The ETS domain transcription factor PU.1 is necessary for the development of monocytes and regulates, in particular, the expression of the monocyte-specific macrophage colony-stimulating factor (M-CSF) receptor, which is critical for monocyctic cell survival, proliferation, and differentiation. The bZIP transcription factor c-Jun, which is part of the AP-1 transcription factor complex, is also important for monocyctic differentiation, but the monocyte-specific M-CSF receptor promoter has no AP-1 consensus binding sites. We asked the question of whether c-Jun could promote the induction of the M-CSF receptor by collaborating with PU.1. We demonstrate that c-Jun enhances the ability of PU.1 to transactivate the M-CSF receptor promoter as well as a minimal thymidine kinase promoter containing only PU.1 DNA binding sites. c-Jun does not directly bind to the M-CSF receptor promoter but associates via its basic domain with the ETS domain of PU.1. Consistent with our observation that AP-1 binding does not contribute to c-Jun coactivation is the observation that the activation of PU.1 by c-Jun is blocked by overexpression of c-Fos. Phosphorylation of c-Jun by c-Jun NH₂-terminal kinase on Ser-63 and -73 does not alter the ability of c-Jun to enhance PU.1 transactivation. Activated Ras enhances the transcriptional activity of PU.1 by up-regulating c-Jun expression without changing the phosphorylation pattern of PU.1. The activation of PU.1 by Ras is blocked by a mutant c-Jun protein lacking the basic domain. The expression of this mutant form of c-Jun also completely blocks 12-O-tetradecanoylphorbol-13-acetate-induced M-CSF receptor promoter activity during monocytic differentiation. We propose therefore that c-Jun acts as a c-Jun NH₂-terminal kinase-independent coactivator of PU.1, resulting in M-CSF receptor expression and development of the monocytic lineage.

The ETS domain transcription factor PU.1 is preferentially expressed in myeloid and B cells (1, 2) and plays a pivotal role in their development (3, 4). Indeed, mice deficient in PU.1 display a complete block in development of monocytes, macrophages, and B cells (5, 6). During hematopoietic development, PU.1 mRNA is expressed at low levels in murine embryonic stem cells and human CD34+ stem cells and is specifically up-regulated upon myeloid differentiation, and down-regulated upon erythroid differentiation (7, 8). PU.1 regulates the expression of almost all characterized myeloid genes, including growth factor receptors, and in particular directs the monocyte-specific expression of the macrophage colony-stimulating factor (M-CSF) receptor (9, 10). Thus, PU.1-deficient hematopoietic cells display minimal expression of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor receptors and no detectable M-CSF receptors (11, 12).

The M-CSF receptor is critical for monocyctic cell survival, proliferation, and differentiation (3, 13). M-CSF is known to augment monocyctic survival and, therefore, to allow macrophage differentiation (14). The responsiveness of hematopoietic progenitor cells to M-CSF is regulated at the level of M-CSF receptor expression (15). Although the important role of the M-CSF receptor for the development of monocytes has been clearly demonstrated, little is known about the signaling molecules or protein-protein interactions that modulate the effect of PU.1 to regulate the M-CSF receptor promoter activity (3, 16).

c-Jun belongs to the bZIP group of DNA binding proteins and is a component of AP-1 transcription factor complexes (17). c-Jun forms homodimers or can heterodimerize with other Jun family members or with other bZIP proteins including members of the Fos and ATF/cAMP response element-binding protein (CREB) families (18, 19). AP-1 has been shown to be involved in many cellular processes including proliferation, differentiation, apoptosis, and stress responses (18). In particular, there is evidence that c-Jun plays a role in monocytic differentiation. c-Jun mRNA is up-regulated upon monocyctic differentiation of bipotential myeloid cell lines (20–22), while stable transfection of c-Jun into myeloid cell lines results in partial differentiation (23, 24).

Although c-Jun and PU.1 are both pivotal for monocyctic development, it is still unclear whether c-Jun is involved in the regulation of the M-CSF receptor, which is critical for monocyctic survival, proliferation, and differentiation. It has been shown that during 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced monocytic differentiation of U937 cells, c-Jun and M-CSF receptor mRNA expression increases (25). However, the monocyte-specific M-CSF receptor promoter (9, 26, 27) contains
no AP-1 consensus binding sites. As c-Jun and the regulation of the M-CSF receptor by PU.1 play important roles in monocytic differentiation, we hypothesized that c-Jun might be involved in the regulation of the M-CSF receptor, not by binding to AP-1 sites, but possibly via a novel mechanism. Therefore, we asked the question of whether c-Jun modulates the ability of PU.1 to transactivate the human monocyte-specific M-CSF receptor promoter.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Cell Culture**—Monkey kidney CV-1 cells (ATCC CCL-70; American Type Culture Collection, Rockville, MD) were maintained in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% calf serum (HyClone, Logan, UT). Murine embryonal carcinoma F9 cells (ATCC CRL-1720; American Type Culture Collection) and human kidney 293T cells (kindly provided by John Blenis, Harvard Medical School, Boston, MA) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (HyClone). U937 cells (ATCC CRL 1593; American Type Culture Collection) were maintained in RPMI 1640 medium (Life Technologies) supplemented with 10% fetal bovine serum, and differentiated with 2 × 10⁻⁷ m TPA (Sigma) (stock solution: 1 × 10⁻³ m in Me₂SO) or vehicle only.

**Reporter Constructs and Expression Plasmids**—The human monocyte-specific M-CSF receptor promoter ranging from bp -88 to +71 with respect to the major monocytic transcription start site (9, 26) was subcloned in the firefly luciferase vector pXP2 (28). pTK with PU.1 sites is a dimer of both PU.1 sites from the granulocyte colony-stimulating factor receptor promoter from bp +28 to +54 (29) subcloned into pTKS1Luc, a pXP2-based luciferase construct with a TATA box only as a minimal promoter (28). pTK with mutated PU.1 sites is a dimer of both mutated PU.1 sites from the granulocyte colony-stimulating factor receptor promoter from bp +28 to +54 (primers: 5'-TCG AGT GGT TCA ACA AAC TTT TGT TGA CAG GAG-3' and 5'-TCG ACT GCT GTC AAC AAA AGT TGA TGA AAC CAC-3') subcloned into pTKS1Luc and was considered for transfection experiments (29). As an internal control plasmid for co-transfection assays, the pRL-null construct driven by the firefly luciferase gene (Promega, Madison, WI) was used (30).

The PU.1 mutants pCDA1/PU.1-1Δ1–133, pCDA1/PU.1-1Δ1–100, and pCDA1/PU.1-1Δ1–70 (31) were kindly provided by Marian Koshland (University of California, Berkeley, CA). The PU.1 deletion mutants pECE-PU.1-1Δ18–160 and pECE-PU.1-Δ54–26 were also generously provided by Marian Koshland and Brian Meyer (Harvard Medical School). pECE-PU.1-1Δ18–160 (32) were also kind gifts from Richard Maki (the Burnham Institute, La Jolla, CA and alanine mutants pECE-PU.1-S41A/S45A and pECE-PU.1-S148A (32) were provided by Joseph Sodroski (Dana Farber Cancer Center, Boston, MA) and Richard Maki (the Burnham Institute, La Jolla, CA) respectively. Expression plasmids for c-Jun (34) and c-Fos (35) were kindly provided by Jianmin Tian and Michael Karin (University of California, San Diego). In vivo labeling and subsequent phosphoamino acid analysis and phosphopeptide mapping. To detect changes in the phosphorylation pattern of PU.1 upon stimulation with activated Ras in vivo, 0.5 µg of pEBG-PU.1 either with 0.25 µg of activated Ras(L61) or with inactive Ras(N17) was transfected into 293T cells using LipofectAMINE Plus (Life Technologies). pGEX-2TK-PU.1 has been described previously (33). pECE-PU.1/Ser251–276 was described previously (36). pSP65-c-Jun and pSP65-c-Fos/S63A/S73A containing serine to alanine mutations in amino acids 281–313, and pSV-SPORT1-c-Jun/Ser281–313, were subcloned into the RI fragment of pBS-PU.1 (38, 39) and activated by Ras (30) into the RI fragment of pBluescript KS + (Stratagene, La Jolla, CA). Then the Nael/NotI fragment of pBS-PU.1 was subcloned into the Smal/EcoRI-cut vector Bluescript KS+ (Stratagene, La Jolla, CA). Then the Nael/NotI fragment of pBS-PU.1 was subcloned into the pMal/NotI fragment of pET28a (37, 38). pSP65-c-Jun containing wild-type c-Jun (34), pSP65-c-Jun/Ser281–313, and pSP65-c-Jun/Ser281–313 containing serine to alanine mutations in amino acids 251–276 were described previously (36). pSP65-c-Jun/Ser281–313 was prepared as described by the manufacturer. U937 cells were transiently transfected by electroporation as described previously (9). Firefly luciferase activities from the constructs PM-CSFR, pXP2, and pTK with PU.1 sites and pTK with mutated PU.1 sites and Renilla luciferase activity from the internal control plasmid pRL-null were determined 24 h after the initiation of the transfection protocols using the Dual-luciferase Reporter Assay System (Promega). Firefly luciferase activities were normalized to the firefly luciferase null values. Results are given as means ± S.E. of at least six independent experiments. The following DNA concentrations of the reporter constructs and expression plasmids were used for LipofectAMINE Plus transfections: 0.3 µg of the human monocyte-specific M-CSF receptor promoter in pXP2, pXP2, the TK promoter with PU.1 sites, and the TK promoter with mutated PU.1 sites, 0.05 µg of pEBG-PU.1, 0.2 µg of the other expression plasmids for PU.1 and PU.1 mutants; 0.25 µg of Ras(L61), Ras(N17), and MEKK1; 0.1 µg of c-Jun, c-Jun mutants, and c-Fos; and the same concentrations of the empty expression vectors as controls, respectively. For electroporation, 10 µg of the firefly luciferase reporter constructs, 5 µg of expression plasmids, and 1 µg of the internal control plasmid were used. pRL-null was chosen as internal control plasmid, because it was not transacti-
lulose (Bio-Rad) for phosphopeptide mapping. After transfer, the 69-kDa GST-PU.1 protein bands were excised. For phosphoamino acid analysis, the samples were boiled at 100 °C for 1 h with 6 M HCl (Pierce), and the presence of serine, threonine, or tyrosine phosphorylation was determined as described (43). To determine the phosphorylated protein residues of PU.1, GST-PU.1 protein bands were digested with 1-chloro-3-tosylamido-7-amino-2-heptanone-treated chymotrypsin (Worthington) and endoproteinase Glu-C (V8 protease) (Boehringer Mannheim) and processed for phosphopeptide mapping as described previously (43).

Western Blot—24 h after the start of transfection, cells were lysed with radioimmunoprecipitation assay buffer. Equal amounts of total protein were separated on 10% SDS-polyacrylamide gels and transferred to Immobilon-P membrane (Millipore). Membranes were incubated with anti-c-Jun antibody (catalog no. SC-45; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-PU.1 antibody (catalog no. SC-352; Santa Cruz Biotechnology), or anti-β-tubulin antibody as an internal control (catalog no. 1111876; Boehringer Mannheim) for 60 min and then with Protein A-horseradish peroxidase conjugate (Amersham, Buckinghamshire, United Kingdom) for 45 min. For U937 cells, an anti-M-CSF receptor antibody (catalog no. SC-692, Santa Cruz Biotechnology) was used. Signals were detected with ECL Western blotting detection reagents (Amersham). In parallel plates, the M-CSF receptor promoter construct was co-transfected, and luciferase activities were determined to ensure that Ras enhances the transactivation function of PU.1 in the particular experiment used for Western blot analysis of c-Jun expression or PU.1 expression and that the transfection efficacy was the same (less than 10% difference between plates) in the particular experiment. Differences in protein expression were quantitated by ImageQuant software (Molecular Dynamics).

RESULTS

**c-Jun Enhances the Ability of PU.1 to Transactivate the M-CSF Receptor Promoter and a Minimal TK Promoter Containing PU.1 DNA Binding Sites Only**—Since c-Jun and the regulation of the M-CSF receptor by PU.1 are both important for monocytic development, we asked the question of whether c-Jun enhances the ability of PU.1 to transactivate the M-CSF receptor promoter. CV-1 cells, which contain c-Jun (Fig. 4B), were transfected with a plasmid containing the human mono-ocyte-specific M-CSF receptor promoter (9, 26) cloned upstream of the luciferase reporter gene along with expression plasmids for PU.1 and c-Jun, and reporter gene expression was determined 24 h post-transfection. Transfection of a c-Jun expression construct significantly enhanced the ability of PU.1 to transactivate the M-CSF receptor promoter (Fig. 1A). Moreover, in c-Jun-deficient F9 cells, PU.1 weakly transactivated the M-CSF receptor promoter (2 fold), while co-expression of c-Jun with PU.1 led to robust transactivation (33-fold) (Fig. 1B). The cooperation of c-Jun with PU.1 is therefore important for M-CSF receptor promoter activity.

We next asked the following questions: (a) whether the binding of PU.1 to DNA was necessary for its activation by c-Jun and (b) whether a PU.1 binding site alone was sufficient for the c-Jun-enhanced PU.1 activation. We observed enhanced PU.1 transactivation mediated by c-Jun using a reporter construct containing four PU.1 binding sites cloned upstream of a minimal TK promoter (Fig. 1C). In control experiments, no effect of c-Jun on PU.1 activity was observed when the PU.1 binding sites were mutated (Fig. 1C). These data indicate that PU.1 binding to DNA is necessary for its activation by c-Jun and that PU.1 binding sites are sufficient to mediate this effect.

**c-Jun Does Not Directly Bind to the M-CSF Receptor Promoter but Associates with the ETS Domain of PU.1**—The activation of PU.1 by c-Jun Is Blocked by Overexpression of c-Fos—To elucidate the mechanism by which c-Jun augments the transcriptional activity of PU.1, we performed experiments to determine whether the activation of PU.1 by c-Jun required the binding of c-Jun:AP-1 complexes to the M-CSF receptor promoter. Since there are no AP-1 consensus sites in the human monocytic M-CSF receptor promoter from bp −88 to +71 with respect to the major monocytic transcription start site (9, 26) (Fig. 1, A and B) or in the TK promoter containing PU.1 sites (Fig. 1C), our data suggested that c-Jun augmentation of PU.1 transactivation was not mediated by DNA binding of c-Jun. In order to formally exclude DNA binding by c-Jun, we performed an electrophoretic mobility shift assay using a bp −88 to +71 M-CSF receptor promoter fragment or a bp −62 to −29 oligonucleotide containing the PU.1 binding site of the M-CSF receptor promoter (9). In vitro translated c-Jun or a mixture of c-Jun and c-Fos specifically bound to a double-stranded AP-1 oligonucleotide probe from the collagenase promoter (Fig. 2A), while no specific binding was observed using the double-stranded bp −88 to +71 M-CSF receptor promoter fragment (Fig. 2A) or a bp −62 to −29 oligonucleotide containing the PU.1 binding site of the M-CSF receptor promoter (data not shown). In control experiments, in vitro translated PU.1 was shown to bind strongly and specifically to the same M-CSF receptor promoter fragments (Fig. 2A). These results indicate that binding of c-Jun to the M-CSF receptor promoter DNA is not required to mediate its activating effect on PU.1.

It has previously been reported that c-Jun can physically and functionally interact with ETS-1, which like PU.1 is an ETS family transcription factor (44). In these same studies, it was reported that c-Jun and PU.1 could physically interact, although the functional consequences of this interaction were not
examined. We were able to confirm that c-Jun did indeed specifically interact with PU.1 in *in vitro* (Fig. 2B). Because c-Jun can bind to PU.1 but does not bind to DNA itself (Fig. 2A), we therefore conclude that c-Jun acts as a coactivator (45) of PU.1.

Since c-Jun can form a heterodimer with c-Fos in AP-1 transcription factor complexes (17–19), we asked the question of whether c-Fos expression could modulate the synergy between c-Jun and PU.1 in F9 cells. Co-transfection of c-Fos did not enhance the synergy between c-Jun and PU.1 but instead completely blocked it (Fig. 2C). c-Fos did not bind to PU.1 (Fig. 2B) and therefore might compete with PU.1 for the binding partner c-Jun. These results are consistent with a model in which c-Jun mediates its effects through direct interactions with PU.1 and not by independent DNA binding to an AP-1 site.

**Phosphorylation of c-Jun by c-Jun NH2-terminal Kinase (JNK) on Ser-63 and -73 Does Not Alter the Ability of c-Jun to Enhance PU.1 Transactivation**—PU.1 (Fig. 3A) and c-Jun (Fig. 3B) are composed of a number of discrete domains. We used deletion mutants to determine which domains of PU.1 and c-Jun are critical for the activation of PU.1 by c-Jun in the context of the M-CSF receptor promoter. The transactivation domain of PU.1 (amino acids 1–118) was necessary for the activation by c-Jun (Fig. 3A). The transactivation domain of c-Jun (amino acids 1–87); the basic domain of c-Jun, which can physically interact with PU.1 (44) and mediates DNA binding to AP-1 sites; and the leucine zipper domain, which is responsible for homodimerization and heterodimerization with c-Fos, were all necessary for the activation of PU.1 by c-Jun (Fig. 3B). The transcriptional activity of c-Jun is increased following phosphorylation on Ser-63 and -73 by JNK (34, 46). To determine whether phosphorylation of Ser-63 and -73 was important for the function of c-Jun as a transcriptional coactivator of PU.1, we used c-Jun constructs in which these sites were mutated to Ala residues. Surprisingly, we found no difference in the ability of wild type c-Jun and S63A/S73A c-Jun to enhance PU.1 transactivation of the M-CSF receptor promoter in F9 cells (Fig. 3B). Furthermore, co-transfection with an activated allele of Ras, which has been demonstrated to enhance the transcriptional activity of c-Jun via phosphorylation by JNK (34, 46), did not enhance the coactivator function of wild type or S63A/S73A c-Jun (Fig. 3C). These data indicate that the coactivator function of c-Jun to enhance the transcriptional activity of PU.1 is independent of JNK phosphorylation.

**Activated Ras Enhances the Transcriptional Activity of PU.1 by Up-regulating c-Jun Expression**—Although Ras signal transduction has been demonstrated to play an important role in myeloid differentiation (47–50), it has not been shown whether Ras increases the activity of the macrophage-specific M-CSF receptor promoter. However, Ras is known to induce the expression of c-Jun (51). Therefore, we asked the question of whether Ras could augment the transcriptional activity of PU.1 and if this was mediated by enhancing c-Jun expression. In fact, activated Ras(L61) enhanced the ability of PU.1 to transactivate the M-CSF receptor promoter in CV-1 cells to a similar degree as c-Jun (Fig. 4A). Furthermore, a dominant negative c-Jun mutant lacking the basic domain, which is required for physical interaction with PU.1 in *in vitro* (44), blocked the activation of PU.1 by Ras (Fig. 4A). In the same experiment, Ras did not change the protein expression of transfected PU.1 (Fig. 4B) but up-regulated endogenous c-Jun protein expression 4-fold (Fig. 4B). Since the transfection efficiency in the CV-1 cells was 40% (Fig. 4B), we estimate that Ras up-regulated c-Jun expression 10-fold in transfected cells.

The c-Jun promoter contains important TPA response elements that preferentially bind heterodimers of c-Jun and ATF-2, both of which are activated upon phosphorylation by JNK (17–19). We therefore tested the effect of another JNK activator, the MAP kinase kinase kinase MEKK1 (40), on the ability of PU.1 to transactivate the M-CSF receptor promoter.
c-Jun Is a Coactivator of PU.1

The transcription factor c-Jun (20–24) and the regulation of the M-CSF receptor by the transcription factor PU.1 (11, 12, 55) both play important roles in the development of the monocytic lineage. Therefore, we asked the question of whether c-Jun could promote the induction of the M-CSF receptor by PU.1. Here we demonstrate that c-Jun enhances the ability of PU.1 to transactivate the M-CSF receptor promoter by PU.1. This effect on the human monocytic M-CSF receptor promoter was blocked by co-expression with a dominant negative c-Jun mutant lacking the basic domain (Fig. 5A). These results indicate that c-Jun function is required for the increase in M-CSF receptor promoter activity observed in myeloid cells differentiated toward the monocytic lineage with TPA.

**DISCUSSION**

The transcription factor c-Jun (20–24) and the regulation of the M-CSF receptor by the transcription factor PU.1 (11, 12, 55) both play important roles in the development of the monocytic lineage. Therefore, we asked the question of whether c-Jun could promote the induction of the M-CSF receptor by PU.1. Here we demonstrate that c-Jun enhances the ability of PU.1 to transactivate the M-CSF receptor promoter, because the human monocytic M-CSF receptor promoter contains no AP-1 consensus binding sites. In contrast, the macrosialin promoter and macrophage scavenger receptor promoter contain PU.1 and AP-1 binding sites, which are critical for their monocytic specificity (56, 57). In fact, our data suggest a novel mechanism by which c-Jun can induce gene expression. c-Jun does not bind to the M-CSF receptor promoter (Fig. 2A) and, moreover, enhances the ability of PU.1 to transactivate a minimal promoter driven by PU.1 sites alone (Fig. 1C). Furthermore, c-Jun physically binds to PU.1 (Fig. 2B). Since c-Jun binds to PU.1 and functionally activates PU.1 without binding to the M-CSF receptor promoter DNA, we conclude that c-Jun acts as a coactivator (45) of PU.1. This is the first report demonstrating a coactivator function for c-Jun.

In fact, MEKK1 enhanced the PU.1 transactivation function to a similar level as Ras (Fig. 4A).

Ras is known to modulate the activity of the ETS domain transcription factors ETS-1 and ETS-2 by phosphorylation (52). However, co-expression of Ras did not alter the phosphorylation pattern of PU.1 in vivo (Fig. 4C), and furthermore, Ras enhanced the transcriptional activity of known phosphorylation site mutants of PU.1 (Ser-41, -45, and -148) (53, 54) similar to wild type PU.1 (data not shown). In conclusion, these data suggest a model in which Ras enhances the transcriptional activity of PU.1 by increasing the expression of its coactivator c-Jun.

**The Expression of the Mutant Form of c-Jun That Lacks the Basic Domain Completely Blocks TPA-induced M-CSF Receptor Promoter Activity during Monocytic Differentiation—** We next asked the question of whether the coactivator function of c-Jun could play a biological role during monocytic differentiation in vivo. We first showed that Ras enhanced the ability of PU.1 to transactivate the M-CSF receptor promoter in myeloid U937 cells (Fig. 5A). U937 cells can be differentiated to monocytic cells upon treatment with TPA, and during this process c-Jun and M-CSF receptor mRNA expression increases (25). TPA increased reporter gene expression from our M-CSF receptor promoter construct (Fig. 5A) and also increased the expression of endogenous M-CSF receptor (Fig. 5B). This effect on the human monocytic M-CSF receptor promoter was blocked by co-expression with a dominant negative c-Jun mutant lacking the basic domain (Fig. 5A). These results indicate that c-Jun function is required for the increase in M-CSF receptor promoter activity observed in myeloid cells differentiated toward the monocytic lineage with TPA.

**FIG. 3. Domains of PU.1 and c-Jun relevant for coactivation of PU.1 by c-Jun.** A, activation of the M-CSF receptor promoter by PU.1 or different PU.1 deletion mutants either with or without c-Jun in CV-1 cells as described in Fig. 1A. Also shown is a schematic representation of the transcription factor PU.1 with transactivation domain (amino acids 1–118), PEST domain (amino acids 118–160), and the ETS DNA binding domain (amino acids 161–255). B, activation of the M-CSF receptor promoter by c-Jun or different c-Jun deletion or point mutants either with or without PU.1 in F9 cells as described in the legend to Fig. 1B. Also shown is a schematic representation of c-Jun mutants, depicting the transactivation domain (amino acids 1–251), basic domain (BD), amino acids 251–281, and the leucine zipper (LZ); amino acids 281–313). C, activation of the M-CSF receptor promoter by PU.1, c-Jun, c-Jun mutated in the JNK phosphorylation sites (Ser-63 and -73), and activated pMT3-Ras(L61) in F9 cells as described in the legend to Fig. 1B.
and not by independent DNA binding to an AP-1 site (Fig. 5C).

Usually, the transcriptional activity of c-Jun is increased following phosphorylation on Ser-63 and -73 by JNK (34, 46). Furthermore, the general coactivator CBP/p300 stimulates c-Jun-dependent transcription, and the c-Jun residues Ser-63 and -73 are required for CBP/p300 stimulation in vivo and CBP/p300 binding in vitro (58). Surprisingly, we found no difference in the ability of wild type c-Jun and the S63A/S73A c-Jun mutant to enhance PU.1 transactivation of the M-CSF receptor promoter (Fig. 3B). Furthermore, co-transfection with an activated allele of Ras, which has been demonstrated to enhance the transcriptional activity of c-Jun via phosphorylation by JNK (34, 46), did not enhance the coactivator function of wild type or S63A/S73A c-Jun (Fig. 3A). Our data indicate that the coactivator function of c-Jun to enhance the transcriptional activity of PU.1 is independent of JNK phosphorylation.

In accordance with its pivotal role in B cell development (5, 6), PU.1 binds to the B cell-specific immunoglobulin kappa (Igκ) 3’ enhancer and can control transcriptional activity (59). The immunoglobulin 3’ enhancer is activated by PU.1, c-Jun, PIP, and c-Fos and contains respective DNA binding sites for these factors (60). In this context, mutants of PU.1 that lack the transcriptional activation domain are as efficient as stimulating enhancer activity as the wild-type PU.1 protein (60). In contrast, the transactivation domain of PU.1 (amino acids 1–118) is necessary for the basal transactivation of the M-CSF receptor promoter by PU.1 (Fig. 3A), and c-Jun cannot exert its coactivator function on PU.1 mutants that lack the transactivation domain (Fig. 3A). These data suggest that the function of different PU.1 domains might vary depending on the cell type (monocyte versus B cell) and on the cooperating proteins (c-Jun as part of the AP-1 transcription factor complex versus c-Jun as a JNK independent coactivator).

How is the coactivator function of c-Jun regulated? Since the coactivator function of c-Jun cannot be regulated via phosphorylation by JNK, the regulation of c-Jun expression might be crucial for the capacity of c-Jun to coactivate PU.1 and induce monocytic differentiation. The Ras signal transduction pathway, for example, induces the expression of c-Jun (51). The Ras family of proteins are GTP-dependent molecular switches that are essential for cell growth and differentiation (61, 62). Ras exerts its effect on cell growth mainly via ETS (63) and AP-1 (64) transcription factors. For example, cells with a null mutation in the c-jun gene lack many characteristics of Ras transformation (64), and dominant negative mutants of ETS-1, ETS-2, or PU.1 with just the DNA binding domain inhibit Ras activation of transcription and revert Ras-transformed cells (63). In particular, Ras has been demonstrated to play an
important role in myeloid differentiation. Macrophage differen-
tiation and M-CSF-dependent survival are altered in trans-
genic mice that express dominant suppressors of Ras signaling
(47), while a number of hematopoietic cell lines undergo sponta-
neous monocytic differentiation in response to expression of
activated Ras (48, 49). In addition, M-CSF, granulocyte-mac-
r phage colony-stimulating factor, or interleukin-3-induced
survival, proliferation, and differentiation of the monocytic
lineage. Ras has not previously been shown to increase
monocytic differentiation in response to expression of
(47), while a number of hematopoietic cell lines undergo sponta-
neous monocytic differentiation in response to expression of
activated Ras (48, 49). In addition, M-CSF, granulocyte-mac-
rphage colony-stimulating factor, or interleukin-3-induced
monocytopoiesis of CD34

tor c-Jun expression, MEKK1 (40), enhanced the PU.1 transacti-

c-Jun (51), and here we demonstrate that Ras induces c-Jun
expression in CV-1 cells during activation of PU.1 (Fig. 4B).
Thus, these data suggest that Ras enhances the transcriptional
activity of PU.1 by up-regulating the expression of its coac-
tivator c-Jun. In accordance with this model, another inducer of
c-Jun expression, MEKK1 (40), enhanced the PU.1 transacti-

Ras modulates the activity of ETS domain transcription fac-
tors such as ETS-1 or ETS-2 by phosphorylation (52). More-
over, the ETS domain factor PU.1, in particular, can be phos-
phorylated by casein kinase (53, 65) or JNK in vitro (66), and
the activity of PU.1 is known to be regulated by phosphoryla-
tion (53, 54, 65). Phosphorylation of PU.1 at Ser-148 is nec-

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