CREB-binding Protein Is a Nuclear Integrator of Nuclear Factor-κB and p53 Signaling*

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Transcriptional coactivators may function as nuclear integrators by coordinating diverse signaling events. Here we show that the p65 (RelA) component of nuclear factor-κB (NF-κB) and p53 mutually repress each other’s ability to activate transcription. Additionally, tumor necrosis factor-activated NF-κB is inhibited by UV light-induced p33. Both p65 and p53 depend upon the coactivator CREB-binding protein (CBP) for maximal activity. Increased levels of the coactivator relieve p53-mediated repression of NF-κB activity and p65-mediated repression of p53-dependent gene expression. Nuclear competition for limiting amounts of CBP provides a novel mechanism for altering the balance between the expression of NF-κB-dependent proliferation or survival genes and p53-dependent genes involved in cell cycle arrest and apoptosis.

Nuclear factor-κB (NF-κB) is an inducible transcription factor that plays an essential role in the regulation of gene expression in response to inflammatory stimuli (1). It is composed of members of the Rel family (p50, p52, p65 (RelA), c-Rel, and RelB), which share a region of homology known as the Rel homology domain capable of directing DNA binding and mediating dimerization. In most cells, NF-κB is found in an inactive form in the cytoplasm bound to an inhibitory protein, IκB. In response to multiple activating signals, the inhibitor is degraded by the ubiquitin-proteasome complex, and NF-κB translocates to the nucleus and induces gene expression. NF-κB components can interact with other DNA binding proteins, as well as with a series of non-DNA-binding coactivator proteins. Among these interactions, the p65 component of NF-κB, like a variety of signal-dependent transcriptional activators, can associate with CREB-binding protein (CBP) or its structural homolog p300 (2, 3).

Activation of NF-κB is associated with resistance to programmed cell death (4–10). Mice with a targeted mutation in the p65 component of NF-κB die before birth with extensive liver cell apoptosis (11). Fibroblasts derived from these mice show increased apoptosis following TNFα stimulation, an effect that can be reversed by overexpression of p65 (4). Inhibition of NF-κB activation increases cell death in response to multiple stimuli (7). Moreover, inhibition of constitutively active NF-κB in lymphoid cell lines causes apoptosis (8). One mechanism by which NF-κB inhibits cell death is to induce the expression of genes that promote resistance to apoptosis. These anti-apoptotic gene include A20 (12), the immediate early response gene IEX-1L (13), as well as TRAF1 (TNFR-associated factor 1), TRAF2, and the inhibitor-of-apoptosis (IAP) proteins c-IAP and c-IAP2 (14). Thus NF-κB can activate a set of genes that function cooperatively to suppress apoptosis.

In contrast to NF-κB’s role in promoting cell survival, the p53 tumor suppressor gene plays an important role in cell cycle arrest or apoptosis in response to various types of stress (15, 16). p53 functions as a transcriptional activator by binding to specific DNA sequence elements (17) and interacting with co-activators, such as CBP (18–21). These interactions appear to be necessary for p53 to function as a transcription factor. p53 increases expression of multiple genes, including the cyclin-dependent kinase inhibitor p21/WAF1/Cip and murine double minute (mdm2) (15, 16, 22, 23). Increased expression of the p21 gene by p53 correlates with cell cycle control by inducing G1 arrest or apoptosis (24). The ability of p53 to induce cell cycle arrest or apoptosis is closely regulated under normal conditions (22, 23). Some oncogenes and stress signals regulate p53 activity through MDM2. This negative regulator of p53 functions in two ways: it binds to the activation domain of p53 and inhibits its ability to stimulate transcription, and MDM2 mediates the degradation of p53. In addition to having a role as a transcriptional activator, p53 represses the expression of multiple genes that lack p53 binding sites (25). In contrast to activation by p53, no common consensus DNA binding site has been identified which correlates with the transcriptional repression. The biological consequences of p53-mediated repression of gene expression are not fully understood.

The divergent roles played by NF-κB and p53 suggest that there might be mechanisms that integrate the activities of these regulatory factors. Some of this control may be provided by the common dependence of both NF-κB and p53-dependent gene expression on limiting levels of transcriptional coactivators. Since transactivation by both p56 and p65 involves CBP, we investigated the role of this coactivator in the p53 and NF-κB signaling pathways. We find that mutual transrepression of these diverse signaling systems results, at least in part, from competition for a limiting amount of this versatile transcriptional coactivator. Interactions between the p65 and p53 signaling pathways mediated by CBP may be an important aspect of regulating the diverse changes in gene expression associated with cell survival.

EXPERIMENTAL PROCEDURES

Cells and Transfections—Saos2 and COS-7 were obtained from the American Type Culture Collection (ATCC). ECV-304 cells were obtained from the European Collection of Animal Cell Cultures (ECACC, Salisbury, Wiltshire, United Kingdom). The cells were cultivated in Dulbecco’s modified Eagle’s medium from Life Technologies, Inc. supplemented with 1% fetal calf serum, 2 mM L-glutamine, and antibiotics. Cells were grown on 10-cm² dishes and cultured at 37 °C in a 5% CO2 incubator. The COS cells were cotransfected with −578 E-selectin

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1 The abbreviations used are: NF-κB, nuclear factor-κB; CAT, chloramphenicol acetyltransferase; CBP, CREB-binding protein; CREB, cAMP-response element-binding protein; TNF, tumor necrosis factor.

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Fig. 1. p65 and p53 mutually repress each other’s transcription in a CBP-dependent manner. A, p65 represses p53-dependent activation of a p21-promoter-reporter. Saos2 cells were cotransfected with 3 μg of a p21 promoter-luciferase reporter, increasing amounts (10 and 20 ng) of p53 alone (lanes 2 and 3), or 20 ng of p53 with increasing amounts (0.1, 0.2, 0.5, 1.0 μg) of a p65 expression vector (lanes 5–8). B, p65 repression of p53-activated gene expression is CBP-dependent. COS cells were transfected with 1 μg of a p21 promoter-luciferase reporter construct, 20 ng of p53 alone (lane 2), or with increasing amounts (0.1, 0.5, or 1.0 μg) of a p65 expression vector, in the absence (lanes 3–5), or presence of, 10 μg of a CBP expression vector (lane 6). C, p53 repression of p65-dependent gene expression is CBP-dependent. COS cells were cotransfected with 2 μg of an E-selectin promoter-CAT reporter construct (−578-CAT), 100 ng of a p65 expression vector alone (lane 2), or with increasing amounts (5, 10, 20, or 35 ng) of a p53 expression vector (lanes 3–6), in the presence or absence of increasing amounts (0.5 and 1.0 μg) of an MDM2 expression vector (lanes 7–9) or increasing amounts (3, 5, 8, or 10 μg) of a p65 expression vector (lanes 10–13). The total concentration of DNA was adjusted to 10 μg/6-cm tissue culture dish with empty pCR3 expression vector. Forty-eight hours post-transfection, luciferase or CAT activities in cell extracts were measured as described previously. Portions of the cellular extracts from A–C were analyzed for p65 and p53 by Western blot analysis ( Insets ). Data presented are representative of at least three independent transfections.

RESULTS AND DISCUSSION

p65 and p53 Mutually Repress Each Other’s Transcription—If CBP functions as a signal integrator for the NF-κB and p53 pathways, there might be mutual transcriptional interference between these two signal-dependent activators. To determine whether the p65 component of NF-κB alters p53 function, a p53 reporter plasmid containing the p21 promoter, a known p53 target gene associated with arrest of the cell cycle, was cotransfected with a fixed amount of p53 and increasing amounts of a p65 expression plasmid. As expected, p53 strongly activates the p21 promoter (Fig. 1A, lanes 2 and 3). Cotransfection of p65 resulted in a dose-dependent suppression of this reporter plasmid in SaoS2 cells (Fig. 1A, lanes 5–8) or in COS cells (Fig. 1B, lanes 3–5). p65-mediated suppression was also seen with a Bax promoter-reporter, a gene that is associated with the induction of apoptosis, as well as an artificial promoter containing only multiple p53 binding sites (data not shown). In contrast to the p53-dependent genes, no repression of a Gal4-dependent promoter-reporter gene was seen (data not shown).

Control studies also demonstrate that overexpressed p65 did not decrease production of p53 from the corresponding expression construct (Fig. 1, A and B, Insets).

To determine whether p53 could alter NF-κB-dependent gene expression, similar studies were done with a NF-κB-dependent E-selectin promoter-reporter construct (2, 28), a fixed amount of p65, and increasing amounts of p53. As predicted, p65 results in an induction of the NF-κB-dependent reporter gene (Fig. 1C, lane 2). Increasing amounts of p53 resulted in a dose-dependent suppression of p65-mediated transactivation (Fig. 1C, lanes 3–6). This effect was reversed by MDM2 (Fig. 1C, lanes 7–9), an inhibitor of p53 transactivation. Control studies demonstrated that overexpression of MDM2 did not alter levels of p65 (Fig. 1C, Insets), although in some experiments it modestly increased p56-dependent gene expression (Fig. 1C, lanes 7–9). MDM2 does not physically interact with p65 (data not shown). The presence of p53 had no effect on nuclear accumulation of NF-κB or DNA binding activity, and it did not physically interact with p65 (data not shown). Additionally, p53 did not increase expression of an inhibitor of NF-κB, IκB-α (data not shown). Thus p65-mediated transactivation activation is repressed by p53, and p53-dependent gene expression is repressed by p65.

CBP Rescues p53 Inhibited p65-dependent Transactivation—One possibility suggested by the preceding findings is that the formation of complexes between either p65 or p53 and specific coactivators would reduce the amount of coactivator available...
for transcriptional activation. If competition for limiting amounts of CBP accounts for the inhibitory effect of p53, then increased levels of the coactivators should restore, or rescue, p56-dependent gene expression. Indeed, the inhibitory effect of p65 on p53-dependent gene expression was completely abolished by cotransfection of a vector expressing CBP (Fig. 1C, lanes 10–13). Control studies demonstrated that CBP overexpression did not alter levels of either p53 (Fig. 1, B and C, insets) or p66 (Fig. 1C, inset). Additionally, overexpression of an irrelevant transcriptional activator, or mutated forms of CBP, did not result in rescue (data not shown). Collectively, these functional studies demonstrate that CBP is limiting for both p56- and p53-dependent transactivation and suggest that CBP can rescue the mutually repressive interaction between the two activators.

**TNF-activated NF-kB Is Inhibited by UV Light-induced p53**—The mutual transrepression of p53- and p65-dependent gene expression described above was observed with overexpressed activators and might not reflect the situation with authentic levels of these transcription factors. To address this important issue, we determined whether TNF-activated NF-kB was capable of inhibiting endogenous p53-mediated gene expression. Endothelial cells transfected with an E-selectin promoter-reporter were treated with TNFα and exposed to increasing amounts of UV irradiation. As expected, TNFα-activated expression of the E-selectin promoter-reporter construct (Fig. 2A, lane 2). UV illumination resulted in a dose-dependent suppression of this activity (Fig. 2A, lanes 3–5). Cotransfection of an MDM2 expression plasmid, while significantly inhibiting a p53 transcriptional response (Fig. 3 and data not shown), increased expression from the NF-κB-dependent E-selectin promoter-reporter (Fig. 2A, lane 6). This suggests that the transcriptional activating capacity of endogenous p53 is required for the suppression of the E-selectin promoter-reporter construct. The inhibitory effect of endogenous p53 on NF-κB-dependent gene expression was completely abolished by cotransfection of a vector expressing CBP (Fig. 2A, lane 7). Control studies indicated that CBP rescued endogenous p53-suppressed NF-κB-dependent gene expression.

**NF-κB Activity Is Suppressed following UV Stimulation**—The findings described above predict that if endogenous NF-κB and p53 are induced simultaneously they would inhibit each other’s transcriptional activity. Because UV irradiation acti-
vates both transcription factors (29), we used it as a stimulus to examine the effect of the signaling pathways on each other. UV exposure strongly induces expression of a p21 promoter-reporter construct (Fig. 3A) in a time-dependent manner. In parallel, UV also activated expression of an E-selectin promoter-reporter gene (Fig. 3B). Cotransfection of an MDM2 expression plasmid blocked the p53 transcriptional response (Fig. 3A), while increasing expression of the NF-κB-dependent reporter plasmid (Fig. 3B). These results are consistent with the proposal that the transcriptional activity of endogenous NF-κB is regulated by p53.

From these studies we suggest that cross-talk between the p53 and NF-κB signaling cascades is mediated by CBP. These findings are consistent with observations that the levels of p300 are limiting relative to those of p65 (30) and that developmental processes are sensitive to the overall gene dosage of either CBP or its homolog, p300 (31). The common dependence of both p65 and p53 on CBP/p300 suggests that the coactivators may integrate multiple signaling pathways that converge on these transcription factors. For example, p53 inhibits AP-1-dependent transcription, and overexpression of p300 abolished the ability of p53 to inhibit AP-1 activity (18). Similar interplay was observed between p53 and E2F through the coactivator p300 (32). NF-κB-dependent gene expression is also regulated by coactivator complexes (38). These assemblies could be used differentially by specific transcription factors, limiting cross-talk between signaling pathways.

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