BRCA1 can stimulate gene transcription by a unique mechanism

Guy Nadeau, Nadia Boufaied, Annie Moisan, Karine M. Lemieux, Charmagne Cayanan¹, Alvaro N.A. Monteiro^{1,2} and Luc Gaudreau⁺

Centre de Recherche sur les Mécanismes d'Expression Génétique, Département de Biologie, Université de Sherbrooke, Sherbrooke, Québec, J1K 2R1, Canada, ¹Strang Cancer Prevention Center, 1230 York Avenue, New York, NY 10021 and ²Department of Cell Biology and Anatomy, Joan and Sanford I. Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021, USA

Received May 3, 2000; revised and accepted August 1, 2000

Most familial breast and ovarian cancers have been linked to mutations in the *BRCA1* gene. *BRCA1* has been shown to affect gene transcription but how it does so remains elusive. Here we show that *BRCA1* can stimulate transcription without the requirement for a DNA-tethering function in mammalian and yeast cells. Furthermore, the *BRCA1* C-terminal region can stimulate transcription of the p53-responsive promoter, *MDM2*. Unlike many enhancer-specific activators, non-tethered *BRCA1* does not require a functional TATA element to stimulate transcription. Our results suggest that *BRCA1* can enhance transcription by a function additional to recruiting the transcriptional machinery to a targeted gene.

INTRODUCTION

BRCA1 mutations are thought to account for ~45% of families with high breast cancer risk and >80% of families with high risk of early-onset breast and ovarian cancer (Easton *et al.*, 1993). The human *BRCA1* gene encodes a 1863 amino acid nuclear protein that has been implicated in DNA repair and transcription activation (see Monteiro, 2000; Welcsh *et al.*, 2000). A role for *BRCA1* in gene transcription has been proposed mainly for the following reasons: (i) the C-terminal portion of *BRCA1*, which bears an excess of negatively charged residues, can activate transcription of a target gene when attached to a DNA-binding domain (DBD) *in vivo* (Chapman and Verma, 1996; Monteiro *et al.*, 1996; Anderson *et al.*, 1998) and *in vitro* using a highly purified system (Haile and Parvin, 1999); (ii) it associates with a form of RNA polymerase II holoenzyme (Scully *et al.*, 1997); and (iii) it modulates the activity of certain transcriptional activators (Somasundaram *et al.*, 1997; Ouchi *et al.*, 1998, 2000; Wang *et al.*, 1998). It is noteworthy that the above mentioned work

implies *BRCA1* as a physiological regulator of p53-dependent genes such as *p21^{WAF}* and *MDM2*.

Here we show that *BRCA1* can stimulate gene transcription of a variety of reporter gene constructs without the requirement for a specific DBD in human and in yeast cells both *in vivo* and *in vitro*. We further show that the *BRCA1* C-terminal region can increase transcription of a reporter gene independently of a functional TATA element.

RESULTS

BRCA1 stimulates transcription without requiring a specific DBD in human cells

While studying the effect of the *BRCA1* C-terminal region on gene transcription, we noticed that expression of a protein fragment (aa 1528–1863) could stimulate gene transcription in transiently transfected human cells. We thus hypothesized that perhaps the activation elicited by a DNA-tethered *BRCA1* chimera (e.g. Gal4–*BRCA1*) might not be solely dependent on a heterologous DBD. To test this hypothesis, we transfected human HCC1937 *BRCA1*^{−/−} breast cancer cells (Tomlinson *et al.*, 1998) with a vector overexpressing Gal4–*BRCA1* (aa 1528–1863) or the *BRCA1* fragment without the Gal4 DBD. As previously reported (Chapman and Verma, 1996; Monteiro *et al.*, 1996; Anderson *et al.*, 1998), the Gal4–*BRCA1* fusion activated transcription from a reporter template bearing four Gal4-binding sites upstream of the *c-fos* minimal promoter fused to the luciferase gene (Figure 1A). Interestingly, the *BRCA1* fragment, without a Gal4 DBD, was also able to enhance the level of transcription several-fold in a dose-dependent fashion. To determine

⁺Corresponding author. Tel: +1 819 821 8000 ext. 2081; Fax: +1 819 821 8049; E-mail: luc.gaudreau@courrier.usherb.ca

whether the effect could be specific to the promoter context of the template, we made use of a promoter template bearing two LexA-binding sites in place of the Gal4 sites, and the *E1B* core promoter in place of the *c-fos* promoter. The results show that even though both the Gal4-BRCA1 fusion and BRCA1 are not expected to bind the promoter template, they are both able to stimulate transcription when overexpressed in the cell (Figure 1B). These results would be expected for an activator that does not require DNA binding to exert its function.

Transcription stimulation of *MDM2* by BRCA1 derivatives

The BRCA1 C-terminal region stimulates transcription of a TATA-mutated promoter

EMBO Reports vol. 1 | no. 3 | 2000 **261**

G. Nadeau et al.

Fig. 2. Transcription stimulation of the *MDM2* promoter by BRCA1 derivatives. HCC1937 cells were transfected with various BRCA1 derivatives (using either 0.5 or 1.0 μ g of DNA) and 25 ng of an *MDM2-luciferase* template (Ouchi *et al.*, 1998). Exons 1–3 (EX1–EX3) and p53-responsive elements (PG) are indicated. The vector control denotes pcDNA3; BRCA1-C denotes BRCA1(1528–1863); BRCA1-C Y1853X is the BRCA1 C-terminal region with the Y1853X mutation; and BRCA1-FL are the full-length molecules. Activation was measured as in Figure 1A.

the TGTA template as compared with the TATA template (Figure 3A, right panel). Introduction of a Y1853X mutation markedly impaired stimulation of transcription.

We next wanted to test whether the BRCA1 C-terminal region could further enhance transcription of a gene activated by artificially recruited TBP. Figure 3B shows that TBP, when fused to the Gal4 DBD, can activate transcription some 15-fold in human HEK-293 cells. When BRCA1(1528–1863) is transfected along with Gal4–TBP, we see an ~3-fold increase in activation by Gal4–TBP. Transfection of the Y1853X BRCA1 mutant did not enhance transcription of Gal4–TBP nearly as efficiently as its wild-type counterpart. These results suggest that BRCA1 can stimulate transcription above levels elicited by artificial recruitment of TBP to the TATA box. We cannot, however, exclude the possibility that BRCA1 further enhances TBP binding to the promoter beyond the recruiting effect of the Gal4 DBD.

BRCA1 stimulates transcription without requiring a specific DBD in yeast cells

To determine whether the effect of BRCA1 on transcription was limited to mammalian cells, we performed experiments in yeast. Figure 4A shows that, when expressed in yeast cells, full-length BRCA1 is able to stimulate transcription (bar 2) of a *GAL1::lacZ* reporter template efficiently. Expression of the Y1853X mutant (Figure 4A, bar 4) in yeast failed to activate transcription, while the C61G mutant (bar 3), also a clinically relevant mutation but located at the N-terminus of BRCA1 (Friedman *et al.*, 1994),

Fig. 3. BRCA1 stimulates transcription independently of TBP binding. (A) BRCA1 can efficiently stimulate transcription in the absence of a functional TATA element. HCC1937 cells were transfected with either a reporter template bearing a functional TATA element or one with a mutated TATA (TGTA) but otherwise identical. The left part of the figure measures the ability of the Gal4 DBD, Gal4–VP16 and Gal4–E2F1 to activate transcription at both templates. The right part of the figure measures the ability of various BRCA1 derivatives (using either 0.5 or 1.0 μ g of DNA) to stimulate both reporters. The vector control denotes pcDNA3; BRCA1-C denotes BRCA1(1528–1863); BRCA1-C Y1853X is the BRCA1 C-terminal region with the Y1853X mutation. Activation was measured as in Figure 1A and B. (B) The BRCA1 C-terminal region stimulates activation elicited by Gal4–TBP. Transiently transfected HEK-293 cells were assayed for luciferase activity using the reporter template of Figure 1A. Either the Gal4 DBD (1–147) (0.2 μ g) or Gal4–TBP (0.2 μ g) was used in the experiment with or without 0.4 μ g of either BRCA1-C derivative (wild-type or Y1853X mutant).

could elicit levels of activation comparable to the wild-type protein. The N-terminal region of BRCA1 seems dispensable for this function, confirming our data from mammalian cells where the C-terminal alone is sufficient for activation. Figure 4B shows that recombinant BRCA1(1528–1863) can stimulate transcription of the *GAL1* promoter *in vitro* using a yeast nuclear extract preparation (Wu *et al.*, 1996). The figure also shows that, as in Figure 1C, BRCA1 can stimulate transcription in a dose-dependent fashion. The Y1853X mutant had no stimulatory effect (Figure 4B, right panel, lane 3).

Fig. 4. BRCA1 can stimulate gene transcription in yeast without the requirement for a DBD. **(A)** *In vivo*. The ability of BRCA1(1–1863) to stimulate transcription in yeast without a DBD was assessed in a yeast strain harboring a *lacZ* reporter template with an ER binding site upstream of the *GAL1* TATA. Activation was measured as arbitrary β-galactosidase units. **(B)** *In vitro*. *In vitro* transcription reactions were carried out with a yeast nuclear extract and *GAL1* DNA template. Primer extensions were carried out as in Figure 1C. Recombinant BRCA1(1528–1863) was added at 200 and 400 ng (lanes 2 and 3, respectively). The right panel depicts a Y1853X control (200 ng, lane 3) along with BRCA1 (200 ng, lane 2) as in Figure 1C.

DISCUSSION

Our results show that the BRCA1 C-terminal region has the surprising ability to stimulate transcription without the need for

a DNA-binding function and, in so doing, exploits a mechanism different from one typified by many enhancer-binding activators. Importantly, these activities were crippled upon introduction of a cancer-associated mutation, Y1853X, which destabilizes the BRCT region leaving one of the two BRCT motifs intact. This intact BRCT could perhaps explain the transcriptional activity of that mutant when it is well overexpressed in some experiments. We have also shown that the C-terminal region of BRCA1, as well as the full-length molecule, could stimulate transcription of a p53-responsive promoter. The experiments described here rely on overexpression of BRCA1 derivatives and could imply a global effect on gene transcription, considering that BRCA1 can activate genes with a variety of promoter contexts. However, we propose that our experimental conditions represent increased local concentrations of BRCA1 under physiological conditions, a condition that we believe bypasses specific targeting of BRCA1 to our reporters, a situation well exemplified by our experiment with the *MDM2* reporter.

Our results showing that BRCA1 does not require a functional TATA element to stimulate transcription could imply, for example, that either BRCA1 stabilizes binding of the transcription machinery (e.g. TFIID) to the mutated promoter or, alternatively, BRCA1 could act independently of TBP binding to DNA to stimulate transcription. Recently, oligonucleotide array-based expression profiling experiments have revealed that BRCA1 expression could efficiently induce transcription of the DNA damage-responsive gene *GADD45* in a p53-independent fashion (Harkin *et al.*, 1999). Interestingly, examination of the *GADD45* proximal promoter region did not reveal the presence of any consensus TATA element (not shown). Thus, the fact that BRCA1 does not require a functional TATA element to stimulate transcription would be relevant in this particular case.

In prokaryotes, there are at least three examples of activators that do not require sequence-specific DNA-binding activity in order to stimulate transcription: (i) DNA-tracking proteins exemplified by the phage T4 Gp54 one-dimensional sliding clamp; (ii) the phage N4 single-stranded binding protein (N4SSB), which interacts with the β' subunit of RNA polymerase and activates σ⁷⁰-type promoters; and (iii) σ⁵⁴-specific activators, which, even if being enhancer-binding proteins, can activate transcription at high concentrations without being DNA tethered (see Ptashne and Gann, 1997; Hochschild and Dove, 1998). It is conceivable that BRCA1 could stimulate transcription by a mechanism similar to any of the bacterial examples listed above. Recent experiments carried out with the TATA-binding protein-interacting protein TIP120 and ABT1 have shown that they too can enhance transcription without requiring a DNA-tethering function (Makino *et al.*, 1999; Oda *et al.*, 2000). Interestingly, our results could explain why BRCA1 activates the *p21WAF1* gene independently of p53 (Somasundaram *et al.*, 1997; Ouchi *et al.*, 1998) and also IFN-γ target genes in the absence of interferon stimulation (Ouchi *et al.*, 2000).

We consider that the mechanism by which BRCA1 stimulates transcription could constitute an advantage in cases where the transcriptional machinery might be paused, for example, at sites of DNA damage in a particular gene, located at a distance from its enhancer sequences. In the latter case, BRCA1 would be able to counteract a transcriptional pause without any interaction(s) with the upstream enhancer. Consistent with this idea is a recent report which suggests that BRCA1 BRCT domains can bind DNA

strand breaks and termini (Yamane and Tsuruo, 1999). Thus, it is conceivable that BRCA1 could recognize damaged DNA at paused transcription sites, and in so doing, increase favorable interactions with the transcriptional machinery thereby further enhancing transcription and perhaps, the transcription-coupled DNA repair process itself. In an alternative mode not mutually exclusive with the previous one, BRCA1 could be targeted to specific genes by interaction with enhancer-binding activators and in so doing, further enhance the transcription process.

METHODS

Transient transfections. Human HCC1937 and HEK-293 cells were used for the transfection experiments and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Cells were plated in six-well tissue culture plates for 24 h before transfection at a density of $1\text{--}2 \times 10^5$ cells/well. Cells were then transfected using either Eugene 6 or Lipofectamine 2000 reagents. After 18–24 h, cells were processed for luciferase assays. Transfection efficiencies, when appropriate, were normalized using the pβgal plasmid; in some experiments, we noticed that BRCA1 increased the activity of our *lacZ* internal control, thus, in many experiments we did not normalize our transfection efficiencies; nonetheless we always measured *lacZ* values to make sure no gross deviations would be observed. Details of plasmid constructions are available upon request.

Recombinant BRCA1 derivatives. Recombinant BRCA1(1528–1863) was expressed in *Escherichia coli* using the pET30a expression vector. The recombinant protein was first chromatographed on Ni-NTA agarose and then further fractionated on an FPLC mono-Q ion exchange column. The Y1853X mutant fragment was also expressed in *E. coli*, affinity purified on Ni-NTA agarose and then subjected to chromatography on a Superdex 200 HR 10/30 column.

Nuclear extracts and *in vitro* transcription. HeLa and HCC1937 nuclear extract preparation and transcription reactions were as previously described (Shapiro et al., 1988). Yeast nuclear extract preparation and transcription reactions were as described in Wu et al. (1996) and supplemented with recombinant TBP and TFIIE. For human *in vitro* transcription experiments, the pGML4 template was used at 100 ng/reaction; pGML4 bears the *AdML* promoter without any enhancer sequences. For yeast *in vitro* transcription experiments, the pGDC01 template was used at 100 ng/reaction; pGDC01 bears the *GAL1* promoter with its native UAS_C. All data were quantified by phosphorimaging.

Yeast manipulations. The yeast strain TGY14 (*MATa*, *ura3-251-373-328*, *leu2*, *pep4.3*) was used and contained a *GAL1::lacZ* reporter gene inserted downstream of an ERE. Competent yeast cells were obtained using the yeast transformation system (Clontech) based on lithium acetate, and cells were transformed according to the manufacturer's instructions. Activity of the reporter was measured by liquid β-galactosidase assays.

ACKNOWLEDGEMENTS

We thank P. Broad, G. Bryant, P. Chambon, M. Chapman, B. Coulombe, M. Erdos, J. Humphrey, M. Larochelle, I. Verma and S. Weintraub for plasmids; N. Caron for advice; and A. Barberis

and T. Urmenyi for critical reading of the manuscript. This work was supported by the MRC of Canada to L.G. A.M. was supported by an NSERC fellowship. L.G. is a scholar of the MRC/CRS Inc. A.N.A.M. is supported by the AMDeC Foundation of New York City (Tartikoff/Perelman/EIF Fund) and by US Army award DAMD17-99-1-9389.

REFERENCES

- Anderson, S.F., Schlegel, B.P., Nakajima, T., Wolpin, E.S. and Parvin, J.D. (1998) BRCA1 protein is linked to the RNA polymerase II holoenzyme complex via RNA helicase A. *Nature Genet.*, **19**, 254–256.
- Bryant, G.O., Martel, L.S., Burley, S.K. and Berk, A.J. (1996) Radical mutations reveal TATA-box binding protein surfaces required for activated transcription *in vivo*. *Genes Dev.*, **10**, 2491–2504.
- Chapman, M.S. and Verma, I.M. (1996) Transcriptional activation by BRCA1. *Nature*, **382**, 678–679.
- Easton, D.F., Bishop, D.T., Ford, D. and Crockford, G.P. (1993) Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. Breast cancer linkage consortium. *Am. J. Hum. Genet.*, **52**, 678–701.
- Friedman, L.S., Ostermeyer, E.A., Szabo, C.I., Dowd, P., Lynch, E.D., Rowell, S.E. and King, M.C. (1994) Confirmation of *BRCA1* by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nature Genet.*, **8**, 399–404.
- Haile, D.T. and Parvin, J.D. (1999) Activation of transcription *in vitro* by the BRCA1 carboxyl-terminal domain. *J. Biol. Chem.*, **274**, 2113–2117.
- Harkin, D.P., Bean, J.M., Miklos, D., Song, Y.-H., Truong, V.B., Englert, C., Maheswaran, S., Oliner, J.D. and Haber, D.A. (1999) Induction of *GADD45* and JNK/SAPK-dependent apoptosis following inducible expression of *BRCA1*. *Cell*, **97**, 575–586.
- Hochschild, A. and Dove, S.L. (1998) Protein–protein contacts that activate and repress prokaryotic transcription. *Cell*, **92**, 597–600.
- Makino, Y. et al. (1999) TATA-binding protein-interacting protein 120, TIP120, stimulates three classes of eukaryotic transcription via a unique mechanism. *Mol. Cell. Biol.*, **19**, 7951–7960.
- Monteiro, A.N.A. (2000) BRCA1: exploring the links to transcription. *Trends Biochem. Sci.*, in press.
- Monteiro, A.N.A., August, A. and Hanafusa, H. (1996) Evidence for a transcriptional activation function of BRCA1 C-terminal region. *Proc. Natl Acad. Sci. USA*, **93**, 13595–13599.
- Oda, T., Kayukawa, K., Hagiwara, H., Yudate, H.T., Masuho, Y., Murakami, Y., Tamura, T.A. and Muramatsu, M.A. (2000) A novel TATA-binding protein-binding protein, ABT1, activates basal transcription and has a yeast homologue that is essential for growth. *Mol. Cell. Biol.*, **20**, 1407–1418.
- Ouchi, T., Monteiro, A.N.A., August, A., Aaronson, S.A. and Hanafusa, H. (1998) BRCA1 regulates p53-dependent gene expression. *Proc. Natl Acad. Sci. USA*, **95**, 2302–2306.
- Ouchi, T., Lee, S.W., Ouchi, M., Aaronson, S.A. and Horvath, C.M. (2000) Collaboration of signal transducer and activator of transcription 1 (STAT1) and BRCA1 in differential regulation of IFN-γ target genes. *Proc. Natl Acad. Sci. USA*, **97**, 5208–5213.
- Ptashne, M. and Gann, A. (1997) Transcriptional activation by recruitment. *Nature*, **386**, 569–577.
- Scully, R., Anderson, S.F., Chao, D.M., Wei, W., Ye, L., Young, R.A., Livingston, D.M. and Parvin, J.D. (1997) BRCA1 is a component of the RNA polymerase II holoenzyme. *Proc. Natl Acad. Sci. USA*, **94**, 5605–5610.
- Shapiro, D.J., Sharp, P.A., Wahli, W.W. and Keller, M.J. (1988) A high-efficiency HeLa cell nuclear transcription extract. *DNA*, **7**, 47–55.
- Somasundaram, K. et al. (1997) Arrest of the cell cycle by the tumour suppressor BRCA1 requires the CDK-inhibitor p21^{WAF1/Cip1}. *Nature*, **389**, 187–190.

- Tomlinson, G.E. *et al.* (1998) Characterization of a breast cancer cell line derived from a germ-line BRCA1 mutation carrier. *Cancer Res.*, **58**, 3237–3242.
- Wang, Q., Zhang, H., Kajino, K. and Greene, M.I. (1998) BRCA1 binds c-Myc and inhibits its transcriptional and transforming activity in cells. *Oncogene*, **17**, 1939–1948.
- Welsh, P.L., Owens, K.N. and King, M.C. (2000) Insights into the functions of BRCA1 and BRCA2. *Trends Genet.*, **16**, 69–74.
- Wu, Y., Reece, R. and Ptashne, M. (1996) Quantitation of putative activator-target affinities predicts transcriptional activating potentials. *EMBO J.*, **15**, 3951–3963.
- Yamane, K. and Tsuruo, T. (1999) Conserved BRCT regions of TopBP1 and of the tumor suppressor BRCA1 bind strand breaks and termini of DNA. *Oncogene*, **18**, 5194–5203.
- Zhang, H., Somasundaram, K., Peng, Y., Tian, H., Zhang, H., Bi, D., Weber, B.L. and El-Deiry, W.S. (1998) BRCA1 physically associates with p53 and stimulates its transcriptional activity. *Oncogene*, **16**, 1713–1721.

DOI: 10.1093/embo-reports/kvd059