[Cell Cycle 5:21, 2501-2508, 1 November 2006]; ©2006 Landes Bioscience

Brief Report

Phospho-Dependent Protein Recognition Motifs Contained in C/EBP Family of Transcription Factors

in Silico Studies

Maria Miller

Correspondence to: Maria Miller; Macromolecular Crystallography Laboratory; National Cancer Institute at Frederick; Frederick, Maryland 21702-1201 USA; Tel.: 301.846.5342; Fax: 301.846.6128; Email: millerm@ncifcrf.gov

Original manuscript submitted: 08/23/06
Manuscript accepted: 09/20/06

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=3421

KEY WORDS

C/EBP transcription factors, protein–protein recognition, Pin1, Polo-like kinase, BRCT

ABBREVIATIONS

BRCT	BRCA1 COOH-terminal
TAD	transactivation domain
bZIP	basic region-leucine zipper
KIM	kinase interaction motif
PBD	polo box domain
DSB	double-strand break
NHEJ	non-homologous end joining

ACKNOWLEDGEMENTS

The author thanks Dr. P.F. Johnson for discussion and critical reading of the manuscript, and Dr. Alex Wlodawer for his interest and support of this project. This work was supported by the Intramural Research Program of NIH, National Cancer Institute, Center for Cancer Research.

ABSTRACT

CCAAT/enhancer-binding proteins (C/EBPs) are transcriptional regulators implicated in cell proliferation, differentiation, survival, and tumorigenesis. Their biological activities require interactions with several protein partners. This report presents insights from in silico analysis aimed at identifying phosphorylation-dependent protein recognition motifs in C/EBPs. (1) All C/EBP variants contain intrinsically disordered Ser/Thr- and Pro-rich segments with potential docking sites for WW and Polo-box domains of prolyl isomerase Pin1 and Polo-like kinases (Plks), respectively. (2) Consensus phosphorylation sequences for Plks are located in a highly conserved region of transactivation domains, suggesting that Plks might modulate transcriptional activities of C/EBPs in a cell cycle-dependent manner. (3) Phosphorylation at these positions, as well as at conserved Ser in the extended basic region, would create phosphoserine-containing motifs (pSXXF/Y/I/L), which could be recognized by BRCT repeats containing proteins such as the PAX-transactivation-dom ain-interacting protein (PTIP), and the breast cancer-associated protein (BRCA1). Proteins containing BRCT domains serve as scaffolds, mediating protein-protein interactions and formation of functional multiprotein complexes involved in DNA repair and cell cycle control. These findings add a new perspective to studies aimed at elucidation of molecular mechanisms underlying the diverse functions of C/EBPs.

INTRODUCTION

C/EBP proteins (C/EBPα, β, δ, ε and γ) bind as dimers to a common DNA sequence motif and perform related functions in regulation of numerous cellular responses (reviewed in ref. 1). They cooperate with each other and with other transcription factors in regulating cell growth and differentiation. Depending on the cell type, C/EBPs are capable of inducing either cell proliferation or cell-cycle arrest (reviewed in refs. 2 and 3). C/EBPα plays a critical role in maturation of adipocytes, granulocytes, and macrophages, induces mitotic arrest during terminal cell differentiation, and can act as a tumor suppressor in some cells. C/EBPα has been implicated as a component of the p53-regulated growth arrest response to DNA damage in epidermal keratinocytes,[4] whereas in prostate cancer cells it interferes with the repair of DNA double-strand breaks (DSB) in response to ionizing radiation (IR).[5] In some tissue types C/EBPα regulates cell growth thorough protein-protein interactions independently of its transcriptional activity. C/EBPβ plays a critical role in proliferation and survival of certain kinds of tumor cells transformed with the H-Ras^{V12} oncogene or downstream effectors such as Raf,[6] and was recently implicated as an essential component of Ras^{V12}-induced senescence in mouse embryo fibroblasts.[7]

The molecular mechanisms of controlling the biological functions of C/EBPs remain poorly understood. No comprehensive searches for interacting proteins have been reported for C/EBP family members other than C/EBPε, and the patterns of posttranslational modifications are only beginning to be elucidated. C/EBPs are components of multiple cellular pathways and interact with multiple protein partners. This suggests that their activities are regulated in part by transient, phospho-dependent intermolecular interactions. In this study, primary structure analysis and data mining were used to identify sites in C/EBPs amenable to phosphorylation, whose modification might regulate their activity either directly or via modulation of interactions with partner proteins. This analysis showed that C/EBPs are natively unstructured proteins able to change conformation upon binding to molecular partners, and revealed several phosphorylation-dependent protein-protein interaction motifs. The pattern of phosphoacceptors in the regulatory regions suggests that C/EBPs may be subjected to an additional, posttranslational level of control by Pin1. Strikingly, serine-containing motifs in conserved regions would conform to recognition

sites for the BRCT-repeats containing adapter proteins, when phosphorylated.

C/EBPs ARE MOSTLY DISORDERED PROTEINS WITH REGIONS THAT FOLD UPON LIGAND BINDING

C/EBPs are modular proteins consisting of a highly conserved C-terminal basic region-leucine zipper (bZIP) domain required for DNA binding, an N-terminal tripartite transactivation domain (TAD), and central regulatory regions (Fig. 1). The N-terminal parts of C/EBPs exhibit little overall sequence homology, except for two segments termed homology box I and homology box II, embedded in the common acidic TADs[8] (Fig. 1A).

Amino acid sequence features of C/EBPs (Table 1), in particular, the low sequence complexity and the high number of polar versus hydrophobic residues, imply that these proteins are intrinsically disordered. Analysis of primary structures using several protein disorder predictors[9-13] revealed the presence of long, structurally flexible segments in all C/EBP proteins, and showed that only the N-terminal part of C/EBPβ has potential to fold into a globular domain. The high content of solvent-exposed residues in C/EBPs indicates that their native, monomeric forms exist mainly as assemblies of extended random coils with limited possibility to form intra-molecular interactions under physiological conditions. In comparison with other family members, C/EBPβ shows the highest propensity to form hydrophobic clusters within its N-terminal region. The charge-hydropathy plot[12] indicates the folded structure for this region, whereas other sequence attributes point to intrinsic disorder (Table 1). It was proposed that such polypeptides may form molten globule-like structures.[14] On the other hand, several regions of C/EBP proteins adopt helical conformation upon binding to the molecular partners. C/EBP monomers readily dimerize thorough leucine zipper motifs, forming coiled-coil structures. Also, the helical fold of the basic regions is induced by binding to the cognate DNA duplex,[15] and the C-terminal sequences following the bZIP domains

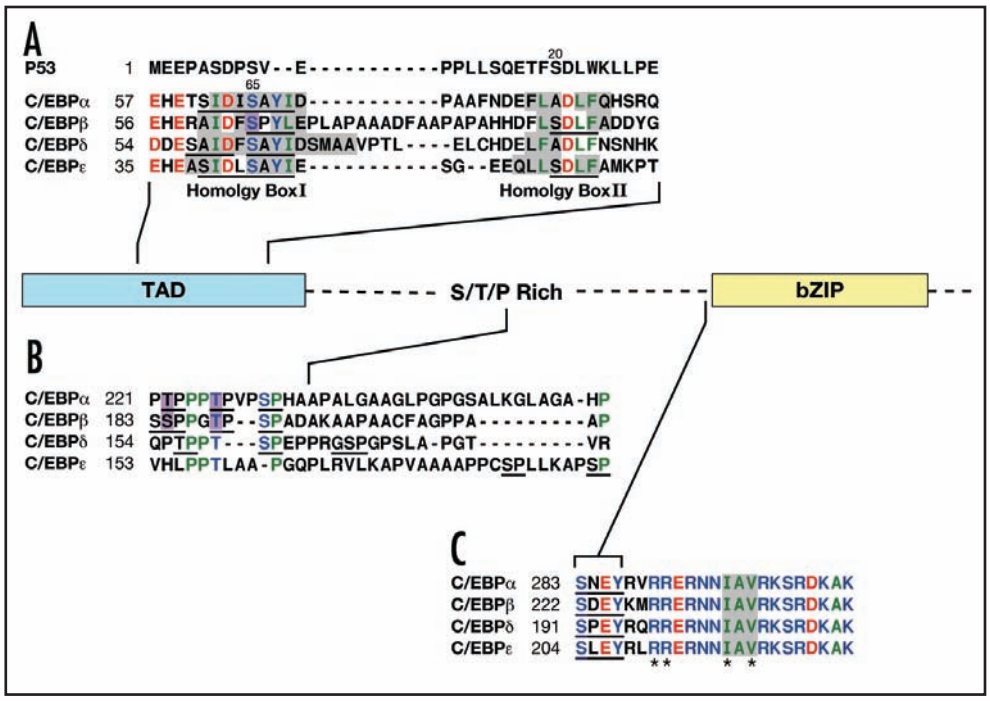

Figure 1. Schematic representation of domain organization of C/EBP family of transcription factors. The murine sequence alignments show: (A) regions comprising homology boxes I and II (note the homology to the region from TAD of p53); (B) parts of S/T/P-rich region; (C) basic regions. Evolutionarily conserved residues are colored (negatively charged in red, polar in blue, and hydrophobic in green). Residues predicted to be partially buried (Predict Protein server[11]) are shaded. Note that S/T/P-rich regions are entirely solvent-exposed. Putative phospho-dependent protein recognition motifs discussed in the text are underlined. Ser/Thr residues known to be phosphorylated are highlighted in magenta. Residues that form the KIM motif are marked by asterisks.

Table 1. **Predictions of disordered regions in C/EBP proteins**

Murine C/EBP Members	Loops/Coil Definition[a,f]	Low Complexity Regions[b,g]	Linker Regions Predicted by Prelink[c]	NORSp[b,h]	Potential Globular Domains[d]	Domains Identified by SMART[e]	Solvent Exposed Residues[b,k]
C/EBPα 358 residues	1-75 92-164 175-202 208-286	28-54 90-135 180-201 217-255	16-53 177-242	95-164 208-288	none	bZIP: 281-345	221 (146)
C/EBPβ 296 residues	1-155 165-212 286-297	37-53 68-87 113-135 169-216	43-52 118-142 170-216	109-224	3-91[i] 1-166[i]	bZIP: 220-284	207 (124)
C/EBPδ 268 residues	1-60 88-136 151-200 251-268	32-46 91-104 150-170 250-262	148-191	1-136	none	bZIP: 189-253	178 (115)
C/EBPε 281 residues	1-36 58-202	165-190	65-74 170-198	69-141	none	bZIP: 202-266	194 (119)

[a]DisEMBL;[9] [b]PredictProtein;[11] [c]PreLink;[13] [d]GlobPlot;[10] [e]Simple Modular Architecture Research Tool;[72] [f]segments of disordered residues based on two state model (Russell/Linding definition); [g]Predicted by SEG;[73] [h]Long regions without regular secondary structure;[74] [i]Kyle/Doolittle definition; [i]Russell/Linding definition; [k]GLOBE, predicted number of exposed residues is compared to number, given in paranthesis, of exposed residues expected for globular protein of the same size

fold upon binding to c-Myb.[16] Homology box II comprises the L/FXXLF motif and corresponds to a "signature helix" found in TADs of many transcriptional activators, including p53[17] (Fig. 1A).

Intrinsically unstructured proteins (or proteins with disordered regions) are very common in eukaryotic cells and are prevalent among proteins involved in cell signaling, and DNA/RNA recognition.[18,19] The intrinsic structural flexibility allows for binding to multiple partners via specific, but low affinity, readily reversible interactions and facilitates dynamic changes in conformation upon complexing different partners. Accordingly, TAD domains of C/EBPs are known to interact with several coregulators, including CBP/p300,[8,20] and with RB protein.[21] The nuclear transport domain overlaps with the DNA recognition helix,[22] whereas the leucine zipper facilitates formation of homo- and heterodimers within the C/EBP family and bZIP transcription factors from different subfamilies. It is noteworthy that the basic region (Fig. 1C) closely resembles the MAP kinase interaction motif (KIM), implying more possibilities to utilize this region. The consensus sequence of KIM, $(R/K)_{2\text{-}3}X_{1\text{-}6}\Psi_A\text{-}X\ \Psi_B$, where X denotes any, and Ψ denotes a hydrophobic residue), has been recognized in the MAPKs-activating kinases, phosphatases, and substrates.[23] The propensity for conformational transition from unordered to partially ordered structures suggests that C/EBPs are particularly adaptable for phosphorylation/dephosphorylation-dependent regulation and function by binding to multiple partners in multi-protein assemblies. Proteins responsible for cellular regulation use specialized recognition modules for mediating intermolecular interactions. Examples of protein interaction domains include SH3 and EVH1, which bind to Pro-rich sequences; SH2, FHA, MH2, 14-3-3, WD40 repeats, WW, Polo-boxes and tandems of BRCT repeats, which recognize phosphorylated peptides (reviewed in ref. 24). These modules retain their unique fold, with exposed ligand-binding surface, when incorporated into a larger polypeptide.

POTENTIAL Pin1 AND POLO-BOX DOMAIN BINDING SITES IN C/EBPs

Unstructured regions are important for protein phosphorylation, and the majority of experimentally determined phosphorylation sites in eukaryotic proteins are located within segments with sequence features indicative of intrinsic disorder.[25] The central regulatory regions of C/EBPs that are exposed to the environment are targets for extensive phosphorylation by a variety of kinases. Phosphorylation of C/EBPα occurs on Ser193, Thr222, Thr226 and Ser248,[26] whereas C/EBPβ is phosphorylated on Thr188, Ser184 and Thr179.[27] Phosphorylation of this region generates motifs that could be recognized by the prolyl isomerase Pin1 (see Fig. 1B). Pin1 contains the catalytic domain, in addition to its phospho-specific WW domain, and catalyzes cis-trans isomerization of pS/pT-P (pS denotes phosphorylated Ser) bonds.[28] The WW domain binds to the same motif and mediates Pin1 interactions with its substrates; therefore, Pin1 requires repetitive pS/pT-P motifs for binding and processive catalysis.[24] Such multiple pS/pT-P Pin1 binding sites were found in the regulatory regions of several proteins.[29,30] Isomerization of the peptidyl-prolyl bond induces conformational changes in target proteins. Several kinases (e.g., ERK2, CDK2) and the PP2A phosphatase are isomer-specific; thus, Pin1 affects phosphorylation status, the stability of its substrates, and protein-protein interactions. Pin1 is an important mitotic regulator and functions as an oncogenic catalyst (reviewed in ref. 31).

Recent studies indicated that biological activities of C/EBPα and -β depend on the phosphorylation status of their S/T-P motifs. This suggests a possible role for Pin1 in an additional post-translational level of control on the C/EBP family. Phosphorylation /dephosphorylation at Ser193 serves as the critical switch of biological activities of C/EBPα. Phosphorylated C/EBPα is a strong inhibitor of cell proliferation in the liver and in myeloid tissues. Cyclin D3-dependent specific phosphorylation at Ser193 by CDK4/CDK6 kinases stabilizes growth-inhibitory C/EBPα-CDK2 (young liver) and C/EBPα-Rb-E2F4-Brm complexes (old liver),[26] whereas removal of the phosphate accelerates cell proliferation via sequestration of Rb.[32] Dephosphorylation of C/EBPα on Ser193 is mediated by the PP2A phosphatase that effectively dephosphorylates only the trans pS/pT-P isomer.[29] It is therefore possible that Pin1 may be an important factor in the regulation of pro-proliferative activities of C/EBPα in post-surgery liver regeneration and, when deregulated, contributes to the development of liver tumors. Indeed, Pin1 has been found overexpressed in a large number of human cancers and in 50% of hepatocellular carcinomas.[33,34] Activation of C/EBPβ depends on the phosphorylation status of the same region. Modification of Thr188 by MAPKs and/or CDKs was proposed to induce exchange of a repressive mediator complex for an activating assembly,[35] whereas subsequent phosphorylation on Ser184 and Thr179 by glycogen synthase kinase 3β (GSK3β) is necessary for acquiring DNA-binding activity in the adipocyte differentiation program.[27] Overall, these data underscore the importance of an additional post-phosphorylation regulatory mechanism of C/EBPs' activities.

Phosphorylation of C/EBPβ Ser184 creates a putative interaction site with Plks. All Plks use the Polo-box domain (PBD) to achieve proper localization in the vicinity of their substrates in a manner similar to Pin1, which uses its WW domain for the same purpose. PBD binds to phosphorylated Ser/Thr sites on substrates or separate docking proteins (ref. 36 and Refs. therein). The optimal phosphopeptide motif recognized by PBDs is SpT/pSP/X, with a strong preference for Ser at the pS/pT (-1) position and a modest preference for Pro at (+1). Analyses of the crystal structures of PBDs with optimal peptides revealed that the presence of Thr or a β-branched, nonpolar residue at (-1) position in the docking site would result in steric clashes with enzyme residues; Ala at (-1) could be incorporated into the complex, but with significant loss of the affinity of binding.[37,38] There are indications that priming phosphorylation is not absolutely necessary when the substrate is present in high concentration.[36]

Inspection of C/EBP sequences shows several putative docking sites for PBDs located in nonconserved regions of the family members. C/EBPα has two evolutionarily conserved pairs of serine residues ($S^{16}S^{17}$ and $S^{348}S^{349}$) located at the N- and C-termini, respectively. There are several possibilities for PBD docking within a S/TP rich region of C/EBPβ, including the optimal $S^{184}SP$ site. In the analogous region of C/EBPδ, a weak binding motif ($G^{167}SP$), present in rat and mouse, is replaced by the preferred SSP sequence in the human homologue. There is only a weak potential conserved binding site—$G^{73}TP$—in C/EBPε, but it is very close to $F^{88}AYP$ sequence, known as the docking motif for ERK, whereas the SSP motif is present only in the human homolog.

PBD is also an autoinhibitory domain, and substrate binding activates the enzymatic activity of Plks.[37] Once bound and activated, Plks can phosphorylate specific sites on the docking protein (processive model), and/or other substrates present in the same location (distributive model).[36] Theoretically, C/EBP variants may

share their docking sites for PBD by forming heterodimers. In this respect, it is worth noting that an SSP motif, conserved among specimens, is contained in the basic region of C/EBPγ, a "short" C/EBP, devoid of TAD, that forms functional heterodimers with all other family members.[39]

HOMOLOGY BOX I FROM C/EBP's TAD CONSTITUTES A CONSENSUS PHOSPHORYLATION SEQUENCE FOR PLK1

Vast accumulated evidence indicates that the interactions between transcription factors and their coactivators are often regulated by phosphorylation of the participating proteins. Phosphorylation of TAD regions may modulate direct binding to protein ligands, as well as intramolecular interactions. It has been demonstrated that the region comprising homology boxes I and II is necessary for the direct binding of C/EBPs to CBP/p300.[20,40] Although the structural basis for this interaction is unknown, it is anticipated that phosphorylation of this region will alter stability of the complex.

Several transcription factors (e.g., c-Jun, Elk-1, LIN-1, SAP-1 and MEF2A) have MAP kinase-regulated TADs, which are preceded by a functional KIM sequence (reviewed in ref. 41). In C/EBPs, the pattern of evolutionarily conserved acidic residues located at positions (-2) with respect to serines within homology box I provides preferred features for phosphorylation by Plk1 (Fig. 1A). The consensus phosphorylation sequence for Plk1 has been identified as E/DXS/TΨ, where X denotes any, and Ψ denotes a hydrophobic residue, respectively.[42] Thus, in addition to the conserved Ser65 (numbering for mouse C/EBPα), C/EBPα, δ, and ε each has a second putative phosphoacceptor site within this region (Fig. 1B). An acidic amino acid at the (+3) position further enhances specificity for Plk1;[42] thus, Ser57 from C/EBPδ should be phosphorylated with the highest efficiency (Fig. 1A). Ser64 of rat C/EBPβ is phosphorylated by the cyclin-dependent kinases CDK2 and Cdc2 in mid-G_1 cells and in cells arrested in the S or M phase.[43] The expression of the Plk1 kinase begins in S phase and its enzymatic activity peaks during mitosis.[44] It is therefore possible that all members of the C/EBP family are phosphorylated in this conserved region during specific stages of the cell cycle. These sites can be phosphorylated by different kinases (see Table 2), but involvement of Plks offers the possibility of regulation in a cell cycle-dependent manner. Substrate specificity for other members of the Plk family has not been well characterized, but was predicted to be similar to Plk1.[36] Based on the homology with the p53 "signature" helix (Fig. 1A), which is phosphorylated on Ser20 by Plk3 and/or Chk2,[45] Ser88 of C/EBPβ and Ser55 of C/EBPε are potential phosphoacceptor sites for the same kinases. Interestingly, the GPS phospho-predictor suite,[46] indicated this region of C/EBPβ as corresponding much better to the specificity of the PKB (also known as AKT) family of kinases. In the case of the p53 tumor suppressor protein, phosphorylation of Ser20 activates its transcriptional activities. Therefore, it would be of great interest to establish whether C/EBPβ Ser88 undergoes modification and which kinase(s) is responsible. Of note, these serines are located in segments predicted to be partially buried (Fig. 1), and might potentially be modified following an initial, "activating" event, such as phosphorylation of C/EBPβ Thr188 by ERK.[27] Perhaps for this reason, the phosphorylation by cyclin-dependent kinases of Ser65 in C/EBPβ was observed in response to Ras signaling, and was delayed relative to the peak of CDK2 activity.[43] Similarities and differences between C/EBP isoforms in the pattern of phosphoacceptors in these regions may be the reason why C/EBPs, which usually function as homodimers, occasionally form heteroduplexes to accomplish specific tasks.[1]

Table 2 **Kinases predicted to phosphorylate serines in conserved regions of C/EBPs by GPS[a]**

C/EBPα		C/EBPβ		C/EBPδ		C/EBPε	
Ser61				**Ser57**		**Ser39**	
GRK	1.72 (1.7)			PKR	11.0 (5.90)	CaM-II	2.62 (2.5)
PLK	1.93 (2.25)			GRK	2.51 (1.70)	PLK	2.07 (2.25)
CK2	1.67 (2.8)			CK2	3.80 (2.80)		
				PLK	2.46 (2.25)		
Ser65		**Ser64**		**Ser62**		**Ser43**	
PLK	1.61 (2.25)	PLK	1.75 (2.25)	PLK	2.25 (2.25)	PLK	1.54 (2.25)
		PKR	10.00 (5.9)				
		CDKs	2.57 (2.5)				
		P34CDC2	1.91 (1.8)				
		Ser88				**Ser55**	
		PKB	4.0 (3.2)			PLK	1.29 (2.25)
		Chk1/Chk2	9.0 (12.0)				
Ser283		**Ser222**		**Ser191**		**Ser204**	
MKKK	9.00 (6.0)	CK1	2.49 (1.95)	MKKK	3.09 (3.0)	MKKK	14.0 (6.0)
GRK	1.91 (1.7)	CK2	3.55 (2.80)	KIS	9.6 (8.0)	GRK	1.85 (1.7)
				CDKs	3.8 (2.5)		
				DNA-PK	2.14 (2.1)		
				Chk1/Chk2	13.0 (12.0)		

[a]GPS: Group-based Phosphorylation Scoring Method.[46] Score of accuracy is given following the name of group of kinases predicted to phosphorylate a particular site. Program suggested cut-offs, corresponding to balanced pair of sensitivity and specificity, are shown in parenthesis.

PHOSPHORYLATION OF SERINES IN CONSERVED REGIONS GENERATES RECOGNITION MOTIFS FOR BRCT DOMAINS

Ser/Thr phosphorylation is a ubiquitous molecular mechanism for regulation of transient protein-protein interactions involved in cellular signaling. Certain phosphoserines are specifically recognized by a tandem pair of BRCT repeats.[24] BRCT domains are present in numerous proteins as multiple tandem repeats—$(BRCT)_2$—of individual BRCT modules. BRCTs are versatile protein recognition modules that, besides binding to the protein targets containing phosphoserine, can mediate protein-protein interactions by specific contacts with BRCT repeats from another protein, and/or with protein domains of different structures (e.g., a complex between $(BRCT)_2$ of 53BP1 and p53 DNA-binding domain). Proteomic screening showed that PTIP-$(BRCT)_2$ and BRCA1-$(BRCT)_2$ bind to a subset of kinase substrates with a strong preference for aromatic and aliphatic residues in the pSer (+3) position.[47] The structural basis for this recognition was provided by crystal structures[48-50] of the BRCA1 tandem BRCT repeats bound to phosphorylated peptides containing pSXXF motifs. These studies identified a pSer-binding pocket on the N-terminal BRCT of the tandem, and a hydrophobic

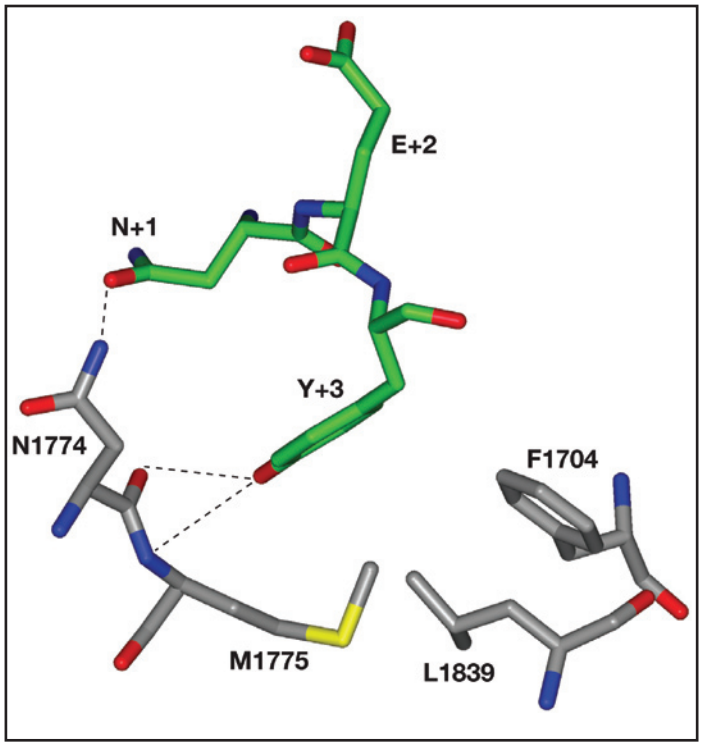

Figure 2. A model of Tyr interactions with the (+3) hydrophobic binding pocket of BRCA1-(BRCT)$_2$. Hydroxyl group of Tyr at (+3) position in respect to pSer makes electrostatic interactions (dashed lines) with the main chain carbonyl and NH groups of BRCA1. Carbon atoms are gray and green for BRCA1 and NEY sequence (derived from C/EBPα) respectively. The model was built with INSIGHT II suite (Accelrys; 2005) using coordinates of the BRCA1-(BRCT)$_2$ bound to an optimized phosphopeptide;[49] (PDB entry: 1T2V).

groove between the two BRCT repeats that recognizes the Phe. Analysis of peptide selections using an oriented peptide library indicated that BRCT domains from MDC1, 53BP1, TOPBP1, DNA Ligase IV, and Rad9p also display distinct, albeit less restrictive, sequence preferences toward phosphoserine-containing peptides.[51] BRCT-containing proteins act as adapters, bringing together proteins involved in cellular events regulating the cell cycle and/or responses to stress (reviewed in ref. 52). In addition to mediating protein-protein interactions, these molecules perform a variety of cellular functions and generate networks of enormous complexity. For example, BRCA1 and P53B1 both display DNA-binding activity, whereas the BRCA1-BARD1 heterodimer forms a potent enzymatic complex exhibiting E3-ubiquitin (E3-Ub) ligase activity.[53] The role of BRCA1 binding partners has been extensively investigated. BRCA1 interacts with proteins that function in the checkpoins activation (e.g., p53, Rb, FancD2, BACH1, CtIP), DNA repair, and regulation of transcription (e.g., histone deacetylases, RNA Pol II, SWI/SNF, C-myc, and Sp1). The N-terminal RING finger domain is responsible for formation of the BRCA1-BARD1 heteroduplex and interacts with BAP1 (BRCA1-associated protein 1) and with E2F1. Central regions contain DNA-binding domain. Through its target proteins, BRCA1 performs a variety of cellular functions in DNA repair and cell cycle regulation (reviewed in refs. 54 and 55).

Phosphorylation of conserved Ser65 in the C/EBP family, as well as Ser61 and Ser39 from C/EBPα and -ε, respectively (Fig. 1A), would generate the pSXXI/L motifs, which could be recognized by a tandem of BRCT repeats from the C-terminal of PTIP in a sequence-dependent manner.[47] C/EBPβ and -ε have putative phosphoserine epitopes, pSXXF, located within the homology box II, which can be recognized by PTIP and/or the BRCA1 protein. In addition to phosphoserine-dependent motifs within TADs, conserved in the C/EBP family sequence, SXEY, located in the extended basic regions (Fig. 1C), matches the primary binding determinants for MDC1-(BRCT)$_2$,[51] if Ser is phosphorylated. However, MDC1-(BRCT)$_2$ has been found to date to interact solely with the phosphorylated H2AX (γH2AX) histone tail, and the reported results irrefutably indicated the importance of the H2AX C-terminus for this association.[56] Based on these considerations and on currently available data, the best candidate for the putative interaction with this site of C/EBPs is BRCA1 protein, which can also recognize the pSXXY sequence.[47] Possible interactions of a Tyr with the hydrophobic pocket of BRCA1-(BRCT)$_2$ are depicted in (Fig. 2). In contrast to MDC1, BRCA1 has been reported to interact with a number of proteins through its BRCT domain, including phosphorylated BACH1,[57] CtIP,[50] and acetyl-CoA carboxylase (ACCA).[58] BRCA1 is a target for several kinases and it is anticipated that phosphorylation on different sites directs BRCA1 to act in different pathways. For example, BRCA1 binds to phosphorylated BACH1 in the S and M phase,[57] and to phosphorylated CtIP in the G$_2$ phase.[50] BRCA1 undergoes phosphorylation during the late G$_1$ and S stages and is transiently dephosphorylated early after the M phase. Thus, the window of opportunity when the two putative partners encounter each other determines the occurrence of a particular association. Although the amino acid sequence surrounding the phosphoacceptor may contribute to the binding affinity to the protein ligand, its primary importance may be to define the specificity of phosphorylating kinase(s). For that reason, care is required in applying the results from in vitro peptide selection studies to the situation in vivo.

What are the implications of these possible associations in regard to biological activities of the C/EBP family of transcription factors? C/EBPs respond to a variety of external stimuli to transactivate target genes, but can also act as repressors and perform functions distinct from their role in gene expression via protein-protein interactions. Depending on the cellular context, they are capable of either promoting or inhibiting cell growth. These diverse activities of C/EBP proteins require transient interactions with numerous protein ligands specific for multiple tissues, cell types, or different nuclear compartments. This implies regulation of C/EBPs actions by adapter proteins. Several observations suggest that ubiquitously expressed PTIP and BRCA1 proteins could act in this capacity.

(1) PTIP is an essential factor in cell division and progression through mitosis,[59] and was shown to bind to TADs of the Pax family proteins and colocalize in the cell nucleus to active chromatin.[60] PTIP inhibits the transactivation activities of Pax2A and Pax2B on the glucagon gene promoter,[61] whereas its *Xenopus laevis* analog, SWIFT, binds to the Smad2-Smad4 complex via its three C-terminal BRCT repeats and acts as coactivator.[62] Thus, localization of the PTIP binding motif in the TAD regions of C/EBPs suggest a possible role of PTIP as their transcriptional coregulator. Reported functional phosphorylation of Ser64 in C/EBP/β,[43](Ser65 in C/EBPα) provides support for this hypothesis. Phosphorylation of Ser64 in C/EBPβ is required for Ras-induced focus formation and transformation of NIH 3T3 cells, and in this context, Ser64 modification seems to be critical for transcription of C/EBPβ target genes. It was demonstrated that C/EBPβ and -δ physically interact with Smad3 and Smad4, and TGFβ signaling through Smad3 inhibits trans-activation functions

of C/EBPs in adipocyte differentiation. Furthermore, this association was significantly stronger in vivo than in vitro, suggesting the participation of an adapter protein.[63] It is thus tempting to speculate that PTIP may facilitate the incorporation of C/EBPβ and -δ into transcriptional complexes with the TGFβ signaling-activated Smads.

The connection between PTIP and C/EBPs actions comes also from the observation that C/EBPα expressing prostate cancer cell lines exhibit impaired ability to repair DSB after DNA damage. Upon irradiation, C/EBPα associates via its C-terminus with Ku70/80 and PARP-1 proteins, initiators of the nonhomologous end joining (NHEJ) pathway of DNA repair.[5] Full length C/EBPα is necessary for this inhibitory effect, suggesting a complex mechanism. On the other hand, PTIP is thought to be an important factor in maintaining genome stability by binding to phosphorylated 53BP1 in response to IR. Subsequently both proteins translocate to sites of DNA damage.[64] Other study showed that 53BP1 plays a major role in NHEJ.[65] Lower levels of PTIP make cells more sensitive to IR,[64] and this correlates with increased sensitivity to radiation of prostate cancer cells. It may be, therefore, worthwhile to check the status of *PTIP* gene mutations in those cells.

(2) BRCA1, which interacts with RNA Pol II holoenzyme, has also been implicated in the regulation of transcription by association with several sequence-specific DNA-binding transcription factors, as well as by binding to repressors like CtiP.[54] For example, in response to MAPK signaling, BRCA1 strongly up-regulates the expression of GADD45 by physical association with OCT-1 and NF-YA transcription factors which directly bind to the OCT-1 and CAAT motifs within the *GADD45* promoter.[66] Interestingly, in human acute promyelocytic leukemia (APL) cells, all-trans retinoic acid (ATRA) induced C/EBPβ and OCT-1 collaborate in up-regulating the expression of PU.1, which is critical for myeloid differentiation. This mechanism underlies successful treatment with ATRA of APL where normal promyelocytic differentiation is blocked.[67] Whether BRCA1 acts as a coactivator in this case remains to be established. C/EBP binding site was found in the vicinity of the E2F element within the *DHFR* promoter, raising a possibility that C/EBPβ exerts its proliferative effects by cooperating with free E2F in activation of expression of the S-phase genes by a not-yet determined mechanism.[3] On the other hand, C/EBPβ is a necessary component of Rb:E2F-dependent, oncogenic Ras-induced senescence.[7] Again, this activity correlates with the possible role of BRCA1 in sustaining G_1 arrest, which can be achieved by BRCA1 binding to and stabilizing hypophosphorylated (i.e., complexed to E2F) Rb protein.[55] Only C/EBPβ and -ε each has a putative site of interactions with BRCA1 within their TADs.

(3) The possibility of creation the BRCA1-$(BRCT)_2$ binding motif via phosphorylation of conserved Ser283 located at the extended basic region (Fig. 1C) is very intriguing. As revealed by the crystal structure analysis, Tyr286 (Tyr285 in rat) plays an important role in maintaining C/EBPα-DNA interface, and Y286A mutant protein exhibits a significant reduction in the binding affinity to the cognate DNA.[68] Thus, temporal binding of pSXEY motif (if it occurs) to BRCT domain probably happens prior to C/EBPs acquiring the DNA-binding ability. Currently, there is no information on how these factors find their DNA targets. It is, therefore, possible that BRCA1 plays a role in recruiting repressed/inactive forms of C/EBPs to specific nuclear compartments, e.g., promyelocytic leukemia (PML) bodies,[69] where they can undergo posttranslational modifications and subsequently colocalize with their partners in the vicinity of chromatin. BRCA1 colocalizes with PML, DNA repair proteins, and displays binding affinity to several proteins known to interact with C/EBPs, including ETF1, p53, Rb and the SWI/SNF complex. Furthermore, accumulated evidence implies that BRCA1 always associates with BARD1 in vivo. Major biological functions of both proteins are mediated by the BRCA1/BARD1 heterodimer and depend on the E3-Ub ligase activity of the complex. This enzymatic activity is abolished by cancer-derived mutations within the RING domain of BRCA1 (Ref. 53 and Refs. therein). It is anticipated that BRCA1/BARD1 may specifically ubiquitylate proteins involved in transcription, cell cycle regulation and DNA repair. However, specific substrates have not been identified. Recently, Polanowska et al[70] demonstrated that the activation of BRCA1-dependent ubiquitylation in response to DNA damage following IR is conserved in *C. elegans*, and Yu et al.[71] showed that BRCA1 catalyzes ubiquitylation of CtIP in a manner that depends on phospho-dependent interaction between CtIP and BRCA1 BRCT domains. Ubiquitinated CtIP associates with chromatin. It would be interesting to know if C/EBPα that is involved in DNA damage response,[4,5] and, may be, other C/EBP proteins are also targets for BRCA1-dependent ubiquitylation.

CONCLUDING REMARKS

The in silico analyses presented here suggest novel ways by which the cellular functions of C/EBPs may be modulated. The predictions of this study that C/EBPs are potential targets for BRCT repeats containing proteins must still be experimentally verified and further work is necessary to elucidate specific links of C/EBPs to their partners. One has to bear in mind that phosphoserine recognition function of BRCT-repeats has been discovered only recently. Not all of the key players have been identified, and their sequence specificity and the full potential for scaffolding remain to be established. As noted by Deng,[55] even in the case of the best studied BRCA1 protein, many questions remain unanswered, and several conclusions from the reported observations seem premature without further scrutiny. Despite enormous progress that has been made over the last decade in our understanding of control of cellular events, the organization of cellular networks in time and space has just begun to be revealed. Nonetheless, evolutionary conservation of Plk phosphorylation motifs, as well as resulting phosphoserine epitopes among C/EBP variants, appear not to be coincidental and offers for the first time a plausible explanation for the role of several evolutionarily conserved residues within the homology regions of C/EBPs. Importantly, exploring possibilities to form transient phospho-dependent interactions via adapter proteins may facilitate further attempts to elucidate molecular events underlying the diverse functions of C/EBP transcription factors.

References

1. Ramji DP, Foka P. CCAAT/enhancer-binding proteins: Structure, function and regulation. Biochem J 2002; 365:561-75.
2. Johnson PF. Molecular stop signs: Regulation of cell-cycle arrest by C/EBP transcription factors. J Cell Sci 2005; 118:2545-55.
3. Sebastian T, Johnson PF. Stop and go: Anti-proliferative and mitogenic functions of the transcription factor C/EBPbeta. Cell Cycle 2006; 5:953-7.
4. Yoon K, Smart RC. C/EBPalpha is a DNA damage-inducible p53-regulated mediator of the G_1 checkpoint in keratinocytes. Mol Cell Biol 2004; 24:10650-60.
5. Yin H, Glass J. In prostate cancer cells the interaction of C/EBPalpha with Ku70, Ku80, and poly(ADP-ribose) polymerase-1 increases sensitivity to DNA damage. J Biol Chem 2006; 281:11496-505.
6. Zhu S, Yoon K, Sterneck E, Johnson PF, Smart RC. CCAAT/enhancer binding protein-beta is a mediator of keratinocyte survival and skin tumorigenesis involving oncogenic Ras signaling. Proc Natl Acad Sci USA 2002; 99:207-12.

7. Sebastian T, Malik R, Thomas S, Sage J, Johnson PF. C/EBPbeta cooperates with RB:E2F to implement Ras(V12)-induced cellular senescence. EMBO J 2005; 24:3301-12.
8. Nerlov C, Ziff EB. CCAAT/enhancer binding protein-alpha amino acid motifs with dual TBP and TFIIB binding ability cooperate to activate transcription in both yeast and mammalian cells. EMBO J 1995; 14:4318-28.
9. Linding R, Jensen LJ, Diella F, Bork P, Gibson TJ, Russell RB. Protein disorder prediction: Implications for structural proteomics. Structure 2003; 11:1453-9.
10. Linding R, Russell RB, Neduva V, Gibson TJ. GlobPlot: Exploring protein sequences for globularity and disorder. Nucleic Acids Res 2003; 31:3701-8.
11. Rost B, Liu J. The Predict Protein server. Nucleic Acids Res 2003; 31:3300-4.
12. Prilusky J, Felder CE, Zeev-Ben-Mordehai T, Rydberg EH, Man O, Beckmann JS, Silman I, Sussman JL. FoldIndex: A simple tool to predict whether a given protein sequence is intrinsically unfolded. Bioinformatics 2005; 21:3435-8.
13. Coeytaux K, Poupon A. Prediction of unfolded segments in a protein sequence based on amino acid composition. Bioinformatics 2005; 21:1891-900.
14. Oldfield CJ, Cheng Y, Cortese MS, Brown CJ, Uversky VN, Dunker AK. Comparing and combining predictors of mostly disordered proteins. Biochemistry 2005; 44:1989-2000.
15. Shuman JD, Vinson CR, Mcknight SL. Evidence of changes in protease sensitivity and subunit exchange rate on DNA binding by C/EBP. Science 1990; 249:771-4.
16. Tahirov TH, Sato K, Ichikawa-Iwata E, Sasaki M, Inoue-Bungo T, Shiina M, Kimura K, Takata S, Fujikawa A, Morii H, Kumasaka T, Yamamoto M, Ishii S, Ogata K. Mechanism of c-Myb-C/EBPβ cooperation from separated sites on a promoter. Cell 2002; 108:57-70.
17. Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ, Pavletich NP. Structure of the *MDM2* oncoprotein bound to the *p53* tumor suppressor transactivation domain. Science 1996; 274:948-53.
18. Dunker AK, Cortese MS, Romero P, Iakoucheva LM, Uversky VN. Flexible nets: The roles of intrinsic disorder in protein interaction networks. FEBS J 2005; 272:5129-48.
19. Minezaki Y, Homma K, Kinjo AR, Nishikawa K. Human transcription factors contain a high fraction of intrinsically disordered regions essential for transcriptional regulation. J Mol Biol 2006.
20. Kovacs KA, Steinmann M, Magistretti PJ, Halfon O, Cardinaux JR. CCAAT/enhancer-binding protein family members recruit the coactivator CREB-binding protein and trigger its phosphorylation. J Biol Chem 2003; 278:36959-65.
21. Chen PL, Riley DJ, Chen-Kiang S, Lee WH. Retinoblastoma protein directly interacts with and activates the transcription factor NF-IL6. Proc Natl Acad Sci USA 1996; 93:465-9.
22. Williams SC, Angerer ND, Johnson PF. C/EBP proteins contain nuclear localization signals imbedded in their basic regions. Gene Expr 1997; 6:371-85.
23. Liu S, Sun JP, Zhou B, Zhang ZY. Structural basis of docking interactions between ERK2 and MAP kinase phosphatase 3. Proc Natl Acad Sci USA 2006; 103:5326-31.
24. Yaffe MB, Smerdon SJ. The use of in vitro peptide-library screens in the analysis of phosphoserine/threonine-binding domain structure and function. Annu Rev Biophys Biomol Struct 2004; 33:225-44.
25. Iakoucheva LM, Radivojac P, Brown CJ, O'Connor TR, Sikes JG, Obradovic Z, Dunker AK. The importance of intrinsic disorder for protein phosphorylation. Nucleic Acids Res 2004; 32:1037-49.
26. Wang GL, Shi X, Salisbury E, Sun Y, Albrecht JH, Smith RG, Timchenko NA. Cyclin *D3* maintains growth-inhibitory activity of C/EBPalpha by stabilizing C/EBPalpha-cdk2 and C/EBPalpha-Brm complexes. Mol Cell Biol 2006; 26:2570-82.
27. Tang QQ, Gronborg M, Huang H, Kim JW, Otto TC, Pandey A, Lane MD. Sequential phosphorylation of CCAAT enhancer-binding protein beta by MAPK and glycogen synthase kinase 3beta is required for adipogenesis. Proc Natl Acad Sci USA 2005; 102:9766-71.
28. Yaffe MB, Schutkowski M, Shen M, Zhou XZ, Stukenberg PT, Rahfeld JU, Xu J, Kuang J, Kirschner MW, Fischer G, Cantley LC, Lu KP. Sequence-specific and phosphorylation-dependent proline isomerization: A potential mitotic regulatory mechanism. Science 1997; 278:1957-60.
29. Zhou XZ, Kops O, Werner A, Lu PJ, Shen M, Stoller G, Kullertz G, Stark M, Fischer G, Lu KP. Pin1-dependent prolyl isomerization regulates dephosphorylation of Cdc25C and tau proteins. Mol Cell 2000; 6:873-83.
30. Monje P, Hernandez-Losa J, Lyons RJ, Castellone MD, Gutkind JS. Regulation of the transcriptional activity of c-Fos by ERK. A novel role for the prolyl isomerase PIN1. J Biol Chem 2005; 280:35081-4.
31. Ryo A, Liou YC, Lu KP, Wulf G. Prolyl isomerase Pin1: A catalyst for oncogenesis and a potential therapeutic target in cancer. J Cell Sci 2003; 116:773-83.
32. Wang GL, Timchenko NA. Dephosphorylated C/EBPalpha accelerates cell proliferation through sequestering retinoblastoma protein. Mol Cell Biol 2005; 25:1325-38.
33. Lu KP, Suizu F, Zhou XZ, Finn G, Lam P, Wulf G. Targeting carcinogenesis: A role for the prolyl isomerase Pin1? Mol Carcinog 2006; 45:397-402.
34. Pang R, Yuen J, Yuen MF, Lai CL, Lee TK, Man K, Poon RT, Fan ST, Wong CM, Ng IO, Kwong YL, Tse E. *PIN1* overexpression and beta-catenin gene mutations are distinct oncogenic events in human hepatocellular carcinoma. Oncogene 2004; 23:4182-6.
35. Mo X, Kowenz-Leutz E, Xu H, Leutz A. Ras induces mediator complex exchange on C/EBP beta. Mol Cell 2004; 13:241-50.
36. Lowery DM, Lim D, Yaffe MB. Structure and function of Polo-like kinases. Oncogene 2005; 24:248-59.
37. Elia AE, Rellos P, Haire LF, Chao JW, Ivins FJ, Hoepker K, Mohammad D, Cantley LC, Smerdon SJ, Yaffe MB. The molecular basis for phosphodependent substrate targeting and regulation of Plks by the Polo-box domain. Cell 2003; 115:83-95.
38. Cheng KY, Lowe ED, Sinclair J, Nigg EA, Johnson LN. The crystal structure of the human polo-like kinase-1 polo box domain and its phospho-peptide complex. EMBO J 2003; 22:5757-68.
39. Parkin SE, Baer M, Copeland TD, Schwartz RC, Johnson PF. Regulation of CCAAT/enhancer-binding protein (C/EBP) activator proteins by heterodimerization with C/EBPgamma (Ig/EBP). J Biol Chem 2002; 277:23563-72.
40. Schwartz C, Beck K, Mink S, Schmolke M, Budde B, Wenning D, Klempnauer KH. Recruitment of p300 by C/EBPbeta triggers phosphorylation of p300 and modulates coactivator activity. EMBO J 2003; 22:882-92.
41. Sharrocks AD, Yang SH, Galanis A. Docking domains and substrate-specificity determination for MAP kinases. Trends Biochem Sci 2000; 25:448-53.
42. Nakajima H, Toyoshima-Morimoto F, Taniguchi E, Nishida E. Identification of a consensus motif for Plk (Polo-like kinase) phosphorylation reveals Myt1 as a Plk1 substrate. J Biol Chem 2003; 278:25277-80.
43. Shuman JD, Sebastian T, Kaldis P, Copeland TD, Zhu S, Smart RC, Johnson PF. Cell cycle-dependent phosphorylation of C/EBPbeta mediates oncogenic cooperativity between C/EBPbeta and H-RasV12. Mol Cell Biol 2004; 24:7380-91.
44. Eckerdt F, Yuan J, Strebhardt K. Polo-like kinases and oncogenesis. Oncogene 2005; 24:267-76.
45. Ando K, Ozaki T, Yamamoto H, Furuya K, Hosoda M, Hayashi S, Fukuzawa M, Nakagawara A. Polo-like kinase 1 (Plk1) inhibits p53 function by physical interaction and phosphorylation. J Biol Chem 2004; 279:25549-61.
46. Xue Y, Zhou F, Zhu M, Ahmed K, Chen G, Yao X. GPS: A comprehensive www server for phosphorylation sites prediction. Nucleic Acids Res 2005; 33:W184-W187.
47. Manke IA, Lowery DM, Nguyen A, Yaffe MB. BRCT repeats as phosphopeptide-binding modules involved in protein targeting. Science 2003; 302:636-9.
48. Clapperton JA, Manke IA, Lowery DM, Ho T, Haire LF, Yaffe MB, Smerdon SJ. Structure and mechanism of BRCA1 BRCT domain recognition of phosphorylated BACH1 with implications for cancer. Nat Struct Mol Biol 2004; 11:512-8.
49. Williams RS, Lee MS, Hau DD, Glover JN. Structural basis of phosphopeptide recognition by the BRCT domain of BRCA1. Nat Struct Mol Biol 2004; 11:519-25.
50. Varma AK, Brown RS, Birrane G, Ladias JA. Structural basis for cell cycle checkpoint control by the BRCA1-CtIP complex. Biochemistry 2005; 44:10941-6.
51. Rodriguez M, Yu X, Chen J, Songyang Z. Phosphopeptide binding specificities of BRCA1 COOH-terminal (BRCT) domains. J Biol Chem 2003; 278:52914-8.
52. Glover JN, Williams RS, Lee MS. Interactions between BRCT repeats and phosphoproteins: Tangled up in two. Trends Biochem Sci 2004; 29:579-85.
53. Boulton SJ. BRCA1-mediated ubiquitylation. Cell Cycle 2006; 5:1481-6.
54. Starita LM, Parvin JD. The multiple nuclear functions of BRCA1: Transcription, ubiquitination and DNA repair. Curr Opin Cell Biol 2003; 15:345-50.
55. Deng CX. BRCA1: Cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. Nucleic Acids Res 2006; 34:1416-26.
56. Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. Cell 2005; 123:1213-26.
57. Yu X, Chini CC, He M, Mer G, Chen J. The BRCT domain is a phospho-protein binding domain. Science 2003; 302:639-42.
58. Magnard C, Bachelier R, Vincent A, Jaquinod M, Kieffer S, Lenoir GM, Venezia ND. BRCA1 interacts with acetyl-CoA carboxylase through its tandem of BRCT domains. Oncogene 2002; 21:6729-39.
59. Cho EA, Prindle MJ, Dressler GR. BRCT domain-containing protein PTIP is essential for progression through mitosis. Mol Cell Biol 2003; 23:1666-73.
60. Lechner MS, Levitan I, Dressler GR. PTIP, a novel BRCT domain-containing protein interacts with Pax2 and is associated with active chromatin. Nucleic Acids Res 2000; 28:2741-51.
61. Hoffmeister A, Ropolo A, Vasseur S, Mallo GV, Bodeker H, Ritz-Laser B, Dressler GR, Vaccaro MI, Dagorn JC, Moreno S, Iovanna JL. The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the *Pax2A* and *Pax2B* transcription factors on the glucagon gene promoter. J Biol Chem 2002; 277:22314-9.
62. Shimizu K, Bourillot PY, Nielsen SJ, Zorn AM, Gurdon JB. Swift is a novel BRCT domain coactivator of Smad2 in transforming growth factor beta signaling. Mol Cell Biol 2001; 21:3901-12.
63. Choy L, Derynck R. Transforming growth factor-beta inhibits adipocyte differentiation by Smad3 interacting with CCAAT/enhancer-binding protein (C/EBP) and repressing C/EBP transactivation function. J Biol Chem 2003; 278:9609-19.
64. Jowsey PA, Doherty AJ, Rouse J. Human PTIP facilitates ATM-mediated activation of p53 and promotes cellular resistance to ionizing radiation. J Biol Chem 2004; 279:55562-9.
65. Nakamura K, Sakai W, Kawamoto T, Bree RT, Lowndes NF, Takeda S, Taniguchi Y. Genetic dissection of vertebrate 53BP1: A major role in nonhomologous end joining of DNA double strand breaks. DNA Repair (Amst) 2006; 5:741-9.
66. Fan W, Jin S, Tong T, Zhao H, Fan F, Antinore MJ, Rajasekaran B, Wu M, Zhan Q. BRCA1 regulates GADD45 through its interactions with the OCT-1 and CAAT motifs. J Biol Chem 2002; 277:8061-7.
67. Mueller BU, Pabst T, Fos J, Petkovic V, Fey MF, Asou N, Buergi U, Tenen DG. ATRA resolves the differentiation block in t(15;17) acute myeloid leukemia by restoring PU.1 expression. Blood 2006; 107:3330-8.

68. Miller M, Shuman JD, Sebastian T, Dauter Z, Johnson PF. Structural basis for DNA recognition by the basic region leucine zipper transcription factor CCAAT/enhancer-binding protein alpha. J Biol Chem 2003; 278:15178-84.
69. Wang J, Shiels C, Sasieni P, Wu PJ, Islam SA, Freemont PS, Sheer D. Promyelocytic leukemia nuclear bodies associate with transcriptionally active genomic regions. J Cell Biol 2004; 164:515-26.
70. Polanowska J, Martin JS, Garcia-Muse T, Petalcorin MI, Boulton SJ. A conserved pathway to activate BRCA1-dependent ubiquitylation at DNA damage sites. EMBO J 2006; 25:2178-88.
71. Yu X, Fu S, Lai M, Baer R, Chen J. BRCA1 ubiquitinates its phosphorylation-dependent binding partner CtIP. Genes Dev 2006; 20:1721-6.
72. Letunic I, Goodstadt L, Dickens NJ, Doerks T, Schultz J, Mott R, Ciccarelli F, Copley RR, Ponting CP, Bork P. Recent improvements to the SMART domain-based sequence annotation resource. Nucleic Acids Res 2002; 30:242-4.
73. Wootton JC. Nonglobular domains in protein sequences: Automated segmentation using complexity measures. Comput Chem 1994; 18:269-85.
74. Liu J, Rost B. NORSp: Predictions of long regions without regular secondary structure. Nucleic Acids Res 2003; 31:3833-5.