Transcription factor CCAAT/enhancer-binding protein β (C/EBPβ) plays an important role in hormone-dependent gene expression. In osteoblasts C/EBPβ can increase insulin-like growth factor I (IGF-I) transcription following treatment with hormones that activate protein kinase A, but little is known yet about the expression of C/EBPβ itself in these cells. We initially showed that prostaglandin E$_2$ (PGE$_2$) rapidly enhances C/EBPβ mRNA and protein expression, and in this study we identified a 3′-proximal region of the C/EBPβ promoter containing a 541-bp upstream sequence that could account for this effect. PGE$_2$-dependent activation of C/EBPβ was blocked by expression of a mutated regulatory subunit of protein kinase A or by mutation of two previously identified cAMP-sensitive cis-acting regulatory elements within the promoter between bp −111 and −61. Nuclear protein binding to these elements was induced by PGE$_2$, required new protein synthesis, and was sensitive to antibody to the transcription factor termed Fos-related antigen 2 (Fra-2). Fra-2 cDNA generated from rat osteoblasts by reverse transcriptase PCR was 95% homologous to human Fra-2, and PGE$_2$ rapidly induced Fra-2 mRNA and protein expression. Consistent with these findings, over-expression of Fra-2 significantly increased C/EBPβ promoter activity in PGE$_2$-induced osteoblasts, whereas expression of Fra-2 lacking its activation domain had a dominant negative inhibitory effect. Together, these results reveal a significant, hormone-dependent role for Fra-2 in osteoblast function, both directly, through its ability to increase new C/EBPβ gene expression, and indirectly, through downstream C/EBPβ sensitive genes.

Many cells and tissues express one or another of the several C/EBPs transcription factor gene family members, termed C/EBPα, -β, -δ, -γ, and -ε (1, 2). Individual C/EBPs can form homodimers or heterodimers and share common DNA binding response elements, consistent with the high degree of homology in their carboxyl termini where their dimerization and DNA binding domains reside. Of these, basal expression of C/EBPβ is high in liver, intestines, differentiating adipocytes, lung, kidney, and spleen, as well as in mononuclear blood cells. However, basal expression of C/EBPδ is relatively low in osteoblasts, but it can be enhanced by treatment with glucocorticoid, PGE$_2$, or 1,25(OH)$_2$ vitamin D$_3$ (3−5).

Because the various C/EBPs are widely expressed, it is no surprise that they direct the synthesis of a large panel of target genes. In osteoblasts either C/EBPα or C/EBPβ, which are variably expressed in several osteoblastic cell models, can in turn activate the expression of several prominent downstream genes, including those encoding IGF-I, IGFBP-5, IL-6, osteocalcin, and cyclooxygenase 2 (4, 6−9).

Earlier we reported that Runx2, a transcription factor essential for osteogenesis (10, 11), is an important, direct regulator of C/EBPδ expression in osteoblasts, by way of a Runx binding sequence located between bp −165 to −159 in the C/EBPδ gene promoter (12). Moreover, through an apparent negative feedback inhibition, the carboxyl-terminal region of C/EBPδ can bind directly to Runx2 and in this way self-limit C/EBPδ expression and activity. Others have reported roles for STAT3, Sp1, and C/EBPβ itself in the regulation of C/EBPδ expression in other cell models (13−16). By contrast, molecular mediators that direct C/EBPβ gene expression have been better established in nonskeletal tissue-derived cells. For example, studies in hepatocytes defined two cAMP-responsive elements (CREs) that are located between bp −121 and −71 in the C/EBPβ promoter and can interact with CREB and C/EBPβ to drive C/EBPβ gene expression. In those cells, lipopolysaccharide increases C/EBPβ expression through shared or distinct elements that require c-Jun and ATF-2, whereas IL-6-dependent induction of C/EBPβ involves an indirect association of STAT3 to these CRE sequences (17−19).

Importantly, agents or events associated with trauma, inflammation, and the acute phase response have critical effects on C/EBPβ synthesis, perhaps to assist the expression of downstream genes associated with recovery and tissue repair (1, 2). In osteoblasts, we earlier reported an increase in C/EBPβ expression in response to PGE$_2$ (3). In the current study, we have characterized the molecular mediators that can account for this effect. We demonstrate the relative importance of a specific protein kinase system and the two previously identified cis-acting CREs that drive new C/EBPβ synthesis. Finally, we show that one trans-acting transcription factor that can control this event in differentiating osteoblasts is distinct from those.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY622611.

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The abbreviations used are: C/EBP, CCAAT/enhancer-binding protein; PGE$_2$, prostaglandin E$_2$; EMSA, electrophoretic mobility shift assay; CRE, cAMP-responsive element; CREB, cAMP-response element-binding protein; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; IL-6, interleukin-6; PTH, parathyroid hormone; Fra-2, Fos-related antigen 2; PKA, protein kinase A; PKC, protein kinase C; STAT, signal transducers and activators of transcription; ATF, activating transcription factor.

W. Chang, A. Rewari, M. Centrella, and T. L. McCarthy, unpublished studies.
EXPERIMENTAL PROCEDURES

Cell Culture—Primary osteoblast-enriched cultures were prepared from parietal bone of 2-day-old Sprague-Dawley rat fetuses (Charles River Breeding Laboratories) by methods approved by the Yale Institutional Animal Care and Use Committee. Bone sutures were dissected, and cells were released from the bone fragments by five sequential collagenase digestions. Cells pooled from the last three digestions express many biochemical features that typify differentiating osteoblasts, including high levels of nuclear factor Runx2, parathyroid hormone (PTH) receptor, type I collagen synthesis, and alkaline phosphatase activity (20–22). They also exhibit an increase in osteocalcin expression in response to vitamin D₃, differential sensitivity to transforming growth factor-β, bone morphogenetic protein-2, and various prostaglandins and form mineralized nodules in vitro (23–28). Cells were plated at 4,000/cm² in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. COS-7 cells (CRL 1651) from the ATCC were cultured in identical medium. Hormone treatments were performed in serum-free medium.

Radiolabeled reporter plasmids—C/EBPβ promoter constructs, prepared from a λ genomic library of rat genomic DNA, were based on earlier reported sequence information (17). Other plasmids were based on rat Fra-2 sequence information in GenBank (accession no. NM_012954), utilizing the EcoRI and XbaI restriction sites (underlined) for directional cloning. An expression plasmid encoding dominant negative Fra-2 was produced from this construct by reverse transcriptase PCR to delete amino acids 208–326, comprising its transactivation domain.

Transfections—Promoter-reporter constructs, gene expression plasmids, or empty parental vectors were pre-titrated for optimal expression of CREs within the C/EBPβ promoter (17) by overlap PCR and mutated oligomer primer pairs. The primers used to mutate CRE1 were: CRE1_Foward, 5’-GGGCGGCCCCGCCGATCCGGCC-3’; and CRE1_reverse, 5’-GGGCGGCGCCGCCCCGGGG-3’. Mutations were introduced in two previously described CREs within the C/EBPβ promoter (17). Hybridization efficiencies were assessed in parallel with positive and negative controls including cDNA inserts encoding rat C/EBPα or rat Fra-2 were transfected into C6 cells (a kind gift from Dr. M. Croce, Duke University) and then supplemented to obtain a final concentration of 5% serum. Cells plated at 30,000/cm² in medium supplemented with 0.8% fetal bovine serum for 16 h (23). The C/EBPα and Fra-2 proteins were detected by Western blot analysis using anti-C/EBPα and anti-Fra-2 antibodies, respectively, and chemiluminescence (3, 31).

Polymerase One-step RT-PCR System (Roche Applied Science) with C.therm. (Promega). 

RESULTS

PGE₂ Induces C/EBPβ Expression—We previously showed that C/EBPβ is an important regulator of IGF-I expression in PFK-activated osteoblasts, by way of a single high affinity C/EBP binding half-site located within exon 1, a transcribed, noncoding, and highly conserved region of the IGF-I gene. C/EBPβ is the principal endogenous C/EBP in unstimulated osteoblasts (4). Close examination of its intronic binding using the IGF-I promoter derived C/EBP binding element, designated HS3D, and nuclear extract from PGE₂-activated osteoblasts, showed two prominent complexes and suggested the presence of multiple proteins. As shown in Fig. 1A, antibodies to either C/EBPβ or C/EBPβ each effectively reduced protein binding to this element. The upper gel shift complex that occurred with C/EBPβ was abolished by anti-C/EBPβ antibody, whereas both complexes were reduced by anti-C/EBPβ antibody, consistent with the importance of heterodimers containing both C/EBPβ isoforms. Treatment with PGE₂ rapidly elevated the levels of C/EBPβ mRNA and protein. A large increase in C/EBPβ mRNA occurred within 1 h of treatment, peaked at 2 h, and declined but remained significantly elevated for at least 24 h (Fig. 1B). By Western immunoblot analysis, a maximal increase in C/EBPβ protein in total osteoblast extract was achieved by 4 h of PGE₂ treatment (Fig. 1C).

Locating the PGE₂-responsive Element in the C/EBPβ Promoter—To locate regulatory elements utilized by osteoblasts after PGE₂ activation, cells were transfected with reporter plasmids encoding progressive C/EBPβ promoter truncations. The C/EBPβ promoter fragments shared a common 3’-end at bp +54 but terminated at bp –2700, –1300, or –541 at their 5’-ends. As shown in Fig. 2A, gene expression through each fragment was significantly induced by 6 h of treatment with PGE₂. Earlier evidence from studies with hepatocytes showed two important cAMP-sensitive elements downstream of bp –541, located between bp –111 and –61 (17), allowing us to
focus our effort more precisely. Indeed, we found that mutation of either or both of these elements within the context of the -541 upstream segment significantly reduced basal C/EBPβ promoter activity in osteoblasts and severely limited the stimulatory effect PGE₂ by 75–80%. To assess the kinase systems responsible for the stimulatory effect of PGE₂, we co-transfected osteoblasts with the fully functional -541 C/EBPβ promoter-reporter construct and either a mutant regulatory subunit of PKA that blocks its activation (PKAreg) or a dominant negative PKC (PKCDN) that exerts broad-spectrum PKC isoform inhibition (34, 35). Expression of the mutant regulatory subunit of PKA completely blocked C/EBPβ promoter activation, whereas expression of the PKCΔIN protein had no effect (Fig. 2B).

Inducible Protein Binding to CRE1 and CRE2—To assess protein binding to these important CRE elements that occur in the C/EBPβ promoter, we performed EMSA with [32P]-labeled oligonucleotide HS3D, corresponding to the C/EBP binding site in the rat IGF-I gene without (0) or with nonimmune rabbit Ig, anti-C/EBPβ (β), or anti-CREB antibody (ab) as indicated. B, total RNA from osteoblasts treated with vehicle (0) or 1 μM PGE₂, for the time periods indicated was fractionated by agarose gel electrophoresis, blotted onto charge-modified nylon, and probed with [32P]-labeled full-length C/EBPβ cDNA. The membrane was stripped and reprobed with low specific activity [32P]-labeled probe for 18 S rRNA. Binding was assessed by autoradiography. C, total osteoblast extracts were fractionated by SDS-PAGE through a 12.5% Laemmli gel under reducing conditions and probed with rabbit anti-C/EBPβ polyclonal antiserum.

appear to have an important effect on PKA-dependent C/EBPβ expression in osteoblasts, and transcription factors other than C/EBP or CREB appear to associate with these elements after treatment with PGE₂.

Fra-2 Binds to CRE1 and CRE2—PGE₂ activated the C/EBPβ promoter through a PKA-dependent event, and we speculated that these CREs might bind AP-1-like factors based on their nucleotide sequences and previous evidence from studies in osteoblasts (36). Select AP-1 transcription factors are expressed during osteoblast differentiation (37–40). Therefore we used a panel of antibodies to various AP-1-binding proteins, C/EBPδ, C/EBPβ, CREB (as shown in Fig. 3), ATF-2, and JunD.7 However, only antibodies specific to the transcription factor Fra-2 effectively modified binding to each of these elements. Again, analogous results occurred with oligonucleotides encoded by element CRE-1 and CRE-2, and data from results obtained using the oligonucleotide specific for CRE-1 are shown in Fig. 4A. Western immunoblot analysis showed that PGE₂ induced a rapid accumulation of Fra-2 (Fig. 4B) that was completely blocked by co-treatment with the protein synthesis inhibitor cycloheximide (Fig. 4C). Using sequence information obtained from GenBank™, we designed primers to synthesize full-length cDNA encoding rat Fra-2 by reverse transcriptase PCR from total osteoblast RNA. The rat Fra-2 sequence that we obtained using the oligonucleotide specific for CRE-1 are shown in Fig. 5. Fra-2 Regulates C/EBPβ Expression in Osteoblasts.
PGE2-treated osteoblasts were examined by EMSA with 32P-labeled B-anti-CREB antibody as indicated. CREB (CB) CRE1 (H) and CRE2 (P) oligonucleotide HS3D (H) that associate with the C/EBPs or CREB (CB), as defined under “Experimental Procedures,” or anti-C/EBPβ (β) or anti-C/EBPδ (δ) antibody as indicated.

Using the rat Fra-2 cDNA as a probe, we then examined Fra-2 mRNA by Northern blot analysis. Fra-2 mRNA levels increased within 30 min of PGE2 treatment, peaked at 1 h, and remained elevated for at least 4 h (Fig. 4D).

Fra-2 Regulates C/EBPβ Promoter Activity in PGE2-induced Osteoblasts—We produced expression plasmid constructs encoding full-length Fra-2 and a carboxyl-truncated dominant negative Fra-2 devoid of its carboxyl transactivation domain (41). The full-length and dominant negative Fra-2 were then co-transfected with either the fully active −541-bp C/EBPβ promoter plasmid or that containing the mutated CRE1 and CRE2 elements. Full-length Fra-2 significantly enhanced PGE2-induced C/EBPβ promoter activation in a concentration-dependent fashion (Fig. 6A), whereas dominant negative Fra-2 significantly reduced C/EBPβ promoter activation by 50% (Fig. 6B). Consistent with the importance of these CREs for Fra-2 activity, full-length Fra-2 failed to enhance gene expression in cells co-transfected to express C/EBPβ promoter that contained the CRE1/CRE2 mutations (Fig. 6A). The dominant negative expression construct encoding Fra-2 also suppressed basal C/EBPβ gene promoter activity (Fig. 6B). This effect may be direct through other currently unidentified Fra-2 binding elements, or it may be indirect through the formation of inactive heterodimer complexes between other important trans-acting factors and the dominant negative Fra-2 protein. In either case, these results confirm that C/EBPβ expression is transcriptionally regulated by PGE2 through the CRE1 and CRE2 AP-1 binding elements and reveal the stimulatory effect at these sites by newly synthesized Fra-2 in PKA-activated osteoblasts.

DISCUSSION

C/EBPβ and C/EBPδ are important components in the development of the acute phase response and participate in the induction of many genes involved in tissue remodeling (1, 2). In osteoblasts, C/EBPs can activate the expression of several important gene products, including IGF-I, IGFBP-5, IL-6, osteocalcin, and cyclooxygenase 2 (4, 6–9). C/EBPδ is the predominantly expressed C/EBP in unstimulated rat and human osteoblasts (4). In this regard, we earlier reported that Runx2, an essential transcription factor required for osteoblast differentiation, is responsible for basal and PGE2-induced C/EBPβ expression (12). Although basal C/EBPβ expression in osteoblasts is relatively low, PGE2 or any hormone, such as PTH, or agent, such as forskolin, that elevates cAMP levels and activates PKA rapidly induces its expression.

A novel role for C/EBPβ in the regulation of cell survival recently has been suggested. In hepatic stellate cells, oxidative stress activates ribosomal protein S-6 kinase, which can phosphorylate C/EBPβ on threonine 217, creating a functional so-called XEXD caspase substrate inhibitory box (42, 43). This evidence shows a direct link between C/EBPβ threonine 217 phosphorylation and an association with procaspase-1 and -8, which inhibits apoptosis. Therefore, C/EBPβ may have profound effects on cell survival as well as gene transcription.

Our study identified two Fra-2-dependent CREs in the C/EBPβ gene promoter and showed that they are responsible for PKA-dependent activation by PGE2 in osteoblasts. These elements were originally identified as CREB-binding elements in hepatocytes, whereas more recent studies indicate a potential auto-regulatory role for C/EBPβ through the more upstream binding sequence that we designated as CRE1 (17, 18). However, we found no CREB, C/EBPβ, or C/EBPδ binding to either CRE in PGE2-treated osteoblasts by EMSA. Moreover, when we transfected osteoblasts to over-express CREB in combination with the C/EBPβ promoter, we did not detect an increase in reporter gene expression. It is important to note that the increase in C/EBPβ expression in IL-6-activated hepatocytes is also thought to occur through these CREs, where the formation of a complex containing activated STAT3 is tethered to an unidentified 68-kDa protein that associates with each of these elements (19). Therefore, multiple observations, including our current findings, show the importance of both CREs in the activation of C/EBPβ gene expression in cells from several tissue sources, albeit through different trans-acting proteins.

In PGE2-activated osteoblasts, Fra-2 binds directly to these CREs in the C/EBPβ promoter. The increase in C/EBPβ gene expression by PGE2 required PKA activation and ongoing protein synthesis, consistent with the low level of Fra-2 in unstimulated osteoblasts and its rapid induction by PGE2. Overexpression of full-length Fra-2 further enhanced C/EBPβ promoter activity in osteoblasts activated with PGE2, whereas truncated, dominant negative Fra-2 severely suppressed this response. These gain- and loss-of-function effects confirm an important if not unique role for Fra-2 in the induction of C/EBPβ expression in differentiating osteoblasts. Furthermore, the activation domain of Fra-2 has several known phosphorylation sites (44–47). Therefore, post-translational kinase-dependent modification may play an important role in Fra-2-dependent C/EBPβ activation in osteoblasts, which will be the subject of our future studies.

The protein sequence predicted from the rat Fra-2 cDNA that we cloned retains 95.4% homology to human Fra-2 and 99% homology to the published rat Fra-2 nucleotide sequence (GenBank™ accession numbers NM_005253 and NM_012954, respectively). However, a comparison among the various Fra-2 sequences available shows that in four of five instances, amino acids predicted by our sequence that differ with the previously reported rat Fra-2 sequence are identical to those that occur in human Fra-2. Thus, some differences may relate to species, strain, or tissue variability. The sequence that we derived from rat osteoblast cDNA has been deposited in GenBank™ (accession no. AF622611).

Analogous to the C/EBPs, CREB, the ATFs, and Ap-1 factors c-Fos and c-Jun, Fra-2 is a member of the bZip (basic leucine...
Fra-2 Regulates C/EBPβ Expression in Osteoblasts

FIG. 4. PGE$_2$ induces Fra-2 expression in rat osteoblasts. A, nuclear extracts from osteoblasts treated for 4 h in serum-free medium with vehicle (0) or 1 μM PGE$_2$ (P) were examined by EMSA with $^{32}$P-labeled oligonucleotide CRE1 as described for Fig. 3 and either nonimmune Ig or anti-Fra-2 antibody (ab). B, total osteoblast extract from vehicle or PGE$_2$-treated osteoblasts for the time periods indicated was fractionated by SDS-PAGE through a 12.5% Laemmli gel under reducing conditions and probed with rabbit anti-Fra-2 polyclonal antiserum. C, total nuclear extracts from osteoblasts treated with PGE$_2$, without or with cycloheximide (Cyclohex), as indicated, were assessed as described in B. D, total RNA from osteoblasts treated with vehicle (0) or 1 μM PGE$_2$ for the time periods indicated was fractionated by agarose gel electrophoresis, blotted onto charge-modified nylon, probed with $^{32}$P-labeled full-length Fra-2 cDNA, and assessed by autoradiography. Parallel samples were stained with ethidium to detect 18 S and 28 S rRNA bands.

FIG. 5. Comparison of amino acid sequences corresponding to Fra-2 from human and rat cells. The sequence of rat osteoblast Fra-2 protein was derived from the cDNA cloned from primary cultures of fetal rat osteoblasts. The predicted protein sequence from rat osteoblast cDNA (Fra$_2$ _Rat OB) was compared with Fra-2 cloned from human monocytic cells (Fra$_2$ _Human _Mono) and rat pineal gland (Fra$_2$ _Rat PIN) using ClustalW software from the European Bioinformatics Institute (www.ebi.ac.uk). By the criteria of this software program, an asterisk below the sequence means that the residues in that column are identical in all sequences in the alignment. A colon below the sequence means that conserved substitutions were observed. A period below the sequence means that semi-conserved substitutions were observed. A period below the sequence means that semi-conserved substitutions were observed.

The zipper) family of transcription factors. Each member of this protein family appears to function as a dimer and can form homodimers or heterodimers with other select family members (1). Polyclonal anti-Fra-2 antibody modified nuclear protein binding to the CREs found in the C/EBPβ promoter by EMSA, but we did not detect C/EBPδ, C/EBPβ, CREB, ATF-2, or JunD in the gel shift complex using specific antibodies and similar methods. Unlike the C/EBPs, the transactivation domain of Fra-2 occurs in the carboxyl-terminal region, which contains potential phosphorylation sites, whereas its leucine zipper dimerization domain is centrally located, and its DNA binding domain resides in the amino-terminal region (41, 48–50). Therefore, this dissimilar organization of Fra-2 protein structure, at least by comparison with the C/EBPs (51, 52), suggests that it may have a restricted pattern of functional binding partners (50, 53, 54). Additional studies will be necessary to determine whether Fra-2 acts as a homodimeric transactivator of C/EBPβ gene expression in osteoblasts or to identify other potential binding partners for Fra-2 in this context.

Expression of Fra-2 during mouse embryonic development...
Osteocalcin is a target gene for C/EBP. Even so, fewer osteocalcin-expressing osteoblasts occurred in deficient mice, but osteoclast number and size were increased. Furthermore, osteoclast death. Osteoblast number was also unaffected in Fra-2-exhibited growth retardation, severe osteoporosis, and perinatal death. Osteoblast number was also unaffected in Fra-2-deficient mice, but osteoclast number and size were increased. Even so, fewer osteocalcin-expressing osteoblasts occurred in Fra-2-deficient animals. This is consistent with the view that osteocalcin is a target gene for C/EBPβ and that loss of Fra-2 would consequently lower the expression of C/EBPβ or its induction by hormones such as PGE₂ and PTH that effect bone remodeling.

In summary, our current studies reveal that C/EBPβ gene expression in osteoblasts is regulated by activation of PKA through two CREs that occur within a downstream region of the C/EBPβ gene promoter and that associate with newly synthesized transcription factor Fra-2. These results, in combination with our earlier studies revealing Runx-2-dependent expression of C/EBPβ, continue to define the complex molecular events (modeled in Fig. 7) that consequently control hormone-dependent changes in expression of the important bone growth factor, IGF-I.

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Fig. 6. Over-expression of native or dominant negative Fra-2 modifies C/EBPβ gene promoter activity in rat osteoblasts. Osteoblasts were transfected with the indicated amounts of pcDNA3 expression plasmids encoding full-length rat Fra-2 DNA (A) or dominant negative Fra-2 (Fra-2-DN) produced by carboxyl-terminal truncation to delete amino acids 208–322 (B) in combination with 250 ng of reporter plasmids driven by either the native 541-bp C/EBPβ promoter fragment or plasmid μ1,2 containing mutations in both CRE1 and CRE2 in a total of 500 μl. Compensating amounts of the parental expression vector pcDNA3 were added to balance the total plasmid load. Cells were treated for 6 h with vehicle (control) or 1 μM PGE₂ as indicated. Reporter gene activity was measured after 6 h of treatment with vehicle (control) or 1 μM PGE₂. Data were corrected for protein content and are the means ± S.E. from 9 or more replicate cultures per condition and 3 or more experiments. *, the stimulatory effect of PGE₂ was significantly different from control (p < 0.05) in cells transfected with 100–150 ng of native Fra-2 and significantly suppressed (p < 0.05) in cells transfected to express 100–300 ng of dominant negative Fra-2.

Fig. 7. PKA-dependent control of IGF-I gene expression in osteoblasts. A, osteoblasts exposed to hormones such as PGE₂ and PTH, having receptors that couple to adenylate cyclase, increase cAMP synthesis and enhance PKA activity, in turn increasing IGF-I mRNA transcription through a C/EBP-sensitive element in exon I within the IGF-I gene. B, previous studies showed that this occurs in part through a translation-independent effect on the activation of pre-existing C/EBPβ and C/EBPβ (central bifurcated arrow) (3, 5) and through a Runx-2-dependent transcriptional effect on new C/EBPβ expression (left column) (12). Studies in this report demonstrate a parallel Fra-2-dependent transcriptional effect on C/EBPβ gene expression (right column) revealing complex re-enforcing effects on IGF-I synthesis through increases in both C/EBP expression and activity. The question mark indicates a current gap in our knowledge about the molecular events that control Fra-2 synthesis.
