Interaction between Interferon Consensus Sequence-binding Protein and COP9/Signalosome Subunit CSN2 (Trip15)

A POSSIBLE LINK BETWEEN INTERFERON REGULATORY FACTOR SIGNALING AND THE COP9/SIGNALOSOME*

Received for publication, June 6, 2000, and in revised form, September 14, 2000 Published, JBC Papers in Press, September 15, 2000, DOI 10.1074/jbc.M004900200

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Interferon consensus sequence-binding protein (ICSBP) is a member of the interferon regulatory factors (IRF) that has a pivotal role in mediating resistance to pathogenic infections in mice and in promoting the differentiation of myeloid cells. ICSBP exerts some of its transcriptional activities via association with other factors that enable its binding to a variety of promoters containing DNA composite elements. These interactions are mediated through a specific COOH-terminal domain termed IAD (IRF association domain). To gain a broader insight of the capacity of ICSBP to interact with other factors, yeast two-hybrid screens were performed using ICSBP-IAD as a bait against a B-cell cDNA library. Trip15 was identified as a specific interacting factor with ICSBP in yeast cells, which was also confirmed by in vitro glutathione S-transferase pull-down assays and by coimmunoprecipitation studies in COS7 cells. Trip15 was recently identified as a component of the COP9/signalosome (CSN) complex composed of eight evolutionarily conserved subunits and thus termed CSN2. This complex has a role in cell-signaling processes, which is manifested by its associated novel kinase activity and by the involvement of its subunits in regulating multiple cell-signaling pathways and cell-cycle progression. We show that in vitro association of ICSBP with the CSN leads to phosphorylation of ICSBP at a unique serine residue within its IAD. The phosphorylated residue is essential for efficient association with IRF-1 and thus for the repressor activity of ICSBP exerted on IRF-1. This suggests that the CSN has a role in integrating incoming signals that affect the transcriptional activity of ICSBP.

Interferon (IFN)* regulatory factors (IRFs) constitute a family of nine cellular transcription factors that share high homology at the first 115 amino acids which comprise the DNA-binding domain and therefore bind to similar DNA elements. These factors mediate numerous biological activities such as, anti-viral activity, IFN signaling, and immunomodulation (1, 2). IRFs are expressed in many cell types and tissues except for IFN consensus sequence-binding protein (ICSBP), also termed IRF-8 and IRF-4, which are expressed specifically in immune cells. IRFs act as transcriptional repressor or activator and the current data suggest that they harbor dual function. This dual functionality is in part due to interactions with different transcription factors resulting in the ability to interact with various promoters leading to alteration in transcriptional activities, i.e. repression or activation (for review, see Ref. 1).

ICSBP is expressed in hematopoietic cells and its expression is further induced upon treatment with IFN-γ (3, 4). Ablation of ICSBP expression results in mice that are deficient in Th1-mediated immune response. Consequently, these mice are susceptible to certain parasitic, bacterial, and viral infections (5). This is attributed in part to lack of interleukin-12 expression thought to be regulated by ICSBP (6, 7). These deficient mice also exhibit a chronic myelogenous leukemia-like syndrome suggesting that ICSBP functions as a tumor suppressor gene (5). Consistent with this is the fact that low levels of ICSBP transcripts were reported in blood samples of chronic myelogenous leukemia patients (8). ICSBP binds to promoters that contain IFN stimulable response element following interaction with IRF-1 or IRF-2 and acts as a transcriptional repressor (9, 10). In addition, ICSBP interacts with non-IRF transcription factors like PU.1 and E47 forming transcriptional activation complexes on different composite DNA elements (11–13). We have characterized the association between ICSBP and IRF-1, IRF-2, PU.1, and E47 (12, 14). Our studies revealed that all these associations are mediated by a conserved domain in ICSBP termed IRFs association domain (IAD) that is shared by all other IRFs excluding IRF-1 and IRF-2, which interact with ICSBP. IRF-4, which is the most conserved IRF member to ICSBP, also interacts with PU.1 and E47 through the IAD but unlike ICSBP it does not interact with IRF-1 and IRF-2 (11, 13). Furthermore, the IAD is also essential for the association of ISGF3γ/IRF-9, a non-tissue-restricted IRF member, with Stat1 and Stat2 to form the ISGF3 complex, which plays a pivotal role in IFN type I signaling (15). Thus, different IRFs utilize the IAD for specific interactions with other factors. This suggests that IRFs-IAD modules are spatially organized like a key lock mechanism; i.e. the overall structure is similar and is determined by conserved residues, while non-conserved resi-

* This work was supported by a grant from G.I.F., the German-Israeli Foundation for Scientific Research and Development, the fund for the promotion of research at the Technion (to B. Z. L.), and the Deutsche Forschungsgemeinschaft (to W. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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¶ The abbreviations used are: IFN, interferon; ICSBP, IFN consensus sequence-binding protein; IRF, IFN regulatory factor; IAD, IRF association domain; CSN, COP9/Signalosome; IVT, in vitro translation; HA, hemagglutinin; PAGE, polyacrylamide gel electrophoresis; GST, glutathione S-transferase; EMSA, electrophoretic mobility shift assay; kb, kilobase(s).
dyes dictate the specificity of interactions. Accordingly, a point mutation within IRFs-IAD in a conserved leucine to proline (Leu₃₃₁ in ICSBP-IAD) was able to ablate the interactions of ICSBP with IRF-1, IRF-2, PU.1, and E47, the interaction of IRF-4 with PU.1 and E47 (12), and the interaction of ISGF3γ with Stat1 and Stat2. This is consistent with a detailed mutational analysis of IRF-4 demonstrating that conserved polar amino acids within two predicted α-helices present in the IAD are essential for protein-protein interactions (16).

The ability of various transcription factors to form combinatorial complexes with different biological activities is a hallmark of eukaryotic gene regulation. Protein-protein interactions play crucial roles in the activity of IRFs and different protein complexes were reported in various immune cells (10, 17, 18). Since ICSBP has an important role in immune cells, we searched for other possible interacting factors that can recruit ICSBP to different promoters or affect its transcriptional activity. Therefore, yeast two-hybrid screens were performed in which the IAD of ICSBP was used as bait against a B-cell cDNA library. Among the specifically interacting clones was Trip15, which the IAD of ICSBP was used as bait against a B-cell cDNA library. Trip15 was therefore termed CSN2 (20, 21). The CSN complex is composed of 8 subunits and was originally identified as a repressor of light controlled development in Arabidopsis thaliana (22). In animals, this complex is localized both in the cytoplasm and in the nucleus, and possesses an associated hemagglutinin (HA) tag and a Not1 restriction site. The amplified fragment was cloned to pGEM-T plasmid and following sequence analysis a Apa1-Nol1 fragment replaced the corresponding fragment in pTarget-ICSBP generating pTarget-ICSBP/HA.

### Yeast Two-hybrid Screens—A GAL4 AD-tagged human B cell line (kindly obtained from Dr. Elledge through Dr. Wallach (26)) was screened for interacting clones with either ICSBP-IAD or ICSBPdel33 as baits which were cloned in the yeast pG769 plasmid. Experimental procedures were performed according to the MATCHMAKER Two-Hybrid System Protocol (CLONTECH) in the presence of 5 μM aminotriazole.

### Northern Blot Analysis—Total RNA was extracted from the various cell lines using Tri-Reagent according to the manufacturer's instructions. Northern blot analysis was performed as described previously (4) with 32P-labeled probes corresponding to a 865-base pair EcoRI and ApoLI fragment from the coding region of CSN2 and a 262-base pair PvuII-BglII fragment corresponding to the COOH-terminal half of human ICSBP.

### In Vitro Transcription and Translation (IVT)—The assays were performed as described before. Plasmids containing the gene of interest under the T7 promoter were linearized downstream of the coding region with the appropriate restriction enzyme. 5 μg of linearized plasmids were in vitro transcribed by T7 RNA polymerase using a commercial kit (Stratagene). Proteins were translated in vitro using the rabbit reticulocyte lysate system (Promega) according to the manufacturer's instructions. To monitor translation efficiency, small scale reactions using [35S]methionine were performed each time and the labeled proteins were separated on 10% SDS-PAGE and subjected to autoradiography.

### GST Pull-down Assay—Escherichia coli BL21 cells harboring the GST-CSN2 fusion construct or just the GST were grown to 0.7 A₅₅₀ at 37 °C and then induced with 0.1 μM isopropyl-1-thio-galactopyranoside for an additional 3 h. Cells were harvested in phosphate-buffered saline, lysed by sonication, and the lysate was cleared by 10 min of centrifugation at 10,000 × g. Cell lysates containing 3–5 μg of fusion protein were incubated with 20 μl of 50% glutathione-Sepharose beads (Amersham Pharmacia Biotech) in a final volume of 500 μl for 1 h at 4 °C with gentle rotation. The beads were then washed 3 times with binding buffer (50 mm potassium phosphate, pH 7.5, 150 mm KCl, 1 mm MgCl₂, 1% Triton X-100, and 10% glycerol), blocked for 30 min with 200 μl of E. coli BL21 cell lysate (1.5 mg/ml soluble proteins), and subsequently 5 μl of IVT [35S]methionine-labeled target protein was added for an additional 60 min. The beads were washed 5 times with 500 μl of binding buffer and the bound proteins were eluted in 15 μl of protein sample buffer and separated by 12% SDS-PAGE. The gel was fixed, dried, and exposed to a x-ray film.

### DNA Transfections, Reporter Analyses, and Immunoprecipitation Assays—NIH3T3 and COS7 cells were transfected by the calcium phosphate-DNA co precipitation method as described previously (27). For reporter gene analysis, NIH3T3 cells were transfected with 3 μg of p-55CIBlue, in which the luciferase gene is driven by the thymidine kinase basal promoter fused to 8 repeats of PRD1, kindly obtained from Dr. Fujita (28), 1 μg of pCH110 (which allows the expression of β-ga-
RESULTS

Interaction between ICSBP and CSN2 (Trip15) Was Identified by Yeast Two-hybrid Screens—ICSBP interacts with different transcription factors such as IRF-1, IRF-2, PU.1, and E47 through the IAD (9–13). To identify other possible interacting factors with ICSBP, yeast two-hybrid screens were employed. The full-length ICSBP missing only the first 33 amino acids and the IAD of ICSBP (amino acids 201–377) were fused to the DNA-binding domain of the yeast transcription factor GAL4 and used as bait against a human B-cell cDNA library fused to the activation domain of GAL4 (for details see “Experimental Procedures”). Yeast H7C7 cells were transformed with the bait constructs and the resulting cells were transformed with the human B-cell library (26). Four interacting clones were identified and tested against a panel of nonspecific baits among which CSN2 (Trip15) was identified as a strong specific interacting component in this assay. The interaction was equally strong with either the full-length ICSBP or just its IAD. The sequence of the cloned CSN2 (Trip15) was identical to the corresponding sequence available in the GenBank™ (accession number number 004236). Support for the specificity of interaction between ICSBP and CSN2 stems from the fact that a mutated IAD of ICSBP, in which Leu331 was mutated to Pro, was incapable of interacting with CSN2 in this assay. This mutation is at a conserved Leu in a predicted a-helix structure in the IAD, essential for the interaction with other factors (12, 16). No interaction with CSN2 was observed if the IAD of ICSBP was swapped with the corresponding IAD of IRF-7 (amino acids 282–462). These data further demonstrate the specificity of interaction between ICSBP and CSN2 in yeast cells, and show that the IAD alone is sufficient for the interaction with CSN2.

CSN2 (Trip15) Is Expressed in Hematopoietic Cell Lines—CSN2 (Trip15) was originally cloned via its ability to interact in a ligand-dependent manner with both the thyroid receptor β and the retinoid X receptor α (19). CSN2 is conserved from fission yeast through plants to mammals. The CSN2 orthologue in Drosophila, Alien, is expressed in muscle attachment sites during embryogenesis (31). Since ICSBP is a nuclear transcription factor with restricted expression to cells of the immune system, we tested whether CSN2 is also expressed in hematopoietic cells. The results of Northern blot analysis of RNA extracted from five different cell lines is shown in Fig. 1. Three bands with estimated molecular size of 5.2, 4.6, and 2.6 kb corresponding to CSN2 mRNA were identified in all tested cell lines of which only HeLa cells are nonhematopoietic. In Molt-4 (T-cells) and in HL-60 (promyelocytic cells) cells, the 4.6-kb band was very faint. The observed intensity of the 2.6-kb band in Namalwa cells (B-cells) was relatively strong as compared with the corresponding band in the other RNA samples. The nature and the variability in the intensity of the various mRNA bands is not clear. The same membrane was also tested for the presence of ICSBP mRNA. As published previously (4, 32), ICSBP was constitutively expressed in Namalwa cells and in U937 cells (promonocytic cells) (Fig. 1, lanes 3 and 4, middle panel). This constitutive expression is further augmented upon treatment of U937 cells with IFN-γ (~10 fold, Fig. 1, lanes 6 and 7, middle panel). However, this treatment had no significant effect on the expression level and pattern of CSN2 (Fig. 1, lanes 6 and 7, upper panel).

In Vitro and in Vivo Interaction between ICSBP and CSN2—To further establish the interaction between CSN2 and ICSBP in vitro, GST pull-down assay was performed. The open reading frame of CSN2 was fused to GST and the recombinant protein from a lysate of expressing bacteria was bound to glutathione-Sepharose beads and the ability to retain 35S-la-
beled ICSBP is shown in Fig. 2. It is clear that ICSBP interacted with GST-CSN2 fusion protein and not with GST alone.

To demonstrate that the interaction between these two proteins also occurs in vivo, COS7 cells were transiently transfected with HA-tagged ICSBP and Myc-His-tagged CSN2 (for details see “Experimental Procedures”). Following transfection, cells lysates were immunoprecipitated with monoclonal antibodies directed against either HA or co-purified with nickel-coated beads through the His tag. The precipitated and purified proteins were subjected to Western blot analysis as indicated in Fig. 3 (lanes A and B). As expected, ICSBP was expressed in the transfected cells and coexpression of CSN2 had no effect on its expression level (Fig. 3A, lanes 1 and 2, respectively). Purification of CSN2 via the His tag led to copurification of ICSBP (Fig. 3A, lane 3). The intensity of the band was weaker than the expression level of ICSBP (compare bands in lanes 2 and 3) implying that not all of the expressed ICSBP is interacting with CSN2. Conversely, purification of CSN2 via its His tag led to the identification of the protein by Western blot (Fig. 3B, lane 6). Immunoprecipitation of ICSBP from cells transfected with both factors led to co-purification of CSN2 (Fig. 3B, lane 7). Interestingly, the intensity of the band corresponding to the co-purified CSN2 was the same as the band corresponding to the purified CSN2 alone (Fig. 3B, compare lanes 6 and 7). This result points out that CSN2 is a limiting factor in comparison to ICSBP and clearly show that ICSBP and CSN2 interact in vivo in COS7 cells. No effect on the expression level of either ICSBP or CSN2 was observed when the two genes were co-transfected to the cells as determined by direct Western blot analysis (Fig. 3C).

ICSBP Interacts with Purified CSN—CSN2 (Trip15) is a component of the CSN, a conserved complex consisting of 8 subunits (20). Many of the CSN subunits have been identified as partaking in signal transduction pathways. For instance, this complex is involved in light-mediated signaling in plants (22). In vitro, the CSN phosphorylates signaling molecules by a yet unidentified kinase (20). Therefore, we tested whether ICSBP can interact with the CSN. Purified CSN was mixed with bacterially expressed ICSBP and the components were separated over glycerol gradient and the collected fractions were analyzed by Western blot (Fig. 4). The CSN complex was found mainly in fractions 6 and 8 as determined with antibodies directed against the seventh component of CSN (α-CSN7). When ICSBP was added to the CSN complex, most of the ICSBP protein was also detected in the same fractions. On the other hand, when ICSBP alone was separated over the gradient it was distributed mainly between fractions 2 and 4 and to a lesser extent in fraction 6. This shift in the distribution of ICSBP on the glycerol gradient when mixed with the purified CSN indicates that the two components are physically associated leading to the redistribution of ICSBP in heavier fractions.

ICSBP Is Phosphorylated by the CSN—Previously, it was demonstrated that purified CSN complex phosphorylates signaling molecules such as c-Jun, IκB, and the p105, precursor of NF-κB (20, 23). Therefore, we tested if CSN can also phosphorylate ICSBP. As seen in Fig. 5, lane 1, ICSBP is indeed phosphorylated by the CSN complex. To identify the phospho-

| Fig. 1. mRNA expression of CSN2 and ICSBP in various cell lines. 20 μg of total RNA was extracted from the indicated cell lines and subjected to Northern blot analysis with a 32P-labeled fragment corresponding to CSN2 (upper panel). The membranes were reprobed with labeled fragment corresponding to human ICSBP (middle panel). Ethidium bromide staining of the 28 S RNA is shown in the lower panel. U937 cells were also treated with 200 units/ml of human IFN-γ (Genzyme) for 5 h prior to RNA extraction. The position of 28 S and 18 S RNA is indicated. | Fig. 2. GST pull-down assay demonstrating the ability of ICSBP to interact with CSN2. Bacterially expressed GST (panel A, lane 1) or bacterially expressed GST-CSN2 (panel B, lane 2) were bound to glutathione-Sepharose beads. The bound proteins were separated by 10% SDS-PAGE and subjected to Coomassie Blue staining to show that equivalent amounts of expressed proteins were used in the assay. The same amounts of expressed proteins, GST (panel A, lane 1) and GST-CSN2 (panel A, lane 2) were incubated with [35S]methionine-labeled IVT ICSBP. The retained proteins were eluted with reduced glutathione, separated by 10% SDS-PAGE together with an identical sample of labeled ICSBP that was not reacted with the glutathione-Sepharose beads (input, lane 3), and the gel was exposed to x-ray film. Arrowheads indicate the position of ICSBP and protein size markers. |
rylated sites in ICSBP, phosphopeptide mapping was performed. Tryptic digest of the phosphorylated ICSBP followed by high performance liquid chromatography separation and peptide sequencing revealed that mainly Ser260 and to a lesser extent Ser 99 were major candidate sites for phosphorylation (data not shown). Using site-directed mutagenesis, Ser 260 was mutated to Ala and the bacterially expressed protein was also subjected to phosphorylation by the CSN complex. Although equal amounts of recombinant ICSBP and recombinant ICSBP-S260A mutant were taken to the assay, the mutated protein was significantly less labeled by the CSN kinase assay (Fig. 5, compare lanes 1 and 2). This supports our finding that the main target for phosphorylation is indeed Ser 260 which is a nonconserved residue among IRFs located within the IAD of ICSBP.

ICSBP-S260A Mutant Possesses a Defective Repressor Activity—Since ICSBP interacts with CSN2, we looked for any effect on ICSBP transcriptional activity. CSN2 had no effect on the repressor activity of ICSBP exerted on IRF-1 and had no effect on transcriptional synergism between ICSBP and either PU.1 or E47 (data not shown). Surprisingly, ICSBP-S260A mutant demonstrated defective repressor activity on IRF-1 as seen in Fig. 6. NIH3T3 cells were transfected with luciferase reporter plasmid driven by the 8xPRDI motif to which both ICSBP and IRF-1 can bind (4, 10). Enhanced luciferase activity was observed when this reporter plasmid is co-transfected with IRF-1 (Fig. 6, lane 4). This transcriptional activation was repressed when ICSBP was co-transfected to the cells (Fig. 6, lane 5) (32). Surprisingly, the ICSBP-S260A mutant was incapable of negating IRF-1 transcriptional activity (Fig. 6, lane 6). Similar repression effect on the basal reporter activity were observed...
for ICSBP or ICSBP-S260A (Fig. 6, lanes 2 and 3). Our results suggest that the mutated ICSBP is defective in its ability to interact with IRF-1 but not with the promoter. This observation is further supported by EMSA described in Fig. 7. As previously shown (14), IRF-1 binds to a 32P-labeled PRDI motif while ICSBP is incapable of binding directly to this element (Fig. 7A, lane 2 and 4, respectively). When the two factors are mixed, a slow migrating band is observed (Fig. 7A, lane 6). This band is a result of protein-protein interaction between the two factors that is mediated via the IAD of ICSBP and a specific interaction module (IAD2) located on IRF-1 (12, 14). The position of the heterocomplex band is also demonstrated by the supershift analysis shown in Fig. 7B. When the ICSBP-S260A mutant is mixed with IRF-1, a significant reduction in the intensity of the heterocomplex band is observed. This band intensity varied between 50 and 70% of the wild type ICSBP/IRF-1 heterocomplex band (Fig. 7A, compare lanes 5 and 6). This reduced ability of the ICSBP-S260A mutant to form heterocomplex with IRF-1 was also observed over a range of protein concentrations (Fig. 7C). Similar results were also seen for the interaction with IRF-2 but not with PU.1 and E47 (data not shown). Taken together, these results imply that ICSBP interacts with the CSN through the association with CSN2. This interaction leads to the phosphorylation of ICSBP in a unique site that is essential for its interaction with other IRFs.

**DISCUSSION**

ICSBP is an essential transcription factor conferring resistance to pathogenic infections through the activation of Th1-dependent (5) and Th1-independent immunity (33, 34). In addition, ICSBP acts as a tumor suppressor gene essential for proper differentiation of myeloid cells (5). Its transcriptional activity is mediated via the interaction with various transcription factors (9–13, 35). These combinatorial interactions render ICSBP the ability to bind to numerous promoters containing composite DNA elements leading to different transcriptional activities. We have shown that the interactions are mediated through a specific conserved domain at the carboxyl terminus termed IAD (12, 14). In this article, ICSBP-IAD was used as bait to screen for other interacting factors in a B-cell cDNA library. CSN2 was identified as a strong and specific interacting factor in this yeast two-hybrid screen. A mutation in a conserved leucine to proline within the IAD of ICSBP that ablated the interaction with IRF and non-IRF transcription factors (12) also ablated the interaction with CSN2 in the yeast cells. The interaction between the two factors was also confirmed in vitro by GST pull-down assay and in vivo by co-immunoprecipitation studies.

CSN2 is highly conserved from fission yeast through invertebrate, plants, and mammals where it is constitutively expressed in different tissues and as shown here in various hematopoietic cell lines (36). Alien is the *Drosophila* orthologue for CSN2 that is expressed in muscle attachment sites during embryogenesis (31). In mice, CSN2 (Cops2) was mapped to chromosome 2 and the reported mRNA sizes were 4 and 2.3 kb (21). Blast homology search against the current GenBank™ revealed that CSN2(Trip15) is located on chromosome 21q22.1 (GenBank™ accession number AP000028) and a closely related gene is located on chromosome 9 (GenBank™ accession number AC007172). However, blast search against the current hts (high-throughput genomic sequences) data also shows that the highest homologous gene is located on chromosome 15q21.2 (GenBank™ accession number AC013452) a location that was predicted following the mouse chromosome mapping (21). This suggests that in humans CSN2 (Trip15) was duplicated relatively late in evolution. In human cell lines, three mRNA bands with molecular sizes of 5.2, 4.6, and 2.6 kb were observed, which were not equally expressed in all the examined cell lines (Fig. 1). This might reflect the existence of three CSN2 (Trip15) loci in the human genome. Two isoforms for CSN7 were identified in mammals (37) and taken together with the presence of multiple CSN2 genes it suggests that CSN may incorporate different isoforms that will render it different characteristics.

Previously, we have shown that IRF-2 and IRF-1 interacts with ICSBP through a conserved module termed IAD2 (12). IAD2 is a predicted PEST domain and therefore rich in proline (P), glutamic acid (E), serine (S), and threonine (T) residues (38). PEST domains were originally believed to be involved in controlling protein instability and in targeting proteins for proteolytic degradation (39). However, some PEST domains can mediate protein-protein interactions as shown initially for the formation of the DNA binding heterocomplex between PU.1 and IRF-4 or ICSBP (12, 40). In addition, we identified two PEST domains in E47 and speculated that one PEST domain confers protein instability and the other is essential for the interaction with ICSBP (12). Interestingly, using the algorithm for searching potential PEST sequences (PESTfinder (38)), the first 60 amino acids of CSN2 were also predicted as a strong PEST domain with a very high score of +10.18. This domain scored much higher than the PU.1 PEST domain and in the same range as that of IRF-1 and IRF-2 (+7.71 for IRF-1, +9.44 for IRF-2, and only +2.15 for PU.1). Each of these PEST domains are of similar size, *i.e.* 42 amino acids in PU.1, and about 55 amino acids in IRF-1 and IRF-2. In the predicted PEST domain of CSN2, the first seven amino acids are not essential for interaction since they were missing from our original yeast two-hybrid clone and were not included in the GST fusion construct. These observations further demonstrate that the IAD of ICSBP interacts effectively with factors that contain PEST motifs.

Unlike the interaction of ICSBP with either IRF-1 and IRF-2, which led to transcriptional repression, and the interaction with PU.1 and E47, which led to transcriptional activation, the interaction with CSN2 had no detectable effect on ICSBP transcriptional activity and its ability to bind DNA either alone or together with IRF-1 or PU.1 (data not shown). This suggests that the interaction with CSN2 do not exert an
Some of the CSN subunits have a role in cell-signaling processes. The largest identified subunit, CSN1 (also termed FUS6 or GPS1), was shown to inhibit JNK1 (c-Jun N-terminal kinase) (44). CSN2 (Trip15) was identified as an interacting component with thyroid hormone receptor and retinoic X receptor (19) with characteristics of a corepressor of nuclear hormone receptor superfamily (45) and c-Jun activation (23). CSN 5, also known as JAB1, JUN activation domain-binding protein 1, was reported to interact with c-Jun and coactivate JUN-mediated gene expression (46). Subunit 6 is identical to VIP (human HIV-1 Vpr interacting protein) that has a role in cell-cycle progression (47). Taken together with CSN associated kinase activity, this suggests that the complex is functioning as a multicomponent signaling complex. A postulated role of CSN in modulating ICSBP activity is illustrated in Fig. 8. Both ICSBP and CSN are co-localized in the nucleus (24, 45, 48). ICSBP is a constitutively expressed transcription factor in hematopoietic cells, and can be present either in an unphosphorylated form or retained by the CSN complex in an “inactive state.” Upon pathogenic infection or IFN stimulation of immune cells, IRF-1, which is an immediately induced gene, may lead to the phosphorylation of ICSBP on Ser260 by the CSN-associated kinase activity leading to its dissociation from the complex and the subsequent interaction with IRF-1 resulting in effective repression. Since both IRF-1 and ICSBP have pivotal roles in immune cells and exert negative activities, the

**Fig. 7. Mutation of Ser260 to Ala affects ICSBP ability to form a DNA binding heterocomplex with IRF-1.** A, IRF-1, ICSBP, and the mutant ICSBP-S260A, were prepared by IVT and their ability to bind DNA and to form heterocomplexes was analyzed by EMSA with 32P-labeled PRDI as the probe. B, EMSA analysis was performed with IVT IRF-1 and ICSBP and the heterocomplex band was identified with antibodies directed against either IRF-1 or ICSBP as described (10). C, EMSA was performed, as described under panel A, with serial dilutions of ICSBP or mutant ICSBP-S260A and constant amount of IRF-1. Following separation of the DNA-protein complexes, the dried gel was exposed to PhosphorImager (Fuji) and the relative intensity of the heterocomplex bands of ICSBP with IRF-1 and mutant ICSBP with IRF-1 is given in arbitrary optical density (OD) units. The protein concentration of ICSBP and mutant ICSBP taken for EMSA under panel A were determined as 4 (in arbitrary units). D, to ensure that equal amounts of either ICSBP and mutant ICSBP-S260A were taken in the various EMSA analyses described under panels A–C, the same IVT reactions were performed in the presence of [35S]methionine and the labeled proteins were separated by 10% SDS-PAGE and the dried gel was exposed to a x-ray film. Arroheads indicate the binding of IRF-1, and heterocomplex (Hetero) bands. Numbers in percentage indicate relative intensity of the heterocomplex bands as determined by PhosphorImager analysis.
Fig. 8. Model for the mode of interaction between ICSBP and CSN2. A, upon viral infection or IFN treatment of immune cells, IRF-1 is immediately induced and activates IFN-stimulated genes that contain IFN stimulable response element in their promoters. ICSBP complex is retained by the CSN through the interaction with CSN2. B, a delayed response to viral infection or IFN treatment results in the activation of the CSN-associated kinase leading to the phosphorylation of ICSBP. C, phosphorylated ICSBP dissociates from the CSN complex, interacts with IRF-1, and represses its activity.
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