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BRCA1 function in transcription

Review Article

Neelima Mondal and Jeffrey D. Parvin

Department of Pathology, Harvard Medical School and, Brigham and Women's Hospital, Boston, MA

Correspondence: Jeffrey D. Parvin, M.D., Ph.D., Brigham and Women's Hospital, Department of Pathology, 75 Francis Street, Boston, MA 02115, USA. Tel: 617-278-0818; Fax: 617-732-7449; E-mail: jparvin@rics.bwh.harvard.edu

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Summary

The breast and ovarian specific tumor suppressor protein, BRCA1, is associated with the transcriptional regulatory machine termed the RNA polymerase II (Pol II) holoenzyme. Experiments support a model in which molecules such as the BRCA1 protein and the CREB binding protein (CBP) function similarly as regulatory targets which link the enhancer-binding regulatory proteins to the basal transcriptional machinery. As an example, phosphorylated CREB protein interacts with the CBP molecule present in the holoenzyme complex and will activate transcription in vitro. We propose a similar model for the BRCA1 protein, but with a different subset of upstream activators functioning via BRCA1 to regulate the Pol II holoenzyme. We have established an in vitro transcriptional assay dependent upon the carboxy-terminal domain of BRCA1 fused to the DNA-binding domain of GAL4. We have also shown that both CBP and BRCA1 are linked to the Pol II holoenzyme complex via a common subunit -- the RNA Helicase A (RHA) protein. CBP binds to an amino terminal domain of RHA, and BRCA1 binds to a separate internal domain of RHA, and truncated RHA molecules have been shown to be dominant negative transcriptional repressors of either CBP or BRCA1. Since most tumor-associated BRCA1 mutations result in the truncation of the BRCA1 protein and loss of its carboxy-terminal holoenzyme interaction domain, it is suggested that an important component of the tumor suppressive function of BRCA1 occurs via the gene expression process.

I. Genetics of BRCA1

The BRCA1 gene was the first identified tumor susceptibility gene specific for breast and/or ovarian cancer (King, 1980). The gene encodes a 220 kd protein which has at its carboxy terminus an acidic domain consistent with a transcriptional regulator (Miki et al, 1994; Futreal et al, 1994). About 5% of breast cancer cases are attributable to the inheritance of a mutant BRCA1 allele. A mutant BRCA1 allele is present among about 50% of families with a predisposition to breast cancer and among about 80% of families with a predisposition to both breast and ovarian cancer (Szabo and King, 1995). Among these families, the genetics of cancer due to BRCA1 are similar to the retinoblastoma (RB) tumor suppressor. Predisposition to cancer is due to inheritance of a mutant allele, and disease occurs when the second allele becomes mutated (Neuhausen and Marshall, 1994; Smith et al, 1992).

The genetic predisposition to breast cancer clearly implicates BRCA1 protein as being important in the etiology of the disease, but these data yield no insights into *how* this protein functions. Data reviewed below will outline new developments from our laboratory on how BRCA1 functions to regulate the process of gene expression.

II. Clues to BRCA1 function

Murine knock-out studies were largely uninformative regarding the function of BRCA1 since nullizygous embryos died at very short gestational ages (Ludwig et al, 1997; Hakem et al, 1996; Hakem et al, 1997; Shen et al, 1998). An interaction with the function of the p53 tumor suppressor was suggested in several of these studies since animals doubly deficient in p53 and in BRCA1 remained alive two embryonic days longer, suggesting an antagonistic genetic interaction between these two tumor suppressors (Hakem et

al, 1997; Shen et al, 1998). Since p53 exerts its tumor suppressing effects in large part by transcriptional regulation, it is possible that BRCA1 is also a transcriptional regulator.

Transcription function of BRCA1 was first suggested in experiments in which the carboxy terminus of BRCA1 was fused to the DNA binding domain of the GAL4 transcription factor and the activation of transcription in transfected cells was observed dependent upon the presence of the GAL4 DNA element in the reporter gene (Chapman and Verma, 1996; Monteiro et al, 1996). Further transcription function of the BRCA1 protein was revealed by experiments in our laboratory in which the master transcriptional regulatory complex known as the RNA polymerase II holoenzyme (see below) was found to have BRCA1 associated with the complex when purified from HeLa or B cell lines (Scully et al, 1997a; Neish et al, 1998).

BRCA1 protein transcriptional function has been associated with the regulation of transcription by p53. Overexpression of full-length BRCA1 was found to potentiate the transcriptional activation of reporter genes controlled by p53 response elements (Ouchi et al, 1998). The p53 protein was shown to bind directly to the amino acid residues 224-500 of the BRCA1 protein (Zhang et al., 1998). These data suggest that BRCA1 may somehow interact with p53 in order to regulate gene expression and thus function in tandem as tumor suppressors. BRCA1 has also been shown to function independent of p53 in regulating the expression of the p21 cell cycle progression inhibitor (Somasundarum et al, 1997). Taken together these data suggest that BRCA1 protein acts as a tumor suppressor protein in concert with the p53 protein. Mechanistically how this occurs is the subject of the model developed in this review. Why such an interaction should result in tumor suppression specifically in breast and ovarian cancer but have less importance in other cancers is unclear.

Other functions of BRCA1 have been identified and will not be reviewed here. These include the association with the RAD51 protein in nuclear dots during the S phase of the cell cycle (Scully et al, 1997b) and BRCA1 regulation of the number of centrosomes and resultant effects on chromosome sorting during mitosis (Xu et al, 1999). It has also been shown that embryonic stem cells nullizygous for BRCA1 have defects in the process of transcription-coupled repair, although it was possible that those results could have arisen from defects in transcription alone (Gowen et al, 1998).

III. RNA polymerase II holoenzyme

Transcription of all protein-encoding genes from an eukaryotic cell into mRNA occurs via the action of RNA polymerase II (Pol II). Pol II cannot function alone, but *in vitro* requires the basal transcription factors TFIID, TFIIB, TFIIF, TFIIE, and TFIIH (reviewed by Orphanides et al, 1996). In order to observe activation of transcription *in vitro* by enhancer-binding proteins even more protein factors are required.

The mRNA-synthesizing enzyme, RNA polymerase II (Pol II) exists in at least two forms: core polymerase and Pol II holoenzyme (Koleske et al, 1994). The core polymerase contains 12 subunits and has a mass of 500 kD. Several basal transcription factors associate with core Pol II in the absence of DNA (Flores et al, 1989; Sopta et al, 1989), possibly foreshadowing the existence of a super complex containing Pol II and other basal factors pre-assembled independent of DNA (**Figure 1**).

The existence of the Pol II holoenzyme was first revealed in a yeast genetic screen for **s**uppressors of **R**NA polymerase **B** mutations which truncated the carboxy terminal domain of the largest RPB1 subunit (Nonet et al, 1989). Nine SRB genes resulted from this genetic screen and the SRB polypeptides were found exclusively in association with the Pol II in a complex termed the holoenzyme (Koleske et al, 1994). Antibodies directed against SRB proteins provide direct evidence for a holoenzyme complex that includes a subset of basal factors, as well as SRB Proteins. The yeast Pol II holoenzyme was independently discovered based upon a biochemical purification of a "mediator" fraction which allowed regulation of transcription in *in vitro* reactions (Kim et al, 1994). In these studies, the association of the yeast Pol II with mediator was required for transcriptional activation.

The yeast RNA polymerase II holoenzyme consists of core Pol II, an SRB/mediator complex, the SWI/SNF chromatin-remodeling complex and subset of basal transcription factors (Wilson et al, 1996). The SRB proteins are considered hallmarks of yeast and mammalian holoenzyme as they are found almost exclusively in the holoenzyme complex and appear to be limiting for holoenzyme formation. The most highly defined yeast SRB/mediator complex consists of polypeptides: SRB2, 4-7. Med1, 2, 4, 6-8, GAL11, Sin4, Rgr1, Rox3 and Pgd1 (Myers et al, 1998).

Yeast SRB10 is a cyclin-dependent kinase that is associated with SRB-8, -9 and -11. These polypeptides are components of the RNA polymerase II holoenzyme purified according to the method of Koleske and Young (1994). At promoters for genes involved in cell-type specificity, meiosis and sugar utilization, SRB10/11 is stimulated to phosphorylate the RNA polymerase II carboxy-terminal domain (CTD) prior to stable pre-initiation complex formation and, by doing so, inhibits transcription initiation (Hengartner et al, 1998).

The Pol II holoenzyme is best characterized from yeast cells, but new studies from our laboratory and others have characterized the Pol II holoenzyme complexes found in human cells (reviewed in Parvin and Young, 1998). Just as in the yeast holoenzyme, the mammalian version includes

SRB/mediator components and basal transcription factors: SRB7 (hSRB7), Rgr1, Med6, Med7, SRB10/11 (CDK8/cyclin C), SWI2 Family member BRG1 (Brm/SWI2 related gene), TFIIF, TFIIE, TFIIH (Parvin and Young, 1998; Neish et al, 1998; Maldonado et al, 1996; Pan et al, 1997; Ossipow et al, 1995). Unlike the yeast holoenzyme, the human holocomplex contains other proteins which have been shown to have coactivator function such as CBP and p300 and also the BRCA1 protein which has been suggested to be a coactivator (Nakajima et al, 1997a; Neish et al, 1998; Ouchi et al, 1998).

IV. Model for activation by phospho-CREB

A number of hormones and growth factors induce the expression of certain genes via a cascade of events by which ligand binding to the cell surface results in a transient increase in the concentration of the second messenger cAMP which leads to the activation of protein kinase A (PK-A) which stimulates the phosphorylation of specific nuclear factors. Genes which respond to cAMP were found to have specific DNA binding elements known as CREs, for **c**AMP **r**esponse **e**lements, and the factor CREB bound to these DNA sequences. Activated PK-A phosphorylates Ser-133 of CREB prebound on its DNA element and this leads to the activation of neighboring genes (Gonzalez and Montminy, 1989). Although phosphorylation has been shown to stimulate a number of nuclear factors via their DNA-binding or nuclear-targeting activities, CREB belongs to a group of activators whose transactivation potential is specifically affected (Gonzalez and Montminy, 1989; Hagiwara et al, 1992).

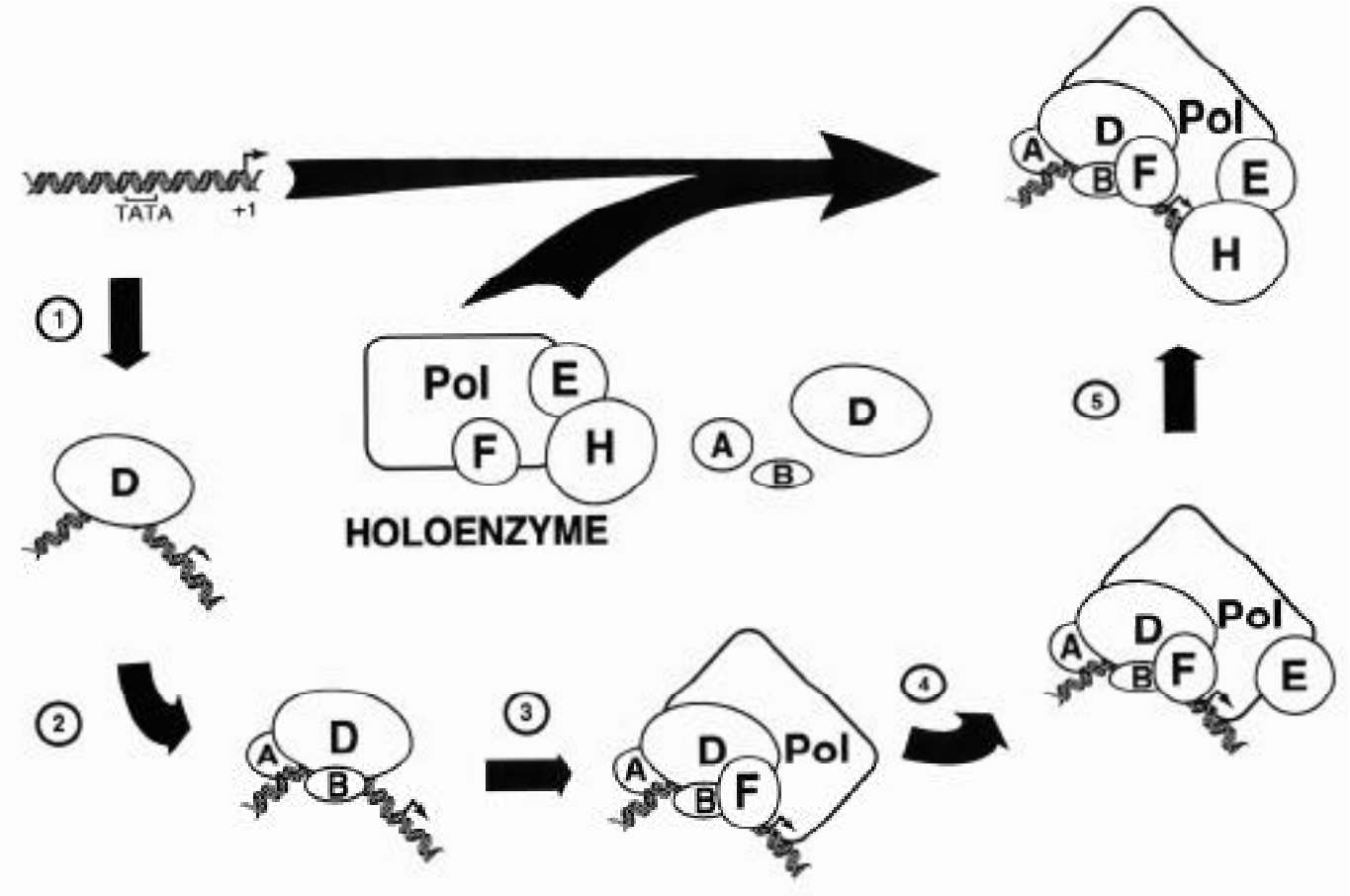

Figure 1. Ordered assembly of individual basal transcription factors versus pre-assembled holoenzyme complex. The topmost left panel shows DNA strand with TATA box element. The small arrow indicates the start site and direction of transcription. TFIID first binds to the TATA box and facilitates the binding of TFIIA and TFIIB. This in turn gives the signal for binding of TFIIF and RNA Pol II (core). This complex then facilitates the binding of TFIIE and finally TFIIH to form the initiation complex for transcription. In contrast the pre-assembled holoenzyme containing TFIIE, TFIIF and TFIIH as well as other subunits not shown, is in fact the basal factor complex, which catalyzes most, or all, of the mRNA transcription in the eukaryotic cell.

The CREB transactivation domain is bipartite, consisting of constitutive and inducible activators, termed Q2 and **k**inase-**i**nducible **d**omain (KID), respectively, which function cooperatively in response to cAMP (Brindle et al, 1993; Quinn, 1993). The glutamine rich Q2 domain has been shown to involve the transcriptional apparatus via a constitutive interaction with the TBP-associated factor hTAFII130 (Ferreri et al, 1994; Nakajima et al, 1997a). In contrast, increased intracellular cAMP levels results in the phosphorylation of the KID domain on serine-133 which modulates association with CREB binding protein (CBP; Arias et al, 1994; Kwok et al, 1994). CBP and its related protein, p300, have since been shown to be coactivators for numerous transcriptional regulators in addition to CREB including c-Jun ATF-2, STAT-2, NF-κB, Mi, and p53 (Kamei et al, 1996; Arias et al, 1994; Hanstein et al, 1996; Gerritsen et al, 1997; Price et al, 1998; Gu et al, 1997; reviewed in Parvin and Young, 1998). For CREB and Mi, it has been shown that phosphorylation of the transcriptional regulator is the essential signal for the activator to interact with CBP/P300 (Chrivia et al, 1993; Price et al, 1998). For other factors, such as NF-κB, nuclear transport may be the regulated step for binding CBP.

We recently showed that CBP was found associated with functional RNA Pol II holoenzyme complex, and the regulation of transcription *in vitro* was dependent upon the CBP-containing Pol II complex (**Figure 2**; Nakajima et al, 1997a). Stimulation of transcription required a phosphorylated Ser-133 CREB as well as interaction with TAF-130 of TFIID. The E1A oncoprotein inhibits transcriptional activation of CREB without interfering with complex formation between phospho (Ser-133)-CREB and CBP. Our *in vitro* transcription and binding data demonstrated that the mechanism by which E1A suppresses CREB-dependent transcriptional activation is by blocking the interaction of CBP with holoenzyme which causes the release of CBP/p300 and thus suppresses transcription (Nakajima et al, 1997a).

CBP and p300 have demonstrated **h**istone **a**cetyl**t**ransferase (HAT) activity (Bannister and Kouzarides, 1996; Orgryzko et al, 1996). It is possible that the enhancer-binding regulator of transcription recruits the Pol II holoenzyme via CBP, or it is possible that the activator recruits the HAT activity to the chromatin in the region of the promoter. The repression of transcription by histone deacetylase supports the role of the HAT activity of CBP (Hassig et al, 1997; Pazin and Kadonaga, 1997). The activation of transcription *in vitro* via CBP has been observed in the absence of the key HAT substrate, histone (Nakajima et al, 1997a), suggesting that at least part of the activity of CBP/p300 is as a holoenzyme-bound target.

The domain of CBP which interacts with the holoenzyme complex was mapped to amino acid residues 1805-1890 (Nakajima et al, 1997a). This domain was used as a probe in far western gels to identify a 140 kd protein which copurified with the holoenzyme complex, and probing of an expression library revealed RNA helicase A (RHA) as a candidate for the subunit in the holoenzyme complex which binds CBP (Nakajima et al, 1997b). RHA (Lee and Hurwitz, 1993) is the homologue of the drosophila maleless protein which regulates the levels of transcription from the X chromosome in fruit flies (Bone et al, 1994). Experiments indicated that CBP amino acid residues 1805-1890 directly contact RHA residues 1-250, and that overexpression in tissue culture of RHA (1-250) resulted in an inhibition of CREB-CBP-dependent transcriptional activation (Nakajima et al, 1997b). Further, the E1A oncoprotein competed with RHA for binding to CBP, suggesting the mechanism by which the truncation mutant functioned as a dominant negative protein. These data support the model presented in **Figure 2**.

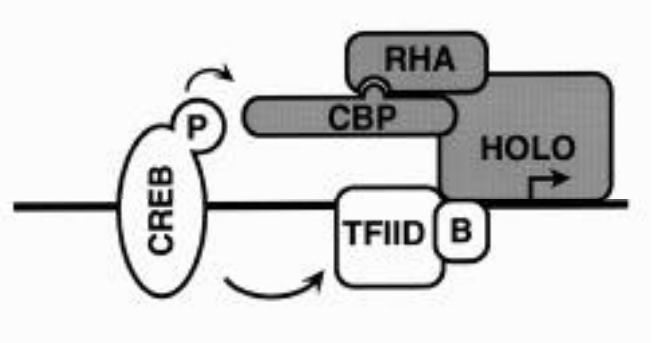

Figure 2. Model for regulation of transcription by phospho-CREB-CBP-holoenzyme. The CREB transcription factor bound to its cognate DNA enhancer element is phosphorylated on the KID domain as a result of elevated intercellular cAMP levels. Phospho-CREB then binds to CBP and recruits the holoenzyme via RHA (1-250) to the promoter. A second activation signal from the Q2 domain of CREB interacts with TFIID. Our data demonstrate that both activation signals are required in order to stimulate high levels of mRNA synthesis

V. BRCA1 is a component of RNA Pol II holoenzyme

We have identified that a significant fraction of the BRCA1 protein in HeLa and B cells is associated with the Pol II holoenzyme complex (Scully et al, 1997a; Neish et al, 1998). The carboxy terminus of BRCA1, when fused to the DNA binding domain of the GAL4 protein, has been shown to activate transcription of GAL4-site dependent reporters in transfected cells (Chapman and Verma, 1996; Monteiro et al, 1996). We demonstrated that this carboxy terminal domain

(residues 1560-1863) could function as an affinity matrix for purification of the holoenzyme and that point mutations in this domain at sites which cause cancer resulted in BRCA1 polypeptide which no longer bound to the holoenzyme with high affinity (Neish et al, 1998).

We found that this carboxy-terminal BRCA1 domain also binds to the RHA subunit of the holoenzyme complex. RHA (1-250) binds to CBP, and a separate RHA domain from residues 235-325 binds to BRCA1 (Anderson et al, 1998). The BRCA1 carboxy terminal domain containing a point mutation at a site associated with cancer binds to RHA with significantly lower affinity suggesting that this interaction is specific. The *in vitro* binding data are supported by directed yeast two-hybrid data which demonstrate the interaction between BRCA1 and RHA occurs in that setting. The BRCA1-RHA interaction is central to the activation of transcription of GAL4-BRCA1 fusions in transfection assays. This was determined using truncated RHA molecules which retain BRCA1 binding, and it was shown that expression of these peptides inhibited GAL4-BRCA1 transcriptional activation (Anderson et al, 1998).

Taken together with the CBP data, it is suggested that BRCA1 functions as an holoenzyme component similarly as does CBP (**Figure 3**). The similar interaction of holoenzyme component RHA with both CBP and BRCA1 suggest analogous function. In addition the findings that full length BRCA1 potentiates activation by the p53 transcriptional activator (Ouchi et al, 1998; Zhang et al, 1998) and that p53 directly contacts BRCA1 residues 224-500 support this model whereby a transcriptional activator, such as p53, binds to the BRCA1 amino terminus and is thus linked with the basal transcriptional machinery in the holoenzyme. Further, other factors may interact in this fashion with BRCA1. For example, the p21 promoter was found to be regulated by BRCA1 via an as yet undetermined transcriptional activator which binds to specific promoter sequences (Somasundarum et al, 1997). The p53 protein could thus interact with the holoenzyme via CBP (Gu et al, 1997) or via BRCA1, raising the issue of why p53 would interact with both CBP and BRCA1. It is likely that interaction with BRCA1 would have a different outcome than activation by p53 via CBP.

VI. Function of BRCA1 using *in vitro* transcription assay

BRCA1 does not bind DNA, but its transcriptional function in transfected cells has been demonstrated when it is linked to a specific DNA binding domain in the GAL4 protein (cited above). The GAL4 DNA binding domain fused to BRCA1 (1560-1863) was purified in bacteria and tested for GAL4-site-dependent transcriptional activation using highly purified basal transcription factors *in vitro* (Haile and Parvin, 1999). When compared with GAL4-VP16, the most powerful transcriptional activator known, the BRCA1 fusion was the more powerful transcriptional activator. This activation by BRCA1 was condition-specific. When certain transcriptional coactivators were present, then the VP16 fusion was more potent. Different constellations of coactivators and TFIIA were checked for activation by GAL4-BRCA1 and GAL4-VP16 and the most striking difference was observed between **P**ositive **C**omponent **4** (PC4) alone versus PC4 plus HMG2. When PC4 alone was used, BRCA1 about 5 fold more effective than VP16, whereas when PC4 plus HMG2 was used, VP16 was twice as effective than was BRCA1. Since PC4 binds to single-stranded bubbles in the DNA, the effect of negative superhelical turns of the template on the transcriptional stimulation was assayed. Activation by BRCA1 was highly dependent upon the supercoiled DNA, whereas the VP16 was more effective on a linear template. These data suggest that VP16 may recruit the TFIIH basal transcription factor to the promoter DNA, and the TFIIH would then generate the bubble of unwound DNA necessary for high levels of transcription. In contrast, BRCA1 would rely upon the supercoiling of the DNA to drive the formation of the transcription bubble. We hypothesize from these data that BRCA1 and VP16 function via different rate-limiting steps leading to the regulation of transcription (Haile and Parvin, 1999).

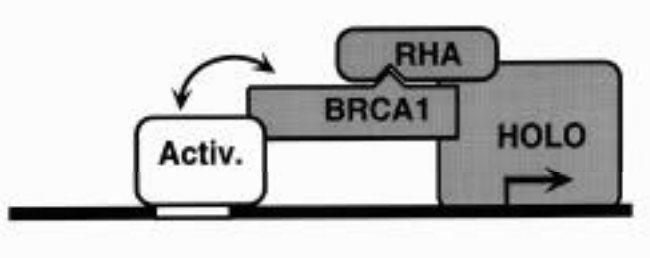

Figure 3. Model for regulation of transcription by BRCA1 as an holoenzyme component. Analogous to the CBP-dependent model outlined in **Figure 2**, activators which binds upstream of promoter to an enhancer element recruit Pol II holoenzyme via BRCA1. Just as CBP signals the holoenzyme via RHA subunit, BRCA1 is modeled to interact with the holoenzyme complex via a second RHA domain. As described in the text, an activator which potentially functions via BRCA1 is p53.

Conclusions.

Data are accumulating to suggest that BRCA1 plays an important role in transcriptional regulation. GAL4-BRCA1 fusions have been shown to activate transcription when expressed in cells or when assayed *in vitro* with purified transcription factors. Full length BRCA1 has been found to potentiate the regulation of transcription by p53 and possibly

via other proteins which function on the p21 promoter. The model presented in this review suggests that BRCA1 functionally links certain upstream enhancer-binding factors to the basal transcription machinery in the holoenzyme. This is similar to a model for CBP function which we demonstrated applies for CREB-dependent transcriptional activation. Ongoing studies are aimed at identifying which factors are being directly contacted by BRCA1 and which amino acid residues of BRCA1 are involved in protein-protein interactions. Further, it may be possible to identify which genes are directly regulated by BRCA1.

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