:1 ratio). After an additional 24 h, lysates were examined by Western blotting for Mirk, endogenous p27, and tubulin as a blotting control.
ating C2C12 myoblasts using RNA interference prevented the induction of myogenin and various contractile proteins and blocked the fusion of myoblasts into myocytes. We found that reduction of Mirk levels by RNA interference in differentiating myoblasts caused a 13-fold decrease in p27 protein levels (Fig. 8) with only a 20% decrease in p27 mRNA levels (not shown). The minimal effect of Mirk on p27 mRNA levels was also seen by microarray analysis (data not shown) and confirmed our earlier results (21), which showed that Mirk did not modulate the activity of a p27 promoter construct. These data indicated that Mirk plays a post-translational role in maintaining p27 stability in post-mitotic myoblasts as well as NIH3T3 cells.

**DISCUSSION**

The current study resolves some apparently conflicting experimental results in the literature. It was clearly demonstrated previously (7) that phosphorylation of p27 at Ser-10 confers increased stability on p27, data which we have duplicated in the current study using NIH3T3 cells arrested in G0. Other investigators (8, 9) have presented equally strong data that phosphorylation of p27 at Ser-10 is required for p27 to bind to the exportin CRM1 and to be translocated to the cytoplasm during G1 where p27 is proteolyzed by the proteasome, and thus shows less stability. Our results indicate that phosphorylation of p27 at Ser-10 has two outcomes which are cell cycle-dependent. Phosphorylation of p27 at Ser-10 during

**FIG. 7. Co-localization of Mirk and p27.** Mirk-EGFP or kinase-inactive YF-Mirk-EGFP were transfected with either p27-DeRed or p27-S10A-DeRed into serum-starved NIH3T3 cells for 16 h and allowed to express in serum-free medium for 24 h. Fluorescent photomicrographs of Mirk alone and p27 alone were made using a simultaneous collection camera, and a merged green/red fluorescence photomicrograph was made. Fluorescence was visualized on a Nikon Eclipse E800 fluorescent microscope (Melville, NY). Images were captured with a Hamamatsu ORCA-ER digital camera (Bridgewater, NJ) and processed with Simple PCI and Adobe Photoshop 5.5 software.

**FIG. 8. RNAi to Mirk decreases p27 abundance in postmitotic differentiated myoblasts.** C2C12 cells were co-transfected with an expression plasmid for Mirk RNAi and EGFP, or vector DNA and EGFP, selected by cell sorting for EGFP, placed in growth medium for 1 day, and then switched to differentiation medium for 2 days to induce Mirk that is expressed at very low levels in proliferating myoblasts. Vc, vector; si, small interfering RNA to Mirk. Cell lysates were examined by Western blotting for Mirk and p27**

$^{10}$**NS, nonspecific cross-reacting protein to demonstrate equal loading and transfer.

$^{10}$**G$_0$ by Mirk/dyrk1B stabilizes p27 and maintains p27 within the nucleus where it can bind to CDK2. In contrast, phosphorylation of p27 at Ser-10 by the KIS kinase in G$_1$ enables p27 to bind to CRM1 and to be transported into the cytoplasm for destruction (6). In support of our conclusions, other investigators (9) have clearly shown that association of p27 with
CRM1 is minimal in G0 and increases markedly during the G1 to S phase progression.

Why are two different kinases needed to perform the same phosphorylation? Mirk and KIS exhibit markedly different transcriptional regulation so they do not occupy the same phase of the cell cycle. Cellular mitogens transcriptionally down-regulate Mirk/dyrk1B (10), whereas mitogens induce the transcription of KIS (6). Mirk levels and activity are highest in G0 (this study) and decline during G1 when KIS levels and activity are rising. Therefore, KIS is not present during G0 to phosphorylate p27. The current study has shown that p27 is not transported to the cytoplasm even after phosphorylation at Ser-10 by Mirk. Thus phosphorylation at Ser-10 is not sufficient to enable p27 to bind to CRM1 during G0. Possibly Mirk and p27 occupy a different part of the nucleus than CRM1 during G0. Mirk is found in 670-kDa complexes within the nucleus together with the Ran-binding protein RanBPM (12, 22). Jab1 functions as an adaptor between CRM1 and p27 (23). The Mirk-containing nuclear complexes have not been completely characterized, but we found in an earlier study that they do not contain Jab1 (22), which is found within the 450-kDa signalosome and smaller complexes (22, 23).

The serine/threonine kinase Mirk/dyrk1B is a RhoA-induced gene that functions in muscle differentiation (10) but is widely expressed at low levels, suggesting that it participates in some general cellular control mechanisms. In the current study we have identified one such Mirk function, phosphorylation of p27 to assist the maintenance of the cell in G0. Other members of the Dyrk/minibrain family include Dyrk1A, which has been implicated in neuronal development through its phosphorylation of cAMP-response element-binding protein (24, 25). Dyrk3 functions in late erythroid progenitor cells to inhibit programmed cell death through activation of the cAMP-response element response pathway (26, 27). The related kinase HIPK2 promotes the pro-apoptotic transcriptional function of the tumor suppressor protein p53 (28, 29).

Although found at only low levels in most normal tissues, Mirk is expressed at high levels in skeletal and cardiac muscle (13, 30). The role of Mirk in muscle differentiation is likely to be multifactorial, involving broad, indirect effects of Mirk acting as a transcriptional activator, as well as more direct effects of Mirk functioning as a substrate-specific kinase. Mirk has been shown to function, in a kinase-dependent manner, as a co-activator of the transcription factor hepatocyte nuclear factor 1α (HNF1α) (11). Mirk binds to HNF1α through its cofactor, DCoH, which binds as a dimer to the unstable HNF1α dimer, thus enabling effective binding of the tetrameric complex to DNA. We speculate that Mirk may act as a co-activator of one of the transcription factors for the myogenin gene. We have also reported recently (22) that Mirk functions to inhibit cell migration. This study identifies an additional function of Mirk/dyrk1B, specific phosphorylation of the CDK inhibitor p27 at Ser-10. p27 acts as a brake on the proliferation program. Mirk phosphorylation of p27 may assist differentiating myoblasts to arrest in G0 by stabilizing p27.

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