Regulation of BRCA1

SIR — *BRCA1*, a gene conferring susceptibility to early onset familial breast and ovarian cancer, has recently been cloned¹. We have found that the 5' end of *BRCA1* lies head to head with the 5' end of the *1A1-3B* gene², with a maximum distance of 295 base pairs (bp) between their putative first exons. This raises the possibility

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

of coregulation of these two genes by a bidirectional promoter and the potential involvement of 1A1-3B in breast and ovarian tumorigenesis.

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 laboratories². *1A1-3B* maps to the same P1 artificial chromosome (PAC) clone as NATURE · VOL 372 · 22/29 DECEMBER 1994

BRCA1, and the two genes are divergently expressed⁴.

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

database the revealed 100% identity to a 131 bp region, 600 bp 5' to our published first exon, with a randomly isolated messen-RNA ger from the myeloblast cell-line KG1, termed ORF (N. Nomura et al., unpublished results; accession number D30756).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 clones4 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) PsttI 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 1A1-3Band 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 genes⁵, of the dihydrofolate reductase gene and the human homologue of the bacterial MutS gene⁶, 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 genes⁵. 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.

sor model for this gene. Melissa A. Brown Hans Nicolai Chun-Fang Xu Beatrice L. Griffiths Karen A. Jones, Ellen Solomon* Somatic Cell Genetics Laboratory Louise Hosking John Trowsdale Human Immunogenetics Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields,

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