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Wael M. ElShamy and David M. Livingston reply:

In response to the correspondence by Kvist *et al.*, we note that there is indeed a ~20% difference in primary sequence between what we have labelled in our paper[1] as 'exon 1c?', the putative exon 1c (compare with Fig. 2a, ref. 1), and the extreme 3′ segment of exon 1a that is reported in the human genome sequence. The ~20% difference raises the question of whether the sequence that we observed in the 5′-end region of *BRCA1-IRIS* mRNA is a polymorphic (or even an *in vivo* edited) version of the published *BRCA1* exon 1a sequence. If it is a polymorphic version of exon 1a, this would support the existence of a common *BRCA1-IRIS/p220* promoter.

Before responding to the interesting question posed by Kvist *et al.* a few points of detail seem worth noting. First, the 46-residue, 5′-end region sequence present in *BRCA1-IRIS* cDNA represents the 5′-most portion of *BRCA1-IRIS* cDNA that we have been able to decode, thus far. It was detected in an experiment in which we also detected the published *p220 BRCA1* exon 1a sequence associated with *p220* messenger. This showed that our methodology was capable of isolating both sets of sequences simultaneously. Furthermore, the *BRCA1-IRIS*-associated sequence has been confirmed in multiple sequencing runs. This sequence, printed in green in Fig. 2a of our paper[1], is free of nucleotide ambiguities (the NCBI database has been updated with this version). Importantly, the existence of this sequence notwithstanding, we do not know the nature of the sequence that immediately adjoins the *BRCA1-IRIS* mRNA cap. Therefore, it is impossible at this point to say where *BRCA1-IRIS* transcription actually commences.

Second, we did detect a sequence (NCBI: AC005332.1) a great distance (~24 megabases) away from *BRCA1* that is 100% identical to the 5′ 14 nucleotides of putative exon 1c (beyond that point there is no apparent homology). Other copies of this 14-nucleotide sequence exist elsewhere in the genome. Moreover, there is a known, duplicated element ~30 kilobases upstream of the 5′ end of the *BRCA1* gene that contains related, albeit degenerate, copies of exons 1a, 1b and 2 of the *BRCA1* gene. Thus, one can speculate there to be sequences that could, hypothetically, serve as alternatives to the intact exon 1a 3′ region sequence as coding elements for the 5′ *BRCA1-IRIS* sequence that we reported.

In this regard, it is unknown to us whether the mRNA from the cloning library we used was derived from single or multiple donors. If it was the latter and the 3′ exon 1a genomic DNA sequence in at least one copy of the gene, represented as cDNA in the library, was identical to that present in *BRCA1-IRIS* mRNA, the possibility that exon 1a is part of the IRIS transcript would gain strength. If it were not, the next question would be whether the duplicated exon 1a sequence upstream of the 5′ end of the *BRCA1* gene is identical to that of our putative exon 1c in the relevant donor gene. If so, one might wonder whether that element participates in *IRIS* transcriptional initiation. Because it is difficult to retrace the steps that led to the commercial cloning library in question, it seems reasonable to leave each of the above-noted possibilities as an open question.

Independent of the exact nature of the full 5′-end sequence of *BRCA1-IRIS* mRNA (which, as we admitted in the paper, may well be incompletely deciphered), we did observe that, in primary, diploid human fibroblasts that were allowed to exit the cell cycle and enter G0 following serum deprivation, both *IRIS* RNA and protein were as abundant as they were in serum-repleted, S-phase cells. This was different from what was observed for both *p220* and *c-myc* mRNA and protein analysed in the same extracts. Both largely disappeared in G0 cells, returning as expected after serum repletion. One interpretation of these results is that the *BRCA1-IRIS* promoter and the *p220* promoter operate differently. Another is that the stability of *BRCA1-IRIS* and *p220* mRNAs is different in G0 cells. Given these results and the apparent differences in sequences in the 5′-end regions of *p220* and *IRIS* mRNA, we suggested the possibility that there may be two promoters that operate differently.

What is apparent is that the detailed nature of the promoter governing *IRIS* transcription is unknown, and clarity on this issue will require considerable future work. We would not argue with the possibility proposed by Kvist *et al.* and did not claim that the *IRIS* promoter is located a vast distance away from the *BRCA1* gene on chromosome 17. There is no direct evidence for this most unusual and unexpected possibility. Rather, given the results summarized above, it seems reasonable to leave open the possibility that the operations of the promoters governing *IRIS* and *p220* transcription are different. The question of where the actual promoter territory relevant to each is physically located is unclear, and we should have emphasized that in the paper.

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Dual role for Bcl-2 in antibody affinity maturation

To the editor:

A recent paper in *Nature Cell Biology* by Youn *et al.* described the suppression of mismatch repair (MMR) activity by Bcl-2, and discussed the possible effect of this on genome stability in oncogenesis[1]. This finding also has fascinating implications for the mechanisms that generate antibody diversity in germinal centres, because the germinal centre reaction characteristically involves downregulation of Bcl-2 to permit the positive selection of high-affinity antibody variants, and the antibody variants are initially created by hypermutation, which involves MMR activity.

Germinal centres form as a consequence of B-cell encounter with T cell-dependent antigens. In this environment, B cells undergo cycles of rapid proliferation, hypermutation

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