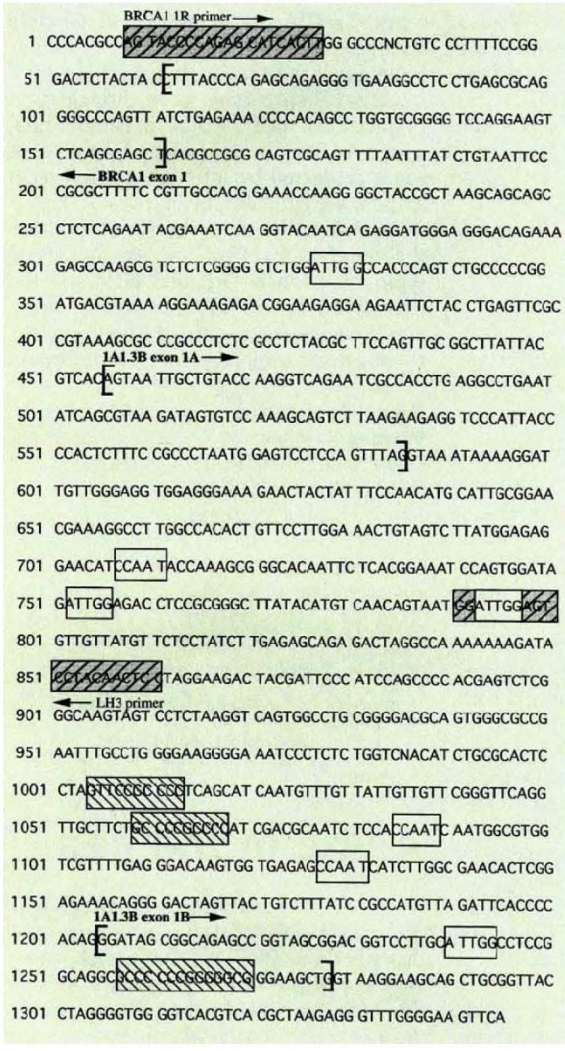
Regulation of *BRCA1*

SIR — *BRCA1*, a gene conferring susceptibility to early onset familial breast and ovarian cancer, has recently been cloned[1]. We have found that the 5′ end of *BRCA1* lies head to head with the 5′ end of the *1A1-3B* gene[2], with a maximum distance of 295 base pairs (bp) between their putative first exons. This raises the possibility of coregulation of these two genes by a bidirectional promoter and the potential involvement of *1A1-3B* in breast and ovarian tumorigenesis.



Sequence analysis of the region between the *BRCA1* and *1A1-3B* genes. Brackets, exon sequences; hatched boxes, positions of primers BRCA1 1R and LH3; open and hatched boxes, positions of CAT and GC boxes, respectively.

During the search for *BRCA1*, we isolated a number of candidate genes[3,4]. One of these, *1A1-3B*, was isolated from a complementary DNA expression library with antisera to the ovarian cancer marker CA125, and was characterized in our laboratories[2]. *1A1-3B* maps to the same P1 artificial chromosome (PAC) clone as *BRCA1*, and the two genes are divergently expressed[4].

The genomic sequence at the 5′ end of the *1A1-3B* gene was cloned and partially sequenced. This sequence contained the previously detected first exon of *1A1-3B* (ref. 2) as well as 800 bp of new sequence upstream. Comparison of this sequence the database revealed 100% identity to a 131 bp region, 600 bp 5′ to our published first exon, with a randomly isolated messenger RNA from the myeloblast cell-line KG1, termed ORF (N. Nomura *et al.*, unpublished results; accession number D30756). ORF also contained sequences identical to exons 2–19 of *1A1-3B*, but not the previously published exon 1 (here named exon 1B). This suggests that an alternative 5′ exon (here named exon 1A), not present in the original *1A1-3B* cDNA clone, is expressed in KG1 cells. This provides evidence for alternative 5′ starts of transcription in the *1A1-3B* gene.

Southern analysis of *BRCA1*- and *1A1-3B* -containing PAC and cosmid clones[4] revealed that the putative first two exons of *BRCA1* and the 5′ region of *1A1-3B* mentioned above (as well as a portion of the *1A1-3B* DNA data not shown) all reside on a single 3-kilobase (kb) *Pst*I fragment. Polymerase chain reaction (PCR) analysis detected a single 750-bp fragment, with the primer pair 1R and LH3 (see figure). This indicated that *BRCA1* exon 1 is centromeric to the *1A1-3B* gene and that the distance between the two is less than 300 bp. To confirm this result, the PCR product and cosmid A11100 were sequenced with primers 1R and LH3. The sequence from these primers overlapped and the 1R- primed sequence read directly into the 5′ region of the *1A1-3B* gene. This result mapped the distance between the two genes as less than 295 bp (see figure). Analysis of the *1A1-3B* gene 5′ region, and the sequence between the putative first exons of *1A1-3B* and *BRCA1*, for potential promoter or enhancer sites revealed seven potential 'CAT' boxes as well as other motifs (see figure). No sequences likely to correspond to a TATA box were found.

The proximity of these two genes raises the possibility of shared promoter and/or enhancer sequences and thus coregulation of expression. There are a number of examples of such bidirectional promoters, including those directing the expression of the $\alpha 1$ and $\alpha 2$ collagen genes[5], of the dihydrofolate reductase gene and the human homologue of the bacterial *MutS* gene[6], of *Surf-1* and *Surf-2* (Ref. 7) and the Wilms' tumour *WT1* and *Wit-1* genes[8,9]. Recent work on the collagen genes provides clear evidence for a bidirectional promoter involving non-TATA elements both between and within the exons of both genes, all of which are critical for the optimal and coordinated expression of both genes[5]. It is therefore conceivable that the 'CAT' boxes and other motifs found between the *1A1-3B* and *BRCA1* genes are important for the expression of both *1A1-3B* and *BRCA1*.

A model of dis-coordinate expression of *BRCA1*/*1A1-3B* would predict a decrease of *BRCA1* expression with the increased expression of *1A1-3B*, as is seen in ovarian cancers. Alternatively, *BRCA1* expression could be quenched by antisense RNA were an additional 5′ exon to lie embedded within the *1A1-3B* gene. Both mechanisms would lead to the effective downregulation of *BRCA1*, and would remain consistent with a tumour- suppressor model for this gene.

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1. Miki, Y. *et al. Science* **266**, 66–71 (1994).
2. Campbell, I.G. *et al. Hum. molec. Genet.* **3**, 589–594 (1994).
3. Jones, K.A., *et al. Hum. molec. Genet.* **3**, 1927–1934 (1994).
4. Brown, M.A. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
5. Heikkila, P., Soininen, R. & Tryggvason, K. *J. biol. Chem.* **268**, 24677–24682 (1993).
6. Shinya, E. & Simada, T. *Nucleic Acids Res.* **22**, 2143–2149 (1994).
7. Lennard, A. C. & Fried, M. *Molec. Cell Biol.* **11**, 1281–1294 (1991).
8. Campbell, C.E. *et al. Oncogene* **9**, 583–595 (1994).
9. Eccles, M.R., Grubb, G., Ogawa, O., Szeto, J. & Reeve, A.E. *Oncogene* **9**, 2059–2063 (1994).