Mirk/Dyrk1B is an arginine-directed serine/threonine protein kinase that is expressed at low levels in most normal tissues but at elevated levels in many tumor cell lines and in normal skeletal muscle. Colon carcinoma cell lines stably overexpressing Mirk proliferated in serum-free medium, but the mechanism of Mirk action is unknown. DCoHm (dimerization cofactor of hepatocyte nuclear factor 1α from muscle), a novel gene of the DCoH family with 78% amino acid identity to DCoH, was identified as a Mirk-binding protein by yeast two-hybrid analysis and cloned. Mirk co-immunoprecipitated with DCoHm and bound to DCoHm in glutathione S-transferase pull-down assays. DCoHm stimulates HNF1α-dependent transcription and enhances its transcriptional activity on the β-fibrogen promoter reporter, and DCoHm had similar activity. Mirk enhanced HNF1α transcriptional activity in a dose-dependent manner, whereas two kinase-inactive Mirk mutants and a Mirk N-terminal deletion mutant did not. Mirk, DCoHm, and HNF1α formed a complex. Mirk bound to a specific region within the CREB-binding protein-binding region of HNF1α and phosphorylated HNF1α at a site adjacent to the Mirk-binding region. Conversely, the HNF1α binding domain was located within the first five conserved kinase subdomains of Mirk. Mirk co-immunoprecipitated with the MAPK kinase MKK3, an upstream activator of p38. MKK3 enhanced Mirk kinase activity and the transcriptional activation of HNF1α by Mirk, suggesting that Mirk, like p38, is activated by certain environmental stress agents. The Mirk-binding protein DCoH has been shown to be selectively expressed in colon carcinoma but not in normal tissue. Mirk may function as an HNF1α transcriptional activator in response to an MKK3-mediated stress signal, and the selective expression of DCoH could restrict the Mirk response to carcinoma cells.

Mirk/Dyrk1B is a serine/threonine protein kinase that is expressed at elevated levels in normal skeletal muscle and certain carcinoma cell lines and at low levels in many normal tissues (1). Colon carcinoma cell lines stably overexpressing Mirk proliferated in serum-free medium (1), but the mechanism of Mirk action that enabled this survival capacity is unknown. Mirk is a member of the Dyrk/minibrain family of dual specificity, tyrosine-regulated, arginine-directed protein kinases (2–4) and is identical to Dyrk1B (5). Mirk/Dyrk1B and the related kinase Dyrk1A exhibit 54% amino acid identity with 90% identity or homology within the conserved kinase domain. Several lines of evidence indicate that Dyrk1A mediates neuronal differentiation. Dyrk1A has been mapped to the Down’s syndrome critical region of chromosome 21, overexpression of Dyrk1A has been found in the Down’s syndrome fetal brain (6), and transgenic mice overexpressing Dyrk1A exhibit cognitive deficits and motor abnormalities characteristic of Down’s syndrome (7). Dyrk1A has been shown to phosphorylate the cAMP-response element-binding protein (CREB) in vivo, leading to the stimulation of subsequent CAMP response element-mediated transcription during neuronal differentiation in hippocampal progenitor cells (8), an activity consistent with its likely mechanism of neuronal maturation in vivo. Because Mirk/Dyrk1B was expressed at elevated levels in normal muscle tissue, we screened a muscle cell cDNA library to detect possible Mirk targets and found that Mirk/Dyrk1B activated the transcription factor HNF1α (hepatocyte nuclear factor 1α) and bound to its cofactor DCoH.

EXPERIMENTAL PROCEDURES

Materials—Antibodies to MKK3 and HNF1 were from Santa Cruz Biotechnology, and antibodies to the flag-epitope were from Sigma. Rabbit polyclonal antibody to a unique sequence at the C terminus of Mirk was raised as described (1). Polyvinylidene difluoride transfer paper Immobolin-P was purchased from Millipore. PLUS reagent and LipofectAMINE were from Invitrogen, all radioactive materials were purchased from PerkinElmer Life Sciences, and ECL reagents were from Amersham Biosciences. All other reagents were from Sigma. We thank Stephen E. Mercer of this institution for GST-Mirk preparations.

Cell Culture—NIH3T3 cells and 293T cells were maintained in Dulbecco’s modified Eagle’s medium containing 7% fetal bovine serum and modified and supplemented as described (9).

Plasmids—pHX9F (Mirk) and pHX9F (kinase-inactive YF Mirk) had been previously generated (1), and all other Mirk expression plasmids, including the pGBRT7-Mirk construct used for the yeast two-hybrid screening, were prepared by Dr. Xiaobing Deng. The (β-28)-luciferase plasmid encoding three tandem repeats of the β-fibrogen HNF1α binding domain in front of a TATA box promoter and a luciferase reporter gene, the expression plasmid pBluescript-DCoH, and the expression plasmid pBluescript-HNF1α were the kind gifts of Dr. G. Crabtree, Stanford University; FLAG-MKK3b and MKK3b(E), each in pcDNA3, were the kind gifts of Dr. J. Han, Scripps Institute. A human muscle cDNA library subcloned into the pACT2 vector was purchased from CLONTECH.

Yeast Two-Hybrid Screening—We are grateful to Dr. David Amberg of this institution for help with the two-hybrid screening. For the bait and a tablet of protease inhibitor; TBST, 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.1% Tween 20; CBP, CREB-binding protein.

* X. Deng, manuscript in preparation.
construct, wild-type full-length Mirk cDNA was subcloned into the pGBK7T vector, because Mirk had no transcriptional activity by itself. The yeast host strain, AH109, contains three different reporter genes that are tightly controlled by the upstream activator sequence, which required GAL4 binding for expression of the reporter gene. AH109 cells were co-transformed with pGBK7T-Mirk and pACT2 muscle cDNA library according to the manufacturer’s protocol. The transformants were plated on -Ade/-His/-Leu/-Trp/X- medium, including 25 mM 3-amino-1,2,4-triasol to screen for the expression of His-3, and incubated at 30 °C for 7–10 days. Subsequently, His+ colonies were replated on -Ade/-His/-Leu/-Trp-X-agar medium, and blue colonies were selected as positive candidates. The plasmids from candidate colonies were harvested as described above. Cells were grown in 5 ml of selective media overnight and collected by pelleting. The pellets were resuspended in 200 µl of lysis buffer and then incubated at 37 °C for 30–90 min after brief vortexing. Lysis buffer consisted of a mixture of 5 ml of SC buffer, 60 µl of 100T zymolyase (10 mg/ml), and 10 µl of β-mercaptoethanol. Following the addition of 400 µl of 0.2 × NaOH/1% SDS solution the lysates were incubated on ice for 5 min and then pelleted, and the supernatants were decanted into fresh tubes, and then redissolved in 50 µl of SDS solution. The lysates were incubated on ice for 5 min and then pelleted, and the supernatants were decanted into fresh tubes and then redissolved in 50 µl of SDS solution. The lysates were incubated on ice for 5 min and then pelleted, and the supernatants were decanted into fresh tubes and then redissolved in 50 µl of SDS solution. The lysates were incubated on ice for 5 min and then pelleted, and the supernatants were decanted into fresh tubes and then redissolved in 50 µl of SDS solution. The lysates were incubated on ice for 5 min and then pelleted, and the supernatants were decanted into fresh tubes and then redissolved in 50 µl of SDS solution. The lysates were incubated on ice for 5 min and then pelleted, and the supernatants were decanted into fresh tubes and then redissolved in 50 µl of SDS solution.

**Immunodetection**—Following treatment as indicated and washing with cold phosphate-buffered saline, cells were lysed in EBC or radio-immune precipitation buffer (1% phosphate-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and the following protease inhibitors: 20 µg/ml leupeptin, 20 µg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, 200 µM sodium orthovanadate, and 20 mM sodium fluoride). Lysates were pelleted in a microfuge for 15 min to remove insoluble material. Depending upon the experiment, 10–50 µg of cell lysate were blotted onto polyvinylidene difluoride membranes after application on SDS-PAGE. The blots were blocked in 5% milk in TBST for 1 h at room temperature, incubated for 1 h at room temperature with primary antibody in TBSST buffer-5% milk, and proteins were subsequently detected by enhanced chemiluminescence. All Mirk blots used an affinity-purified polyclonal antibody directed to the Mirk unique C terminus. Band density in autoradiograms was measured using a Lacie Silverscan and Silverscan III software and analyzed by the IP Lab Gel program.

**GST Pull-down Experiments**—Mirk and DCoHm were produced in vitro by a coupled transcription and translation system (Promega) using 1 µg of plasmid per reaction. Translation took place in the presence of 2 µl of 10 µl of [35S]methionine. The labeled Tnt proteins and the GST-fusion proteins were incubated together in binding buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, pH 8.0, and 0.1% Nonidet P-40) overnight and washed six times in the same buffer before analysis by SDS-PAGE and autoradiography.

**RESULTS**

Mirk Binds to the HNF1α Dimerization Cofactor DCoHm—To discover the function of Mirk, we used the yeast two-hybrid assay to find Mirk-binding proteins. We isolated a novel gene of the DCoH family (11) family, DCoHm, by yeast two-hybrid analysis using full-length Mirk as bait. Yeast two-hybrid could be used for the isolation of DCoHm. HNF1α is a transcription factor found in endoderm-derived tissues, including kidney, liver, intestine and pancreas, where it may function in maintaining the differentiated phenotype (12). HNF1α is also a tissue-specific transcription factor regulating glucose metabolism-related genes in the liver (11), insulin and insulin-like growth factor 1 (IGF-1) (13) in the pancreatic-beta cells, and the protooncogene c-Src in the intestine (14). DCoH binds as a dimer to the unstable HNF1α dimer and enables effective binding of the tetrameric complex to DNA (15). DCoH enhances HNF1α transcription activity 2–3-fold by stabilizing this complex (11). The DCoH family is highly conserved. DCoHm has 78% and 88% amino acid identity to human and chicken DCoH, respectively (Fig. 1). DCoHm was considered likely to have activity similar to other members of the DCoH family, because it retained the amino acids necessary for transcriptional activation of HNF1α, His-62, His-63, and His-80 as well as amino acid Phe-67, which mediates binding to HNF1α (16). The interaction of Mirk and DCoHm was confirmed in yeast AH109 cells by a 1.5–2-fold activation of a β-galactosidase reporter gene localized downstream of DCoHm (data not shown). Protein interaction was also demonstrated by co-immunoprecipitation of in vitro [35S]-labeled DCoHm with Mirk using antibody to the unique C terminus of Mirk (Fig. 2) with preimmune serum (P) used in the control. The last lane shows 30% of input DCoHm. The physical association between DCoHm and Mirk shown by co-immunoprecipitation experiment forms the basis between DCoHm and Mirk in the yeast two-hybrid analysis.

**Mirk Enhances the Transcriptional Activity of HNF1α**—Because DCoHm functions to stabilize HNF1α and increase its transcriptional capacity, we next tested whether Mirk altered the activity of HNF1α. HNF1α is novel for the increases in activity of 60-fold the activity of the reporter construct (β-28)-Luc, which consists of three copies of the HNF1α binding element, whereas...
DCoH further enhanced this activation about 2-fold (16). We assayed for the effects of Mirk on HNF1α transcriptional activation of (−28)_3-Luc in transient transfection assays in NIH3T3 cells, which exhibit low levels of endogenous DCoH. DCoH alone, Mirk alone, or DCoH plus Mirk were unable to activate (−28)_3-Luc. In contrast, the expression of HNF1α alone was enough to activate the reporter construct 20-fold, and co-expression of HNF1α and DCoH activated the reporter 30-fold (Fig. 3A, first six lanes). Increasing amounts of Mirk were co-transfected with expression plasmids for HNF1α and DCoH, which resulted in a dose-dependent activation of HNF1α, up to five times the activation induced by HNF1α and DCoH and 7.5 times the activation by HNF1α alone (Fig. 3A, last four lanes). Therefore, Mirk can substantially increase the transcriptional activity of HNF1α and may function as a co-activator of HNF1α in vivo.

Mirk is a serine/threonine protein kinase activated by autophosphorylation on tyrosine (1). Was the capacity to phospho-ratylate HNF1α necessary for the activation of this transcription factor by Mirk? Wild-type Mirk increased HNF1α activation 3-fold when co-transfected with DCoH and HNF1α, whereas the co-transfection of two Mirk kinase-inactive mutant constructs, Mirk-YF and Mirk-KR (1), had no effect (Fig. 4A), demonstrating that the kinase activity of Mirk is essential for its ability to increase HNF1α function. Further mutational analysis of Mirk was performed. Mirk is composed of a central conserved kinase domain flanked by non-conserved N-terminal and C-terminal sequences (1). Mirk deletion constructs were made; Nmirk consists of amino acids 1–629, and Cmirk consists of amino acids 1–435. The deletion of the N-terminal region of Mirk completely suppressed the activation of HNF1α by Mirk, whereas the deletion of the Mirk C terminus had little effect (Fig. 4A).
ΔN-KR, would act as a dominant negative, but the double mutant did not completely suppress HNF1α activity and simply maintained it at background levels. These co-transfection experiments (Fig. 4A) had included DCoH. We next compared the effect of wild-type and mutant Mirk on HNF1α activation in the absence of DCoH. Wild-type Mirk enhanced HNF1α
transcriptional activation in a dose-dependent manner in the absence of DCoH, whereas kinase-inactive YF-Mirk and the Mirk deletion mutant ΔMirk had no activity (Fig. 4B). These data indicate that Mirk kinase activity was essential for a transcriptional co-activator of HNF1.

Synergism between Mirk and MKK3E—Transcription factors are often activated through a kinase cascade such as the well-known MAP kinase superfamily. Mirk was shown to be a sub-strate of extracellular signal-related kinase 2 (ERK2), c-Jun NH2-terminal kinase (JNK1), and p38 in vitro (1), suggesting that one or more MAP kinases might activate Mirk in vivo, allowing Mirk to then activate HNF1α. We screened a series of MAP kinase kinases and their activators for their ability to substitute for Mirk in the activation of HNF1α. MKK3E was expressed in 293T cells, immunoprecipitated, and added to kinase mixtures containing Mirk, although similar amounts of MKK3E, MKK3, and Mirk were synthesized in the lysates (Western blot of total lysates shown in lower two panels). One representative experiment of four repeat experiments with the same cells was shown if >5%. H, HNF1α, Mirk; YF, Mirk-YF; KR, Mirk-KR; ΔN, ΔMirk; ΔC, ΔC-Mirk; ΔE, MKK3E.

MKK3E directly activated Mirk by phosphorylation. 3) MKK3E activated p38, which in turn phosphorylated and activated Mirk.

MKK3E Binds to Mirk and Activates It—To test this hypothesis, we initially determined whether Mirk and MKK3E interacted directly by co-immunoprecipitation experiments. 293T cells were co-transfected with either MKK3E or MKK3E together with either wild-type Mirk, kinase-inactive YF-Mirk, or epitope-tagged vector, and Mirk was immunoprecipitated after expression (Fig. 6A). A distinct band of MKK3E was observed in both the immunoprecipitates of wild-type Mirk and kinase-inactive Mirk. Much less (6–7%) wild-type Mirk associated with Mirk, although similar amounts of MKK3E, MKK3, and Mirk were synthesized in the lysates (Western blot of total lysates shown in lower two panels, Fig. 6A). Therefore, MKK3E associated directly with Mirk, and any effect MKK3E exerted on HNF1α could be mediated through exogenous or endogenous Mirk (Fig. 5, lanes 2 and 3, respectively). Because MKK3 is a potent activator of p38 (21), it was also possible that MKK3 activates Mirk. MKK3E was expressed in 293T cells, immunoprecipitated, and added to in vitro kinase reaction mixtures containing recombinant purified Mirk or recombinant purified p38, as indicated, with [γ-32P]-ATP and MBP as substrates. The reaction mixtures containing [γ-32P]-ATP were analyzed by SDS-PAGE and autoradiography (Fig. 6B). Mirk phosphorylation of MBP was increased ~50% in the presence of MKK3E, although MKK3E itself did not phosphorylate MBP. As a control, MKK3E was shown to increase the activity of a small concentration of p38 as an MBP kinase ~50% (Fig. 6B, last two lanes). Therefore, MKK3 binds to Mirk and increases Mirk kinase activity. Because MKK3 mediates various environmental stress signals, it is possible that MKK3 increases the function of Mirk as a transcriptional activator of HNF1α in
response to certain stresses. Stably overexpressed Mirk enables cells to proliferate and remain viable in serum-free conditions (1), possibly because Mirk activates the transcription of survival factors such as insulin-like growth factor 1 through the activation of HNF1α.

Mirk Binds to a Specific Region within the CBP-binding Region of HNF1α—We next determined the regions of Mirk and HNF1α necessary for their interaction. Glutathione S-transferase pull-down assays confirmed a three-part association between DC0Hm, Mirk, and HNF1. Both DC0Hm and Mirk were 35S-labeled by coupled transcription and translation reactions, incubated with either GST-HNF1 or GST coupled to beads, and the bead eluates were examined by PAGE followed by autoradiography. Coomassie Blue staining of reaction input. B, GST pull-down assays of 35S-prelabeled Mirk binding to GST-HNF1 deletion mutants. An autoradiogram of SDS-PAGE of bound proteins is shown. Representative experiments of replicate studies are shown.

Next, a series of deletion mutants of HNF1α were generated, and GST-pull-down assays were repeated with 35S-labeled Mirk (Fig. 7B). Mirk bound to HNF1α fragments encompassing amino acids 1–203 and 1–283 but not to fragments of the N terminus (amino acids 1–111) or C-terminal regions (amino acids 283–481 and 481 to 628). Therefore, the minimal region of Mirk/HNF1α binding encompassed HNF1α amino acids 112–203. This region was similar to the minimal region for HNF1α interaction with the co-activator CBP (22), amino acids 95–295. We next determined which region of Mirk was necessary for binding to HNF1α by performing GST pull-down assays with deletion constructs of Mirk (Fig. 8). The constructs consisted of ΔN, which lacked the conserved N terminus; ΔN2, deleted of the nonconserved N terminus; ΔN2Mirk (amino acids 233–629) cleaved of the nonconserved N terminus and the first five subdomains of the conserved kinase domain and the ATP binding site K140; Δ233–435, a partial kinase-domain deletion of amino acids 233–435 that retained the N and C termini; ΔCMirk (amino acids 1–435 with the nonconserved C terminus deleted); and C, an unrelated control protein p53. There were equivalent amounts of Mirk deletion products added to the GST pull-downs (Fig. 8, lower panel). However, ΔN2Mirk bound very poorly to GST-HNF1α (1–203) compared with the other deletion constructs and to full-length Mirk (Fig. 8, lane 1). Therefore, the Mirk region of amino acids 111–233 encompassing the first five conserved kinase subdomains was essential for Mirk binding to HNF1α.

Mirk Phosphorylates HNF1α—A direct interaction between Mirk and HNF1α occurred in the absence of DC0Hm, shown in the GST pull-down assays (Fig. 7A, lane 5). These results suggested that DC0Hm simply facilitated Mirk association with HNF1α and that HNF1α might be the immediate target of Mirk. This was shown to be the case, because Mirk phosphorylated GST-HNF1α in vitro in the absence of DC0Hm while exhibiting no kinase activity on GST itself. Left panel, autoradiography of SDS-PAGE kinase reaction products; right panel, Coomassie Blue staining. B, Mirk phosphorylates HNF1α at amino acid 249 within the minimal region for HNF1α interaction with co-activator CBP. HNF1α was mutated to S249A, then wild-type GST-HNF1 and mutant GST-HNF1S249A were phosphorylated in vitro with recombinant Mirk. Upper band, autoradiography of SDS-PAGE kinase reaction products; lower band, Western blot for GST demonstrating equal loading. Both in vitro phosphorylation experiments were repeated four times with similar results.

Mirk Protein Kinase Is a Transcriptional Activator of HNF1α

**FIG. 7.** A, GST pull-down assays confirmed a three-part association between DC0Hm, Mirk, and HNF1. Both DC0Hm and Mirk were 35S-labeled by coupled transcription and translation reactions, incubated with either GST-HNF1 or GST coupled to beads, and the bead eluates were examined by PAGE followed by autoradiography. CB, Coomassie Blue staining of reaction input. B, GST pull-down assays of 35S-prelabeled Mirk binding to GST-HNF1 deletion mutants. An autoradiogram of SDS-PAGE of bound proteins is shown. Representative experiments of replicate studies are shown.

**FIG. 8.** GST pull-down assays of 35S-prelabeled Mirk deletion mutants bound to the N terminus of HNF1α, GST-HNF1α (1–203). Top, autoradiogram of proteins bound to GST; bottom, autoradiogram of total input Mirk deletion proteins. One of two replicate experiments is shown.

**FIG. 9.** A, in vitro kinase assay demonstrated that Mirk phosphorylated GST-HNF1α in vitro in the absence of DC0Hm while exhibiting no kinase activity on GST itself. Left panel, autoradiography of SDS-PAGE kinase reaction products; right panel, Coomassie Blue staining. B, Mirk phosphorylates HNF1α at amino acid 249 within the minimal region for HNF1α interaction with co-activator CBP. HNF1α was mutated to S249A, then wild-type GST-HNF1 and mutant GST-HNF1S249A were phosphorylated in vitro with recombinant Mirk. Upper band, autoradiography of SDS-PAGE kinase reaction products; lower band, Western blot for GST demonstrating equal loading. Both in vitro phosphorylation experiments were repeated four times with similar results.
Mirk/Dyrk1B, like its family member Dyrk1A, is now shown to function as a transcription factor activator. There is strong evidence that Dyrk1A functions in neurogenesis and brain development (6, 7, 23, 24). Dyrk1A activity was induced during the differentiation of immortalized hippocampal progenitor cells, and the addition of the neurogenic factor basic fibroblast growth factor to these cells resulted in the specific binding of Dyrk1A to CREB, the phosphorylation of CREB by Dyrk1A, and the stimulation of CRE-mediated gene transcription (8). Mirk/Dyrk1B is also expressed in the brain, but its highest expression is in skeletal muscle (1). Screening a normal skeletal muscle cDNA library led us to discover that Mirk binds to a novel member of the highly conserved DCOH family, DCOHm. DCOH binds as a dimer to the unstable HNF1α dimer and enables effective binding of the tetrameric complex to DNA (15). DCOH enhances HNF1α transcription activity 2–3-fold by stabilizing this complex (11), and Mirk increased this activity a further 5-fold. The interaction between DCOHm and Mirk was confirmed by co-immunoprecipitation studies and GST-pull down assays. Mirk was shown to substantially increase the transcription activity of HNF1α in transient transfection assays and may function as a co-activator of HNF1α in vivo. Mirk did not require DCOH to active HNF1α. Mirk, through the N terminus of its conserved kinase domain, bound to HNF1α at a site within its CBP binding domain and then directly phosphorylated HNF1α adjacent to the binding site but still within the CBP binding domain. Mirk kinase activity was required for transcriptional activation of HNF1α, because kinase-inactive mutants were also unable to activate HNF1α.

HNF1α mediates the transcription of several genes that potentially could contribute to the differentiation and growth of normal muscle cells through Mirk interaction with DCOHm. However, Mirk is expressed in several cell lines established from solid tumors, including colon, lung, and ovarian, with much less expression in leukemia and lymphoma-derived cell lines (1). DCOH and Mirk are both expressed in colon carcinomas. DCOH was not found in normal colon tissue but was found in every colon carcinoma examined by immunohistochemistry (17), and Mirk was found in each of seven colon carcinoma cell lines (1). Mirk, DCOH, and HNF1α were found to form a complex in vitro, thus Mirk may complex with the DCOH/HNF1α tetramer in vivo. Therefore, in many colon carcinomas Mirk and DCOH are co-expressed and may function as an activating complex for HNF1α to induce ectopic gene expression. The expression of some of these genes may contribute to the ability of cell lines with stably overexpressed Mirk to maintain serum-free proliferation (1).

Recombinant Mirk is a constitutively active kinase. However, its kinase activity on MBP and its transcriptional activation of HNF1α was enhanced by MKK3, an upstream activator of the stress-activated MAP kinase p38 (21). MKK3 also directly bound to Mirk in vivo as demonstrated in co-immunoprecipitation experiments. MKK3 is activated by phosphorylation at Ser-189 and Thr-193 within a P-activation loop of its conserved kinase subdomain VIII (21) by upstream kinases in response to stress agents, p38, in turn, is activated by dual phosphorylation at threonine and tyrosine within the TPY motif in conserved kinase subdomain VIII (25–27). However, Mirk/Dyrk1B’s activation domain is YQY, which is at a position located within conserved subdomains VII and VIII of the catalytic domain homologous to the p38 activation domain (1). Recent studies of the related Dyrk1A have shown that only the second tyrosine in the activation domain, Tyr-321 and not Tyr-319, was autophosphorylated, and the mutation of Tyr-319 to Phe failed to reduce kinase activity (28). MKK3 possibly activates Mirk by phosphorylating it at the second tyrosine in its activation domain. Autophosphorylated tyrosine residues were also found in the N terminus of Dyrk1A, and deletion of the N terminus suppressed kinase activity (28) as deletion of the N terminus of Mirk suppressed its transactivator activity (Fig. 4). Dyrk1A is an arginine-directed kinase (4), and comparison of the Dyrk1A and ERK2 catalytic cores revealed that Tyr-321 of Dyrk1A can interact with Arg-325 and Arg-328 (28). There are also two comparable arginine residues upstream of the YQQ activation motif of Mirk. Although Dyrk1A and Mirk/Dyrk1B can autophosphorylate in bacteria, greater phosphorylation was seen when both proteins were expressed in mammalian cells. MKK3 binding sites and phosphorylation sites on Mirk must be mapped to uncover the precise relationship between MKK3 and Mirk.

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Mirk Protein Kinase Is Activated by MKK3 and Functions as a Transcriptional Activator of HNF1 α
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Additions and Corrections

Vol. 277 (2002) 25040–25046

Mirk protein kinase is activated by MKK3 and functions as a transcriptional activator of HNF1α.

Seunghwan Lim, Kideok Jin, and Eileen Friedman

Page 25045, Fig. 9: The mutant in panel B is S247A, not S249A. All references in the text to Ser-249 should be Ser-247. The corrected Fig. 9 and its legend are shown below.

A  Mirk Phosphorylates HNF1 in Vitro
    GST-HNF1 - + - +
    GST + - + -
    HNF1 autorad
    GST CB

B  Mirk In Vitro Kinase Assay on ΔHNF1 (1-283)
    Wt S247A autorad
    WB: GST

Fig. 9. A, in vitro kinase assay demonstrated that Mirk phosphorylated GST-HNF1 in vitro in the absence of DCoHm while exhibiting no kinase activity on GST itself. Left panel, autoradiography of SDS-PAGE kinase reaction products; right panel, Coomassie Blue staining. B, Mirk phosphorylates HNF1 at amino acid 247 within the minimal region for HNF1 interaction with co-activator CBP. HNF1 was mutated to S247A, then wild-type GST-HNF1 and mutant GST-HNF1S247A were phosphorylated in vitro with recombinant Mirk. Upper band, autoradiography of SDS-PAGE kinase reaction products. Lower panel, Western blot for GST demonstrating equal loading. Both in vitro phosphorylation experiments were repeated four times with similar results.

Vol. 278 (2003) 48162–48168

Structural insight into modest binding of a non-PXXP ligand to the signal transducing adaptor molecule-2 Src homology 3 domain.

Tomonori Kaneko, Takashi Kumasaka, Tadashi Ganbe, Takao Sato, Keji Miyazawa, Naomi Kitamura, and Nobuo Tanaka

Page 48162: The volume number and year printed in the top righthand corner are incorrect. The corrected line should read:

Vol. 279, No. 48, Issue of November 28, pp. 48162–48168, 2003.

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.
Molecular basis for the direct inhibition of AP-1 DNA binding by 15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$.

Dolores Pérez-Sala, Eva Cernuda-Morollón, and F. Javier Cañada

Page 51256, Fig. 5C: The structure of PGA shown in this figure is not correct. The correct panel C is shown below:

![Corrected Structure of PGA](image)

Fig. 5

Evidence that dioxygen and substrate activation are tightly coupled in dopamine $\beta$-monooxygenase. Implications for the reactive oxygen species.

John P. Evans, Kyunghye Ahn, and Judith P. Klinman

Page 49693: Under “Synthesis of $\beta$-$\beta$-Difluorophenethylamine Hydrochloride,” end of line 12 should read, “After 24 h of stirring . . . .”

Page 49696, Scheme 3, top right: “Cu$_{\beta}$(I)$\cdot$O$_2$” should read “Cu$_{\beta}$(I)(O$_2$)$_2$.”

Page 49697: Third line from top left should read, “a pre-equilibrium formation of Cu(II)$\cdot$O$_2$ from O$_2$ . . . .”

Page 49697, 16th line from bottom left: “between Cu(1)$\cdot$O$_2$ and . . . .” should read, “between Cu(1)(O$_2$)$_2$ and . . . .”

Transcriptional control of the arginine/lysine transporter, Cat-1, by physiological stress.

James Fernandez, Alex B. Lopez, Chuanping Wang, Rangnath Mishra, Lingyin Zhou, Ibrahim Yaman, Martin D. Snider, and Maria Hatzoglou

Dr. Hatzoglou’s name was misspelled. The correct spelling is shown above.