We recently reported that cAMP suppresses apoptosis in colon cancer cells and induces cellular inhibitor of apoptosis protein-2 (c-IAP2) via a cAMP-responsive element (CRE), suggesting a mechanism for chemoprevention of colon cancer by non-steroidal anti-inflammatory drugs.

In this study, we used T84 human colon cancer cells to define the pathway by which increases in cAMP induce c-IAP2 expression. Treatment with several different cAMP agonists stimulated phosphorylation of CRE-binding protein (CREB) and activated expression of c-IAP2 in a CREB-dependent manner. Studies with pharmacological inhibitors revealed that cAMP-dependent phosphorylation of CREB required activation of ERK1/2 and p38 MAPK but was largely independent of protein kinase A. Immunoblots and transcriptional reporter assays using specific inhibitors, as well as expression of constitutively active forms of MEK1 and MKK3, showed that c-IAP2 induction by cAMP is regulated predominantly through ERK1/2 and p38 MAPK and suggested involvement of p90 ribosomal protein S6 kinase and mitogen and stress response kinase-1 as well. Consistent with these results, we found that cAMP-dependent suppression of apoptosis was blocked by treatment with inhibitors of ERK1/2 and p38 MAPK. We conclude that cAMP can induce c-IAP2 expression in colon cancer cells through CREB phosphorylation and CRE-dependent transcription in a manner that involves activation of ERK1/2 and p38 MAPK. These results emphasize that activation of kinases other than protein kinase A can mediate the actions of agents that increase cAMP, particularly in the regulation of CREB-dependent events.

Suppression of normal apoptotic pathways contributes to tumor progression and confers resistance to cytotoxic anticancer drugs and radiation (1). The second messenger cAMP has antiapoptotic actions (2–4), and its primary effector enzyme, PKA,1 is a target for cancer therapy (5–7). Prostaglandin E2 (PGE2), which is formed from arachidonic acid by cyclooxygenases, binds to G2-coupled receptors and increases intracellular cAMP concentration (8). Cyclooxygenase levels are high in human colon cancers (9), and cyclooxygenase inhibition prevents cell proliferation and promotes apoptosis (10, 11). Recently we reported that increases in cAMP levels promoted by PGE2, or other agents that raise cAMP inhibit apoptosis in colon cancer cells through the induction of c-IAP2, therefore suggesting a novel mechanism of cancer chemoprevention by non-steroidal anti-inflammatory drugs (12).

Inhibitors of apoptosis proteins (IAPs) are characterized by a domain of about 70 amino acids, termed the baculoviral IAP repeat (BIR), based on the original discovery of these apoptosis suppressors in the baculoviral genome (13, 14). IAP family proteins have potentially important roles in the regulation of apoptosis and tumorigenesis (15, 16). The expression of survivin, one of the IAP family proteins, is significantly increased in several human cancers (17). Although eight human IAPs have been identified (18), c-IAP1/BIRC2 and c-IAP2/BIRC3 are the only IAPs that appear to be part of a signaling complex recruited to the cytoplasmic domain of the type 2 TNF receptor (19). In addition, c-IAP2 was also suggested to be a causative gene of mucosa-associated lymphoid tissue lymphoma and to have a role in carcinogenesis and tumor progression (20). The active forms of caspase-3 and -7 are directly inhibited by c-IAP2 (19), which can also prevent the proteolytic processing of procaspase-3, -6, and -7 by blocking the cytochrome c-induced activation of procaspase-9 (21).

Expression of c-IAP2 is regulated through multiple regulatory elements in its promoter region. A NF-κB binding site is essential for induction by TNFα in Jurkat T cells (22) and radiation in human embryonic kidney 293 cells (23). Dexamethasone induces c-IAP2 expression through a putative glucocorticoid response element in A549 human lung cancer cells (24), while a cAMP-responsive element (CRE) has an essential role in induction of c-IAP2 expression by cAMP in T84 colon cancer cells (12).

This paper is available online at http://www.jbc.org
In this study, we analyzed the signal transduction pathways that mediate induction of c-IAP2 in response to increases in cAMP in T84 cells. We show that cAMP-promoted phosphorylation of CREB and transcriptional activation of c-IAP2 appear in large part to be mediated by activation of ERK1/2 and p38 MAPK, perhaps acting via p90RSK and MSK1.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Reagents**—Anti-c-IAP2 polyclonal antibody and anti-actin monoclonal antibody (mAb) were from Chemicon International (Temecura, CA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Polyclonal antibodies against CREB, phospho-CREB, phospho-MSK1, phospho-p90RSK, p44/42 MAPK, p38 MAPK, and phospho-p38 MAPK and the MEK1/2 inhibitors PD98059 and U0126 were obtained from Cell Signaling Technology Inc. (San Diego, CA). mAb against Rap1 was from BD Bioscience Pharmin. Cholera toxin (CTX), 8-CPT-cAMP, forskolin (Fsk), human recombinant TNFα, phorbol 12-myristate 13-acetate, H89, (R)-cAMPS, SB203580, and SB202190 were from Calbiochem. PGE2 was from Sigma. 8-CPT-2'-O-Me-cAMP was purchased from BIOLOG Life Science Institute (Bremen, Germany).

**Plasmids**—pCMV-HA (vector control), pCMV-CREB (wild-type CREB), pCMV-KCREB (dominant negative form), and pCMV-CREB (expression institute (Bremen, Germany).

**FIG. 1.** cAMP induces c-IAP2 expression primarily through PKA-independent pathways. A, T84 cells, transfected with pGL3-c-IAP2-WT-Luc and pSV40-RL-Luc, were stimulated for 6 h with PGE2, Fsk, or 8-CPT-cAMP (CPT) or for 12 h with CTX at the indicated concentrations. Cell extracts were assayed by a dual luciferase assay. Results represent relative increases compared with untreated controls and are mean ± S.E. (n = 3; *, p < 0.05 versus controls, respectively). B, T84 cells, transfected with pGL3-c-IAP2-WT-Luc and pSV40-RL-Luc, were treated with H89 (10 μM) or (R)-cAMPS (Rp, 100 μM) for 1 h and stimulated for 6 h with 8-CPT-cAMP (CPT, 100 μM) or 12 h with CTX (250 ng/ml). CTX and CPT both significantly increased c-IAP2 promoter activity in the absence or presence of inhibitors (p < 0.05). Results shown represent relative promoter activity compared with untreated cells without inhibitors as 100% and are mean ± S.E. (n = 3; *, p < 0.05; N.S., not significant). C, T84 cells were treated for 1 h with H89 (10 μM) or (R)-cAMPS (Rp, 100 μM) and further stimulated for 6 h with 8-CPT-cAMP (CPT, 100 μM) or 12 h with CTX (250 ng/ml). Cells were lysed in SDS sample buffer, and proteins were analyzed by immunoblotting with the indicated antibodies. The bottom graphs show densitometric results of the respective blots normalized against actin levels and expressed relative to untreated controls and are mean ± S.E. (n = 3; *, p < 0.05; N.S., not significant). CTX and CPT both significantly increased c-IAP2 protein expression in the absence or presence of inhibitors (p < 0.05). D, T84 cells, serum-starved for 12 h, were treated with H89 (10 μM) or (R)-cAMPS (Rp, 100 μM) for 1 h and stimulated with Fsk (1 μM) or 8-CPT-cAMP (CPT, 100 μM) for 3 min. Cell extracts were assayed for PKA activity using the PopTag assay. Results represent relative changes in PKA activity compared with untreated controls and are mean ± S.E. (n = 3; *, p < 0.05 versus Fsk- or CPT-treated cells without inhibitors, respectively). IB, immunoblot; pAb, polyclonal antibody.
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S133A (dominant negative form) were purchased from Clontech. pUSE-MEK1 (S185D/S286D) (dominant active form) was purchased from Upstate Biotechnology (Lake Placid, NY). pBabeHygro-MKK3A (S133A) (dominant active form) was a gift from Dr. Peiqing Sun (25). pGL3-c-IAP2-WT-Luc (−1931 to +27) and pGL3-c-IAP2-CREII-Luc (−87 to +27) were described previously (12).

Cell Culture—T84 human colon epithelial cells were cultured in 50% Dulbecco’s modified Eagle’s medium, 50% Ham’s F-12 medium supplemented with 5% newborn calf serum and 2 mM L-glutamine. Chinese hamster ovary (CHO) cells were cultured in Ham’s F-12 medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine.

Immunoblot Analysis—for detection of phosphorylation with a phospho-specific monoclonal antibody. The dual luciferase assay was conducted with the indicated antibodies. Specific binding was visualized by Super Signal West Dura extended duration substrate (Pierce) and quantified by using an EpiChemi II darkroom image analyzer (Uvina UV Products).

Dual Luciferase Assay—T84 cells were grown in 24-well plates were transfected using the FuGENE 6 transfection reagent (Roche Applied Science) with pGL3-c-IAP2 (−1931 to +27) or pGL3-c-IAP2-CREII-Luc (−87 to +27), pRL-SV40-Luc (Promega, Madison, WI) carrying Renilla luciferase under the control of a constitutively active SV40 promoter as a transfection control, and CREB, MEK1, or MKK3 expression vectors as indicated in the figure legends. The dual luciferase assay was conducted with a dual luciferase reporter assay system (Promega).

PKA Activity Assay—Kinase activity of PKA was assayed with the PepTag® assay (Promega). Briefly T84 cells serum-starved for 6 h were treated for 1 h with H89 (10 μM) or (Rp)-cAMPS (100 μM) and stimulated with forskolin (Fsk) or 8-CPT-cAMP for 3 min. Cells were washed with phosphate-buffered saline twice and lysed in lysis buffer (1% Nonidet P-40, 150 mM NaCl, 0.5 mM Na3VO4, 10 mM NaF, 1 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). For detection of other proteins, cells were lysed in 1× SDS sample buffer and sonicated twice for 15 s. Proteins were separated by SDS-PAGE and analyzed by immunoblotting with the indicated antibodies. Specific binding was visualized by Super Signal West Dura extended duration substrate (Pierce) and quantified by using an EpiChemi II darkroom image analyzer (Uvina UV Products).

ERK1/2 and p38 MAPK Kinase Assay—Kinase activities of ERK1/2 and p38 MAPK were assayed with a p44/42 MAPK assay kit (non-radioactive) and p38 MAPK assay kit (non-radioactive) (Cell Signaling Technology, Inc.), respectively. Briefly T84 cells serum-starved for 6 h were preincubated with inhibitors for 1 h followed by incubation with 8-CPT-cAMP for 30 min. Cells were lysed in lysis buffer and centrifuged at 20,000 × g for 10 min. Supernatants were incubated with immobilized anti-phospho-p44/42 MAPK or anti-phospho-p38 MAPK mAb for 4–12 h. Precipitated kinases were incubated with Ekl or activating transcription factor-2 (ATF-2) fusion protein for 30 min in kinase reaction buffer containing 200 μM ATP, and phosphorylation of fusion proteins was detected by immunoblotting with phosphospecific antibodies.

Rap1 Pull-down Assay—Cells were serum-starved overnight and incubated with inhibitors for 1 h followed by further stimulation as indicated in the figure legends. Cell lysates were prepared in lysis buffer (1% Nonidet P-40, 25 mM HEPES (pH 7.4), 150 mM NaCl, 10% glycerol, 1 mM EDTA, 10 mM MgCl2, 0.5 mM Na3VO4, 10 mM NaF, 1 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride) and centrifuged at 20,000 × g for 1 min. The supernatants were incubated with 15 μg of an agarose-conjugate of Ral GDS-Rap binding domain (Upstate Biotechnology) for 45 min, and the beads were washed with lysis buffer three times. The precipitates were separated by SDS-PAGE and analyzed by immunoblotting with anti-Rap1 mAb.

Analysis of Apoptosis—T84 cells were incubated with inhibitors for 1 h followed by further incubation with CTX or 8-CPT-cAMP for 1 h. Cells were stained with staurosporine for 12 h and lysed in lysis buffer after centrifugation of cells at 250 × g for 5 min, supernatants were isolated and assayed with the Cell Death Detection enzyme-linked immunosorbent assay PLUS kit (Roche Applied Science).

Caspase-3 Assay—Caspase-3 activity was assayed with a colorimetric assay kit (R&D Systems, Minneapolis, MN).

Data Presentation and Analysis—Results are shown as mean ± S.E. of at least three experiments as indicated in the figure legends. Comparisons between experimental groups were by paired or unpaired t test as appropriate with p < 0.05 considered significant.

RESULTS

CAMP Induces c-IAP2 Expression Predominantly through PKA-independent Pathways in T84 Colon Epithelial Cells—We have previously shown that increases in cellular cAMP levels can transcriptionally induce c-IAP2 expression in T84 cells (12). To characterize the underlying mechanisms, we investigated the effects of multiple agents that raise cellular cAMP concentrations to regulate the activity of a full-length transcriptional reporter of c-IAP2. PGE2 binds to Gs-coupled EP2 and EP4 receptors on colon epithelial cells and increase intracellular cAMP concentration (26, 27). Fsk directly stimulates adenyl cyclase activity and increases cAMP production. We also used CTX, which catalyzes the ADP-ribosylation of Gα, leading to the activation of adenylyl cyclase, and the membrane-permeant cAMP analog 8-CPT-cAMP. Stimulation of T84 cells with all four agonists that raise intracellular cAMP increased c-IAP2 promoter activity in a concentration-dependent manner (Fig. 1A).

To begin to define the downstream signaling mechanism for the cAMP-promoted increase in c-IAP2 promoter activity, we first focused on the role of PKA, the major effector kinase that mediates the action of CAMP. Surprisingly two different pharmacological inhibitors of PKA, H89 (10 μM) and (Rp)-cAMPS (100 μM), showed only limited inhibition (~25%) of cAMP-dependent increases in c-IAP2 promoter activity (Fig. 1B) and
protein expression (Fig. 1C), although increases in PKA activity after Fsk and 8-CPT-cAMP stimulation were effectively inhibited by the same concentrations of these agents (Fig. 1D). These data suggested that cAMP-induced c-IAP2 expression is predominantly regulated by PKA-independent pathways in colon epithelial cells. Subsequent studies were designed to examine this possibility.

**cAMP Stimulates c-IAP2 Promoter Activity through Phosphorylation of CREB**—Our previous data indicated that a CRE in the c-IAP2 promoter region is necessary for induction by cAMP (12), suggesting that CREB may be a prominent factor in controlling cAMP-dependent c-IAP2 transcription. To test this idea directly, we performed co-transfections with a minimal c-IAP2 transcriptional reporter that contains the proximal CREB phosphorylation (Fig. 2B) and data not shown). Together these data indicate that phosphorylated CREB plays an essential role for c-IAP2 gene transcription in response to increases in cAMP.

**CREB Phosphorylation Is Mediated by ERK1/2 and p38 MAPK**—Phosphorylation of CREB is required for its transcriptional activation (30). Although PKA is an important kinase for CREB phosphorylation, we found that in T84 cells the PKA inhibitors H89 and (R)<sub>p</sub>-cAMPS only slightly suppressed its cAMP-dependent phosphorylation (Fig. 2B). Therefore, we hypothesized that in this system other kinases, such as p90RSK, a kinase downstream of ERK1/2 (31), and MSK1, a kinase downstream of ERK1/2 and p38 MAPK (32), both of which can phosphorylate CREB in response to increases in cAMP.

Phosphorylation of p90RSK promoted by 8-CPT-cAMP was inhibited by the MEK1/2 inhibitor U0126 (10 μM), while it was slightly, but not significantly, increased by the p38 MAPK inhibitor SB202190. 8-CPT-cAMP-promoted phosphorylation of MSK1 was inhibited moderately by both U0126 and SB202190 and more strongly by the combination of these agents (Fig. 3). The PKA inhibitors (R)<sub>p</sub>-cAMPS (Fig. 3) and H89 (data not shown) did not affect Fsk- or 8-CPT-cAMP-stimulated phosphorylation of p90RSK or MSK1. These results suggest that cAMP activates p90RSK through ERK1/2 and
MSK1 through ERK1/2 and p38 MAPK in T84 cells and that activation of these kinases occurs independently of PKA.

Importantly CREB phosphorylation promoted by 8-CPT-cAMP (Fig. 3) or Fsk (data not shown) was significantly suppressed by U0126 or SB202190 alone, and this suppression was even more pronounced after addition of both inhibitors. Addition of (Rp)-cAMPS, which by itself only minimally decreased CREB phosphorylation (Fig. 2, B and 3, lane b), together with U0126 and SB202190 caused only modest and not significant further inhibition beyond that produced by the latter agents (Fig. 3, lanes e and f). Similar results were obtained by using other inhibitors for ERK1/2 and p38 MAPK, i.e. PD98059 and SB203580, respectively (data not shown). These data suggest that ERK1/2 and p38 MAPK are involved in the cAMP-dependent phosphorylation of CREB.

cAMP Activates ERK1/2 and p38 MAPK in T84 Cells—Because the results in Fig. 3 suggested the involvement of ERK1/2 and p38 MAPK in the phosphorylation of p90RSK and MSK1, we directly examined the activation of ERK1/2 and p38 MAPK in response to agents that raise cAMP levels. Treatment of T84 cells with PGE2, Fsk, or 8-CPT-cAMP enhanced phosphorylation of ERK1/2 and p38 MAPK within 30 min as did TNFa as a positive control (Fig. 4A). Furthermore in vitro kinase assays demonstrated that increased phosphorylation of ERK1/2 and p38 MAPK after 8-CPT-cAMP stimulation was accompanied by activation of these kinases. Thus, activated-ERK1/2 or p38 MAPK immunoprecipitated from 8-CPT-cAMP-stimulated cell lysates showed an increased ability, relative to control cells, to phosphorylate their respective substrates, Elk or ATF-2 fusion proteins (Fig. 4, B and C). Increased ERK1/2 kinase activity was completely inhibited by treatment with U0126 but was insensitive to PKA inhibitors (Fig. 4B), documenting further that ERK1/2 activation is PKA-independent in T84 cells. In contrast, in CHO cells ERK1/2 activation by cAMP is a PKA-dependent event because H89 inhibited ERK1/2 phosphorylation induced by 8-CPT-cAMP stimulation (Fig. 4E), a finding consistent with prior reports (34). The PKA inhibitors also failed to inhibit the cAMP-dependent increase in p38 MAPK activity (Fig. 4C). Taken together, these results support the conclusion that increases in cAMP activate both ERK1/2 and p38 MAPK in T84 cells, and these activations appear to occur independently of PKA.

In addition to PKA, another recently recognized effector of cAMP action is Epac, a guanine nucleotide exchange factor for the small GTPase Rap, which is able to regulate ERK1/2 activity in some cells (35, 36). Because our results showed that cAMP-promoted ERK1/2 activation is a PKA-independent event, we examined the ability of 8-CPT-2'-O-Me-cAMP, a specific activator of Epac that activates Rap in CHO, PC12, and human embryonic kidney 293T cells (34), to stimulate ERK1/2 activation in T84 cells. We found that 8-CPT-2'-O-Me-cAMP failed to activate ERK1/2 and p38 MAPK in these cells (Fig. 4, D and E), although as a positive control, this analog was able to activate Rap1 (Fig. 4F), consistent with the conclusion that cAMP-promoted ERK1/2 activation in T84 cells occurs via an Epac-independent pathway.

cAMP Induces c-IAP2 Expression through the Activation of ERK1/2 and p38 MAPK—We next examined the involvement of ERK1/2 and p38 MAPK in cAMP-dependent transcriptional activation of the c-IAP2 gene. Stimulation of c-IAP2 promoter activity by CTX and 8-CPT-cAMP (see Fig. 1A) was significantly diminished by adding the MEK1/2 inhibitor U0126 and the p38 MAPK inhibitor SB202190, and this inhibition was even greater by combined treatment with both inhibitors (Fig. 5A). Consistent with this, increased c-IAP2 protein expression after stimulation with CTX and 8-CPT-cAMP was also markedly inhibited by the combination of inhibitors of MEK1/2 and p38 MAPK (Fig. 5B). Addition of (Rp)-cAMPS yielded no significant further inhibition of c-IAP2 expression, providing addi-
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Fig. 6. cAMP regulates apoptosis through ERK1/2 and p38 MAPK. T84 cells were incubated for 1 h with H89 (10 μM), (Rp)-cAMPS (Rp, 100 μM), SB202910 (SB, 20 μM), and/or U0126 (U, 10 μM) as indicated followed by further incubation for 1 h with CTX (250 ng/ml) or 8-CPT-cAMP (CPT, 100 μM). Cells were stimulated with staurosporine (200 nm) for 12 h, and oligonucleosome release into the cytoplasm was assayed by enzyme-linked immunosorbent assay (A and B), or for 6 h, and caspase-3 activity was assayed by enzyme-linked immunosorbent assay (C). A and C, results represent relative increases compared with untreated cells without inhibitors, respectively, and are mean ± S.E. (n = 3; *, p < 0.05 versus staurosporine-treated cells without CTX or CPT stimulation; #, p < 0.05). B, results represent relative levels of 8-CPT-cAMP-mediated inhibition of DNA fragmentation induced by staurosporine compared with CPT- and staurosporine-treated cells without inhibitors as 100% and are shown as mean ± S.E. (n = 3; *, p < 0.05 versus CPT- and staurosporine-treated cells without inhibitors).

Fig. 7. Model of cAMP-mediated c-IAP2 induction. PGE2-, CTX-, and Fsk-stimulated increases in cAMP activate PKA, ERK1/2, and p38 MAPK followed by p90RSK and MSK1 activation. The actions of cAMP on ERK1/2 and p38 MAPK activation are likely to be indirect as indicated in the figure with two arrows. CREB, phosphorylated in response to activation of p90RSK and MSK1, and to a lesser degree PKA, then induces c-IAP2 expression through CRE within the proximal c-IAP2 promoter region. c-IAP2 protein inhibits activated caspase-3 and apoptosis. AC, adenyl cyclase.

ERK1/2 and p38 MAPK play a pivotal role in the cAMP-promoted induction of c-IAP2 via increased phosphorylation of CREB.

cAMP Regulates Apoptosis in Colon Epithelial Cells through ERK1/2 and p38 MAPK—To assess the biological significance of the involvement of ERK1/2 and p38 MAPK in the c-IAP2 induction stimulated by cAMP, we assayed T84 cells for apoptosis. Treatment of T84 cells with CTX or 8-CPT-cAMP inhibited apoptosis induced by staurosporine as assessed by quantitative analysis of DNA fragmentation. This inhibition was abrogated by adding a combination of inhibitors of ERK1/2 and p38 MAPK (Fig. 6A). When we examined these inhibitors individually, U0126 and SB202190 as well as H89 but not (Rp)-cAMPS, each significantly blunted the 8-CPT-cAMP-promoted inhibition of staurosporine-induced apoptosis (Fig. 6B). The difference between the responses to H89 and (Rp)-cAMPS might relate to the ability of H89 to inhibit not only PKA but also MSK1 (38). U0126 and SB202190 also blocked the inhibition of caspase-3 activity produced by CTX or 8-CPT-cAMP treatment of T84 cells (Fig. 6C); this effect was not further enhanced by addition of (Rp)-cAMPS. These results are consistent with the action of c-IAP2 in binding to the active form of caspase-3 and thereby diminishing its activity (15) and with results of experiments shown above for the role of kinases other than PKA in regulation of c-IAP2 expression. Fig. 7 shows a model that summarizes these pathways.

DISCUSSION

Apoptosis is a complex cellular process that is regulated by a balance of stimulatory and inhibitory pathways. The ability of cAMP to inhibit apoptosis might result from a blockade of proapoptotic pathways, a stimulation of antiapoptotic pathways, or a combination thereof. While some of the proapoptotic members of the Bcl-2 family, such as BAD, have been reported to be phosphorylated and inactivated by PKA, thereby resulting in inhibition of apoptosis (40–43), we recently identified an alternative mechanism for cAMP-mediated antiapoptosis: an inhibition of apoptosis in intestinal epithelial cells by agonists that increase cAMP and induce c-IAP2 via a CRE in the c-IAP2 promoter (12). We showed that c-IAP2 induction by increases in cAMP levels inhibits apoptosis that occurs in response to anti-Fas antibody (extrinsic apoptotic pathway) or staurosporine (intrinsic apoptotic pathway). The results thus suggested a novel mechanism for the cancer-chemopreventive effect of nonsteroidal anti-inflammatory drugs, which inhibit cyclooxygenases and decrease PGE2 synthesis (12).

In the current studies, we have identified new aspects of the
signal transduction pathway by which increases in cAMP induce c-IAP2 expression in colon cancer cells (Fig. 7). The data show that CREB phosphorylation is a key step but that the phosphorylation appears to occur by kinases other than the expected involvement of PKA. Activation of p90RSK and MSK1, which follow ERK1/2 and p38 MAPK activation, appears to be involved in both CREB phosphorylation and c-IAP2 induction. The current data also suggest that agents that raise intracellular cAMP and induce c-IAP2 expression in T84 cells do not appear to act via ATF-2 and CCAAT/enhancer-binding protein, other transcriptional factors that bind to CRE, because we found minimal phosphorylation of ATF-2 and CCAAT/enhancer-binding protein after cAMP treatment.

Conventional ideas emphasize that cAMP-mediated transcriptional responses involve the ability of PKA to phosphorylate CREB (30). However, the data shown here provide evidence for an alternative mechanism whereby cAMP promotes phosphorylation of CREB through ERK1/2 and p38 MAPK and perhaps other protein kinases as well. Previous data have implicated a role for p90RSK in the phosphorylation of CREB promoted by certain growth factors (44, 45). The potential role of p38 MAPK in cancer expression, mitosis, motility, and metabolism to apoptosis. The mechanisms of MAPK activation.

Conventional ideas emphasize that CREB phosphorylation is a key event in the induction of c-IAP2 via multiple protein kinases in addition to PKA. The data also suggest that kinase inhibitors, other than inhibitors of PKA, might be able to blunt cAMP-promoted antiapoptosis. As an alternative approach, one might consider a CRE decoy system in which a decoy oligodeoxynucleotide carrying a CRE can be used to inhibit CRE-directed gene transcription and tumor growth without affecting normal cell growth (39).

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