prompts speculation that overexpression of transgenes in localized areas of the heart may be useful in correcting local cardiac abnormalities. These findings extend beyond the immediate results to challenge us to explore the potential for gene therapy in the prevention of cardiac arrhythmias. Such research may lead to the dawn of 'molecular electrophysiology'.

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Pointing (zinc) fingers at BRCA1 targets

A long search for the mechanism by which BRCA1 regulates DNA damage-inducible genes leads to a novel zinc finger protein, ZBRK1.

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Susceptibility to breast cancer has long been known to possess a genetic component. Some of the genes implicated in the development of breast cancer include the tumor suppressors *p53* and *CHK2* in the Li-Fraumeni syndrome, ataxia telangiectasia (AT) mutated (*ATM*), *PTEN* in Cowden syndrome and the *BRCA1* and *BRCA2* genes in familial breast cancer. Individuals who inherit a mutant *BRCA1* gene have a high lifetime risk of developing breast and/or ovarian cancer. Surprisingly, *BRCA1* and *BRCA2* mutations do not occur in sporadic forms of breast cancer. This is unlike other tumor suppressor genes such as *APC*, *p16INK4A*, *Rb* or *p53*, in which mutations occur in sporadic as well as familial forms of cancer. In recent years, however, it has become clear that tumor-suppressing proteins function within cellular pathways that negatively regulate cell cycle, cell death, angiogenesis, DNA damage response or repair processes. Thus the attempt at understanding the biology and mechanism of action of BRCA1 as a major contributor, at least to familial breast cancer risk, has been met with great interest and the hope of understanding more about sporadic breast cancer.

When the breast and ovarian cancer susceptibility gene was discovered almost seven years ago, the lack of any discernable motifs or domains in the predicted protein sequence hindered the immediate characterization of the protein's function[1]. Study of the subcellular localization of BRCA1 protein and colocalization with Rad proteins identified a function in the DNA damage response[2]. This finding was substantiated by the partial knockout of BRCA1 (deletion of exon 11) that resulted in genomic instability of cells derived from these embryos, which died *in utero*. On the other hand, BRCA1's acidic carboxy terminus and ability to bind to a number of proteins involved in transcription led to the suggestion that it played a role in transcriptional control.

The involvement of BRCA1 in transcriptional regulation has been well-studied, revealing its ability to bind transcriptional regulatory proteins (such as STAT1, Myc, p53 and estrogen receptors) and proteins involved in chromatin remodeling (such as BRG1, RbAP46/48-HDAC, p300/CBP) (Fig. 1). Expression of several cellular regulatory proteins have also been shown to be activated or repressed by BRCA1, such as the cyclin-dependent kinase inhibitor $p21^{WAF1}$, cyclin B1 and the cellular transcription factor early growth response factor 1 (EGR1). Unfortunately, this line of study has been criticized due to the lack of evidence that BRCA1 is able to physically associate with the regulatory regions of the genes it appears to control.

This missing link between BRCA1 and DNA binding has finally been uncovered by Zheng *et al.*[3] and reported in the October issue of *Molecular Cell*. Using a yeast two-hybrid screen that used BRCA1 amino acids 1–1142 as bait, a novel protein, named ZBRK1, has been identified and shown to physically interact with BRCA1. Upon sequence analysis, ZBRK1 appears to be a transcription factor, due to the presence of eight consecutive Krupple-like C2H2 zinc finger motifs and a KRAB domain. The presence of these domains not only suggests that ZBRK1 can regulate transcription, but also that it may be a repressor. Indeed, ZBRK1 was found to repress transcription of GADD45, a gene whose transcription is controlled by BRCA1 (ref. 4,5). This repressive effect disappeared when reporter assays were performed in BRCA1-deficient mouse embryo fibroblasts, confirming the requirement for BRCA1 in GADD45 transcriptional repression.

An indication of the physiological relevance of a particular protein–protein interaction is whether the interaction is altered in cancer cells, such as in the case of the tumor suppressors pRb and p53. Adding clinical relevance to this functional interaction, certain tumor-derived mutations of BRCA1 are incapable of both interacting with ZBRK1 and functioning as a corepressor with ZBRK1 in *Brca1*$^{-/-}$ cells. The study of Zheng *et al.*[3] continued where other studies of BRCA1 in transcriptional control fell short, demonstrating the presence of BRCA1 in nuclear complexes that bind a ZBRK1 DNA consensus sequence. After screening an oligonucleotide library, the authors discovered a DNA sequence capable of binding directly to the ZBRK1 protein. Zheng *et al.* then showed that this sequence was present in the third intron of GADD45. By using antibodies that recognize BRCA1, the authors were able

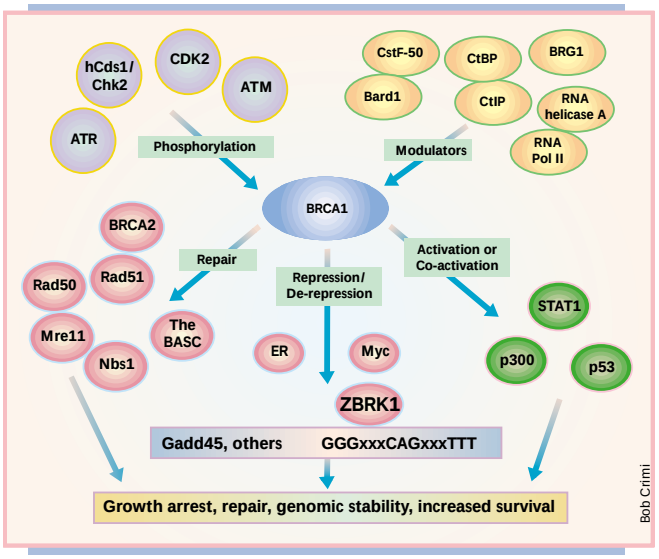

Fig. 1 Activity of tumor suppressor BRCA1 in cellular DNA damage repair and transcription. BRCA1 binds to a number of proteins and can be regulated by phosphorylation in response to DNA damage. BRCA1 can be phosphorylated by kinases following DNA damage, including ATM and CHK2. The BRCA1 protein is recruited to transcription or DNA repair complexes. BRCA1-containing DNA repair complexes (Rad50/Mre11/Nbs1) have been observed in mammalian cell nuclei after DNA damage. BRCA1 has also been biochemically linked to chromatin remodeling (BRG1) and transcription regulatory factors (RNA polymerase II and RNA Helicase). In addition, BRCA1 can coactivate transcription through interactions with a number of sequence-specific or non-sequence-specific transcription factors (STAT1, p300 and p53) and appears to also repress transcription through c-Myc, the Estrogen receptor (ER) or ZBRK1. The recently reported ZBRK1-BRCA1 containing DNA complex is the first example of BRCA1 recruitment to any DNA transcription response element[3]. Altered levels of BRCA1 can derepress ZBRK1 targets such as GADD45 to upregulate gene expression leading to downstream effects in growth arrest and the maintenance of genomic integrity.

to show that BRCA1 formed a complex with this DNA fragment, bridged through ZBRK1. Mutation of this sequence abolished both BRCA1 and ZBRK1 binding, and resulted in de-repression of reporters from either of these proteins.

Interestingly, repression of GADD45 by BRCA1 and an associated protein was not exactly expected. After all, two reports in the past year had identified GADD45 as a gene whose transcription is activated by BRCA1 (Ref. 4, 5). In fact, the authors themselves published a paper proposing that phosphorylation of the BRCA1-associated protein CtIP by the DNA damage activated kinase ATM allows activation of GADD45 by dissociating it from the BRCA1 complex[6].

This discrepancy was addressed by the use of cell lines that express BRCA1. While *Brca1*$^{-/-}$ cells were a platform for observing repression of GADD45 via BRCA1 overexpression, performing the same assay in cells that contained functional BRCA1 resulted in derepression of the reporter construct. The authors suggest that in normal cells, BRCA1 and ZBRK1 bind to the ZBRK1 consensus sequence and actively repress transcription of GADD45. Only after DNA damage occurs do the protein and phosphorylation levels of BRCA1 increase. This could result in a change in affinity for the ZBRK1 repressor or change the overall function of the interaction to that of an a transcriptional activator. Overexpression of BRCA1 in a BRCA1-proficient cell line may mimic this process. Future studies into this interaction may reveal exactly what happens to the BRCA1-ZBRK1 interaction after DNA damage, and whether BRCA1 phosphorylation has any effect on its ability to repress GADD45 transcription when bound to ZBRK1.

Finally, the authors took advantage of two recent studies that utilized cDNA array screens[4,5] to identify changes in gene expression after overexpression of BRCA1. Using their consensus DNA-binding sequence as a search qualifier, the authors discovered at least ten genes in addition to GADD45 that possess the ZBRK1 binding sequence (or a minor modification thereof) somewhere within their transcriptional regulatory region, and this list included genes that were found in these array screens to be both induced and repressed.

Therefore, it appears that BRCA1 may control transcription of at least a subset of its downstream effectors through interaction with ZBRK1. It will be interesting, however, to find out how this interaction is able to direct repression or activation and how it may be regulated by DNA damage. BRCA1 is known to be phosphorylated upon the induction of several types of DNA damage or cell cycle progression by kinases such as hCds1/CHK2, ATM, ATR and Cdk2 (Fig. 1). BRCA1 phosphorylation results in changes in both cellular distribution and protein–protein interactions. It would not be surprising to find that the functional interaction between BRCA1 and ZBRK1 changes after modification of either protein,with respect to the cell cycle or DNA damage response.

Perhaps the change in CtIP binding to BRCA1 confers an activating signal to the BRCA1-ZBRK1 complex. Another possibility could lie in recent findings that p53 represses BRCA1 expression[7,8]. After DNA damage, wild-type p53 protein accumulates and eliminates BRCA1 expression, which might allow a derepression of the *Gadd45* promoter in intron 3.

Nevertheless, the implication of BRCA1 in transcriptional control is growing steadily and can now be incorporated into models (Fig.1) to explain the role of BRCA1 as a tumor-suppressing protein. Though it is clear that BRCA1 is physically localized to complexes involved in DNA repair (such as Rad50/Mre11/Nbs1 and Rad51/BRCA2), BRCA1 also seems to regulate transcription through direct interactions with several transcriptional activators and repressors.

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