Exon-skipping in *BCR/ABL* is induced by *ABL* exon 2

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The *BCR/ABL* fusion gene is pathognomonic for chronic myelogenous leukaemia (CML). We have previously reported alternative splicing of *BCR/ABL*, as indicated by the detection of both p190- and p210-encoding transcripts, in about 60% of CML patient samples. These exon-skipping events involved the joining of *ABL* exon 2 to variable upstream *BCR* exons. Similarly, *ABL* exon 2 is alternatively spliced to either of two upstream *ABL* exons (1a or 1b) in *c-ABL*. We have constructed *BCR* and *BCR/ABL* minigenes to study this phenomenon in more detail. These constructs were transfected into various cell types and splicing was assessed by reverse transcriptase PCR. Whereas the

basic *BCR* minigene expressed exon-inclusive transcripts only, insertion of genomic DNA spanning *ABL* exon 2 induced exon-skipping but only when expressed in the CML cell lines K562 and EM3. In this study we localized the required sequence element to *ABL* exon 2 itself. These results mimic the splicing phenotype displayed by most CML patients. We propose a model where a *trans*-factor present in some CML cells interacts with *ABL* exon 2 pre-mRNA to promote skipping of upstream *BCR* exons.

Key words: alternative, CML, minigene, splicing.

INTRODUCTION

Chronic myelogenous leukaemia (CML) is a clonal disorder caused by the neoplastic transformation of a pluripotent haematopoietic stem cell (reviewed in [1]). The hallmark of CML is the presence of the Philadelphia chromosome (also known as Ph⁺), the result of a t(9;22)(q34;q11) reciprocal translocation and subsequent fusion of the *c-ABL* proto-oncogene on chromosome 9 with the *BCR* gene on chromosome 22 [2–6].

There are two major forms of the *BCR/ABL* fusion gene resulting from the joining of *ABL* exon 2 to different exons of the *BCR* gene. One fusion involves breaks within the major breakpoint cluster region (M-bcr) of *BCR*, which includes introns 13 and 14, and leads to the production of an 8.5 kb transcript coding for a 210 kDa protein (p210) [7]. In CML, most translocations involve the M-bcr of *BCR*. The other fusion gene is the result of breaks within the first intron of *BCR* (the minor breakpoint cluster region, m-bcr) and leads to the expression of a smaller 7.5 kb transcript that codes for a 190 kDa (p190) protein [8] (Figure 1). Other, less common, fusions have also been reported (reviewed in [9]). Whereas both *BCR/ABL* fusion proteins display activated tyrosine kinase activity, the p190 form, more frequently associated with acute lymphoblastic leukaemia, is more potentially transforming. The p190 *BCR/ABL* fusion more efficiently transforms cells in culture [10–12]. p190 *BCR/ABL* transgenic mice develop leukaemia with a higher penetrance and shorter latency than p210 *BCR/ABL* transgenic mice [13–18]. Also, p190 *BCR/ABL* transgenic mice always develop acute leukaemia while some p210 *BCR/ABL* mice develop a myelodysplastic syndrome resembling human CML [18].

Generation of the p210-encoding *BCR/ABL* transcript from the Philadelphia chromosome requires splicing of the 3'-most *BCR* exon to *ABL* exon 2. This can involve the excision of greater than 200 kb of pre-mRNA, including *ABL* exons 1a and 1b [19]. Alternative splicing of the p210 *BCR/ABL* transcript has

also been reported in which alternative *BCR* exons 1a or 1b are utilized [20], as both *BCR* promoters can be active. *ABL* exon 2 can also alternatively splice to *BCR* exon 13 by skipping exon 14 [21]. In both cases the result is a variant p210-encoding transcript produced from a Philadelphia chromosome with a break in the M-bcr. Recently, we, and others, have reported the co-expression of both the p210- and p190-encoding *BCR/ABL* transcripts in CML patient samples [22–24]. We believe this co-expression to be the result of alternative splicing from a single *BCR/ABL* allele [24].

Alternative splicing serves as a means by which a single gene can code for multiple proteins and provides an important level of post-transcriptional regulation. Control of alternative splicing is generally mediated through the interaction of *trans*-acting splice regulators with *cis*-elements present within the nascent pre-mRNA transcript [25,26]. In some cases it is the presence or absence of particular splice-regulatory proteins [27,28], while in others it may be the relative abundance of multiple splice-regulatory proteins that determines the pattern of splicing [29]. Aberrant splicing has been associated with several pathologies including cancer [30–33] and amyotrophic lateral sclerosis [34]. Alternative splicing of fusion transcripts associated with cancer has been reported in desmoplastic small round cell tumour [35], T-cell acute lymphoblastic leukaemia [36,37] and acute myeloid leukaemia [38,39]. Alternative splicing of fusion transcripts in these malignancies could be the result either of the juxtaposition of sequences not normally present within the same transcript or of the expression of splice-regulatory factors that direct this alternative splicing, or a combination of both.

In this study we have set out to develop a model for the alternative splicing of *BCR/ABL* detected in CML patient cells. We have constructed *BCR/ABL* minigenes that displayed exon-skipping only when expressed in CML cells. This exon-skipping was dependent on the presence of *ABL* sequences, which we have localized to *ABL* exon 2. We propose a model where transacting

Abbreviations used: CML, chronic myelogenous leukaemia; M-bcr, major breakpoint cluster region; m-bcr, minor breakpoint cluster region; RT, reverse transcriptase.

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Figure 1 Genomic organization of the *BCR/ABL* fusion gene at the Philadelphia chromosome translocation breakpoint

The p210-encoding transcript is produced from chromosomes with a break in the M-bcr while the p190-encoding transcript is usually produced from chromosomes with a break in the m-bcr. It is possible to produce a p190-encoding transcript from a Philadelphia chromosome with an M-bcr junction through alternative splicing. Such an exon-skipping event would remove as much as 300 kb of pre-mRNA.

factors present in some CML cells interact with *ABL* exon 2 pre-mRNA to promote exon-skipping. This leads to expression of the more potently transforming p190 isoform.

MATERIALS AND METHODS

Detection of *BCR/ABL* and *BCR* transcripts

Peripheral blood or bone-marrow samples from patients with a referring diagnosis of CML were obtained for molecular studies as part of a diagnostic workup. Normal peripheral blood leucocytes were obtained from healthy volunteers. Red cells were lysed and total RNA was extracted from the white-cell pellet, as well as from control cell lines, using the Qiagen RNeasy kit according to the manufacturer's instructions. Total RNA (500 ng) was subjected to reverse transcription using random hexamers and Moloney murine leukaemia virus reverse transcriptase (RT). Following this, 10% of the cDNA was used in a nested PCR using primers specific for *BCR* exons 1 (GCA GCT CCA ATG AGA ACC TC) and 15 (CCC AAA GGA ATC CAC CTC CA followed by ATC CAC CTC CAG GGT GCA GT) or *BCR* exon 1 (ACC ATC GTG GGC GTC CGC AAG A followed by AGA TCT GGC CCA ACG ATG GCG AGG GC) and *ABL* exon 2 (TGA TTA TAG CCT AAG ACC CGG A followed by ATC TCC ACT GGC CAC AAA ATG CAT ACA). External amplifications were heated to 94 °C for 5 min and then subjected to 26 cycles of 94 °C for 30 s, 63 °C for 30 s and 72 °C for 15 s followed by a 5 min soak at 72 °C. Prior to the nested PCR, 10 µl of the external reactions were diluted to 1 ml with H₂O. Internal reactions were heated to 94 °C for 5 min and then subjected to 30 cycles of 94 °C for 30 s, 65 °C for 30 s and 72 °C for 15 s followed by a 5 min soak at 72 °C in a Thermal Cycler (Perkin Elmer, Rexdale, ON, Canada). Products were resolved on 1% agarose gels.

Cell lines

The CML cell lines K562 and EM3 were maintained in RPMI 1640 (10% fetal bovine serum), as was the histiocytic lymphoma line U937. The adherent cell lines, 293T (simian virus 40 large TAG-transformed human kidney) and CV1 (African Green

monkey kidney), were maintained in α -modified Eagle's medium (10% fetal bovine serum).

Construction of *BCR* and *BCR/ABL* minigenes

In order to construct the basic *BCR* minigene three fragments were inserted into a pBlueScript (pBS; Stratagene) backbone in a step-wise fashion (see Figure 2). First a 4218 bp genomic *BCR* *SacI/EcoRI* fragment containing the entire *BCR* promoter [40–42], as well as the first exon of *BCR* and the first 1500 bp of intron 1 was cloned into pBS. Next, a 2606 bp *BglII/BamHI* fragment of genomic DNA spanning *BCR* exons 12 and 13 was blunted and cloned into the *EcoRV* site of pBS downstream from the *BCR* exon 1 fragment. This *BCR* exon 12/13 fragment did not include the transcriptional repressor located within intron 13 of *BCR* [42]. For the final step PCR primers were designed to amplify a product from the eukaryotic expression vector pSG5 (Stratagene) consisting of rabbit β -globin intron, 3' splice acceptor site and exon fused to the 3'-untranslated region, and poly(A⁺) signal of polyoma large T antigen. These primers were designed to include a *KpnI* site, which was used to clone the product into the polylinker *KpnI* site present in the pBS portion of the minigene construct. A 1359 bp genomic fragment spanning *ABL* exon 2 was then amplified from human genomic DNA using primers appended with *ClaI* sites. This fragment was cloned into pBS for manipulation and subcloned into the unique *ClaI* site downstream of *BCR* exon 13 to create the *BCR/ABL* minigene.

Generation of *ABL* mutants

The 3' splice site of *ABL* intron 1 was replaced with the 3' splice site of *BCR* intron 11 using the PCR (Figure 2). Two products were amplified from the cloned *ABL* genomic fragment described above using primers specific for sequences flanking the 3' splice site, which were appended with overlapping sequences derived from the 3' splice site of *BCR* intron 11 (GTT GGA AAA GAG GTG CAC CAA TTG GTA GTT TTA TTC TCA GAA GCA), a vector (pBS)-specific primer to amplify the upstream intron (GTG CAC CTC TTT TCC AAC CTC CCA GAA GCC CTT CAG CGG CCA GTA) and a vector (pBS)-specific primer to amplify *ABL* exon 2 plus down-

Figure 2 Schematic diagram of *BCR* minigene, 3' splice sites and *ABL* genomic inserts

(A) Basic *BCR* minigene consisting of the *BCR* promoter, exon 1 and 1500 bp of intron 1 linked to a 2606 bp genomic fragment spanning *BCR* exons 12 and 13 (B12 and B13) and a rabbit β -globin terminal exon with the large T antigen poly(A⁺) signal. *ABL* genomic fragments were inserted at the unique *Cla*I site (*). Minigene transcripts were detected by RT-PCR. Primer sites are shown (arrows). (B) Alignment of 3' splice sites upstream of *ABL* exon 2 in *BCR/ABL* pre-mRNA. Those of the upstream *BCR* introns have shorter polypyrimidine tracts with more interruptions than that of *ABL* intron 1, which has an unusually long and uninterrupted polypyrimidine tract. Upper-case letters indicate pyrimidines and lower-case letters indicate purines. (C) Three versions of the *ABL* genomic insert used in this study. The 3' splice site of *ABL* intron 1 was replaced with the *BCR* intron 11 3' splice site (°; see B) to generate the splice-site-switched insert while *ABL* exon 2 was replaced with *BCR* exon 13 to generate the exon-switched insert. wt, wild-type.

stream intron (partially overlapping *BCR* intron 11 sequences are underlined). These two products were then isolated and allowed to anneal at the overlapping *BCR* intron 11 sequences with which they were appended and extended to blunt ends with *Pfu* polymerase. A fused splice-site switched product was then amplified using *Pfu* polymerase and the flanking vector (pBS)-specific primers. Similarly an exon-switched mutant was generated from three PCR products, one corresponding to *BCR* exon 13 (with primers GTT TCA GAA GCT TCT CC TG and CTT CCT TAT TGA TGG TCA GC), one derived from *ABL* intron 1 which was amplified with a *BCR* exon 13-appended primer [GAT GTC AGG GAG AAG CTT CTG AAA CCT GGA AGA GAA AGG GGG GAA and a vector (pBS)-specific primer; *BCR* exon 13 sequence underlined], and a fragment derived from *ABL* intron 2 amplified with a *BCR* exon 13-appended primer [ATT CCG CTG ACC ATC AAT AAG GAA GGT AAA AGG GTT GTG GGC AGC and a vector (pBS)-specific primer; *BCR* exon 13 sequences underlined]. These three products were allowed to anneal at the over-

lapping *BCR* exon 13 sequences, extended to blunt ends and a fused exon-switched product was amplified using pBS-specific primers and *Pfu* polymerase. Both mutant *ABL* fragments were flanked by *Cla*I sites that were used to insert them into the *BCR* minigene, creating splice-site-switched *BCR/ABL* and exon-switched *BCR/ABL* minigenes, respectively.

Transfections and RT-PCR

Transient transfections of 293T and CV1 cells were performed using Qiagen Superfect reagent according to the manufacturer's instructions. Transient transfections of K562, EM3 and U937 cells were performed using Qiagen Effectene reagent according to the manufacturer's instructions. Briefly, 10 μ g of minigene plasmid was mixed with the appropriate transfection reagent and either incubated with 10⁵ cultured cells for 3 h and then replaced with fresh culture medium (Superfect transfections) or left in the medium (Effectene transfections). Transfection efficiencies of

30 % (293T and CV1 cells with Superfect) and 5 % (K562 and U937 with Effectene) were achieved with other constructs expressing green fluorescent protein under these conditions (results not shown). Post-transfection (18 h) total RNA was collected using the Qiagen RNeasy kit. Total RNA (500 ng) was reverse transcribed using oligo(dT) primers and Moloney murine leukaemia virus RT: 10 % of the resulting cDNA was subjected to nested PCR using primers specific to *BCR* exon 1 (GCA GCT CCA ATG AGA ACC TC followed by ACA GTC CTT CGA CAG CAG CA) and rabbit β -globin (GCA CAA TAA CCA GCA CGT TG followed by ATA ACC AGC ACG TTG CCC AG) to detect minigene-specific transcripts. External amplifications were heated to 94 °C for 5 min and then subjected to 26 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 10 s followed by a 5 min soak at 72 °C. Prior to the nested PCR, 10 μ l of the external reactions were diluted to 1 ml with H₂O. Internal reactions were heated to 94 °C for 5 min and then subjected to 30 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 10 s followed by a 5 min soak at 72 °C in a Thermal Cycler (Perkin Elmer). Products were resolved on 2.5 % agarose gels.

RESULTS

Exon-skipping is confined to the translocated allele of *BCR*

We investigated whether co-expressing leukaemic cells displayed a gross dysregulation of splicing that dysregulated the splicing of both alleles of *BCR* or whether the alternative splicing was confined to *BCR* exons in the context of the fusion transcript. The splicing of the germ-line allele of *BCR* was assessed by RT-PCR using primers specific for *BCR* exons 1 and 15 (Figure 3). As *BCR* exon 15 is not translocated to the Philadelphia chromosome this primer pair detects transcripts derived from the germ-line allele only. By RT-PCR we were able to detect multiple isoforms of *BCR/ABL* in both CML patient samples and the CML cell line K562 but failed to detect alternatively spliced forms of *BCR* in any of the same samples (Figure 3). These data

indicate that alternative splicing was confined to those *BCR* exons found in the context of the *BCR/ABL* fusion transcript.

Construction of *BCR* and *BCR/ABL* minigenes

We hypothesized that the juxtaposition of *ABL* sequences and *BCR* exons induces exon-skipping. To test this hypothesis we constructed a *BCR* minigene into which various *ABL* genomic fragments were inserted (Figure 2). In order to detect exon-skipping events similar to those detected in CML patient samples we constructed an unusually large minigene with 4 or 5 exons (three from *BCR* and one from rabbit β -globin, plus or minus an *ABL* exon). The expression of the minigene transcript, which included those *BCR* exons present on the classic Philadelphia chromosome minus exons 2–11, was driven by the *BCR* promoter [40–42] rather than a strong viral promoter in order to mimic the expression level of the *BCR/ABL* fusion gene. The transcriptional repressor reported to lie within *BCR* intron 13 [42] was not included. As the *BCR* promoter used in our minigene constructs was relatively weak, minigene transcripts were detected using nested RT-PCR primers specific for *BCR* exon 1 and the terminal rabbit β -globin exon. Since the alternative splice-forms detected in patient samples were the results of various *BCR* exons being joined to *ABL* exon 2, we decided initially to generate a *BCR/ABL* minigene containing *ABL* exon 2. A genomic fragment spanning *ABL* exon 2 was amplified from human genomic DNA and inserted downstream of *BCR* exon 13 in the sense or antisense orientation (Figure 2).

Alternative splicing is dependent on *ABL* sequences and a CML cell type

When our first minigene constructs were transfected into K562 cells (CML) and 293T (Ph⁻-negative) cells we were able to detect

Figure 3 Detection of *BCR/ABL* and *BCR* transcripts by RT-PCR

(A) Germ-line *BCR* transcripts were detected using primers specific for *BCR* exons 1 and 15 (flanking checked region representing *BCR* exons 2–14). Alternatively spliced forms were not detected in any sample (lanes are as listed for B). (B) *BCR/ABL* transcripts were detected using primers specific for *BCR* exon 1 and *ABL* exon 2. p210-encoding transcripts (upper arrow) were detected in all five CML patient samples (lanes 1–5) and in the CML cell line K562 (lanes 6). Four CML patients (lanes 1, 2, 3 and 5) displayed a p190-encoding transcript as well (lower arrow). One CML patient in this group expressed a different, alternatively spliced form (*, middle arrow, lane 4, *BCR* exons 1, 9, 10, 11, 12, 13, *ABL* exon 2). Only the p190-encoding transcript was detected in SupB15 (Ph⁺-positive acute lymphoblastic leukaemia cell line, lanes 7). *BCR/ABL* transcripts were not detected in normal peripheral blood leucocytes (lanes 8–10). R, reagent control.

Figure 4 RT-PCR detection of minigene transcripts

Minigene transcripts were detected by RT-PCR following transfection of the CML cell line K562 or the Ph⁻ negative cell line 293T. The *BCR* minigene produced exon-inclusive transcripts only (lanes 1, A-type transcript). Insertion of *ABL* exon 2 spanning genomic fragment (lanes 2) induced exon-skipping when expressed in K562 only. Upper band in lane 2 is exon-inclusive B-type transcript. Arrows indicate alternatively spliced forms seen in lane 2 for K562 (upper arrow indicates C-type and D-type transcripts that co-migrate; lower arrow indicates E-type transcript). Insertion of *ABL* genomic fragment in the antisense orientation (lanes 3) produces A-type transcripts. All products were cloned and sequenced for identification. M, mock transfection; R, reagent control. Long black boxes, *BCR* exon 1; black squares, *BCR* exons 12 and 13; white boxes, *ABL* exon 2; shaded boxes, β -globin exon.

alternatively spliced forms only in the CML cell line K562 (Figure 4). Also, exon-skipping was only detected when *ABL* sequences were inserted in the sense orientation. Alternatively spliced forms were isolated and sequenced for identification. Three isoforms; BCR1/12/13/globin, BCR1/12/globin and BCR1/ABL2/globin, were detected in transfected K562 cells (Figures 4 and 5) as well as the CML cell line EM3 (results not shown) but were not seen in 293T (Figure 4), U937 (Figure 5) or CV1 cells (results not shown).

Exonic sequences are required for induction of exon-skipping

Mutant versions of the genomic fragment encompassing c-*ABL* exon 2 were generated and inserted into the *BCR* minigene in order to identify the minimal *ABL* sequences required to induce exon-skipping. The 3' splice site of *ABL* intron 1 has an unusually long and uninterrupted polypyrimidine tract [21]. To determine whether this splice site played a role in alternative splicing we replaced it with the consensus-like 3' splice site from *BCR* intron 11 using PCR to generate 3' splice-site switch *BCR/ABL* (Figure 2). When a *BCR/ABL* minigene containing this mutant *ABL* insert was transfected into K562 cells, alternative splicing was still detected (Figure 5), indicating that this unusual *ABL* intron 1 splice site was not required for the induction of alternative splicing. The resulting PCR products were subcloned and sequenced to confirm the presence of both the BCR1/12/13/

Figure 5 Exon-skipping requires *ABL* exon 2

BCR/ABL minigenes with mutant *ABL* genomic inserts were transfected into the CML cell line K562 and the non-CML cell line U937. Minigene transcripts were detected by RT-PCR. In both cell lines the *BCR* minigene produced an exon-inclusive A-type transcript (lanes 1). The insertion of an *ABL* exon 2-spanning genomic fragment (lanes 2) led to the production of an exon-inclusive B-type transcript in each cell line (upper band) while the K562 cells produced alternatively spliced transcripts as well (upper arrow indicates C-type and D-type transcripts; lower arrow indicates E-type transcript). Replacement of the unusual 3' splice site in the *ABL* genomic insert failed to alter this alternative splicing seen in K562 cells (lanes 3). Replacement of *ABL* exon 2 with *BCR* exon 13 abolished exon-skipping in K562 cells (lanes 4) leading to the production of F-type transcripts in both cell lines. Replacement of *ABL* exon 2 with *BCR* exon 13 in the antisense direction led to the production of A-type transcripts (lanes 5). M, mock transfection; R, reagent control. Symbols for the different exons are as for Figure 4.

globin and BCR1/ABL2/globin splice forms (results not shown). We also replaced *ABL* exon 2 with *BCR* exon 13 (flanked by *ABL* introns 1 and 2) to determine whether *ABL* exon 2 itself was required for the induction of exon-skipping in the minigene transcript (Figure 2). When a *BCR/ABL* minigene containing this insert (exon-switched *BCR/ABL*) was transfected into K562 cells we were no longer able to detect alternatively spliced forms of the minigene transcript (Figure 5). Again, the resulting PCR product was sequenced to confirm its identity (results not shown). This result indicates that *ABL* exon 2 is required for the induction of alternative splicing in the context of the *BCR/ABL* minigene transcript.

DISCUSSION

Alternative splicing of the *BCR/ABL* transcript is induced by *ABL* exon 2

We have previously reported the detection of p190- and p210-encoding *BCR/ABL* transcripts in CML patient samples. We

Figure 6 Proposed model for the alternative splicing of the *BCR/ABL* transcript

We propose that the internal *BCR* exons are spliced together prior to the excision of the large first introns of *BCR* and *ABL* respectively. This may occur through the formation of two large internal 'exons' when exons 2–8 and 9–14 are joined due to their relative proximity. We propose further that a *trans*-acting factor active in some CML cells interacts with *ABL* exon 2 RNA to facilitate the joining of *BCR* exon 1 and *ABL* exon 2 generating a p190-encoding fusion transcript.

hypothesized that this co-expression was due to alternative splicing of a *BCR/ABL* pre-mRNA as these patients displayed a single Philadelphia chromosome per cell and because we could detect additional alternatively spliced forms in some samples [24]. It is interesting to note that in reports of the rare variant e19/a2 *BCR/ABL* translocation (creating a *BCR* exon 19–*ABL* exon 2 junction), alternatively spliced forms that resulted from exon-skipping of upstream *BCR* exons were detected as well [43,44], implying that *ABL* exon 2 can affect the splicing of various upstream *BCR* exons. In this study we have examined the mechanism underlying this exon-skipping phenomenon. We failed to detect alternative splicing of the wild-type *BCR* transcript produced from the germ-line allele of *BCR* expressed in both patient samples and the CML cell lines K562 and EM3. This observation implied that the alternative splicing of those *BCR* exons present on the Philadelphia chromosome was affected by their context within the *BCR/ABL* fusion transcript. To study the effect of *ABL* sequences on the splicing of *BCR* exons we constructed a series of *BCR* and *BCR/ABL* minigenes. Through the use of this model we determined that the juxtaposition of *ABL* exon 2 with upstream *BCR* exons induced exon-skipping but only when the minigene was expressed in the CML cell lines K562 and EM3 (results not shown). Thus our minigene constructs serve as a model for the alternative splicing phenomenon that we observe in many of our CML patients.

Alternative splicing of several genes has been associated previously with tumourigenesis [30–33,35–39]. In some cases this leads to the production of isoforms that may have differing transforming abilities [35] or may affect the response of the malignant cells to environmental signals [30], whereas in other cases the expression of a particular alternatively spliced form may have prognostic value [31]. As well, it has become clear that alternative splicing plays an important role in the regulation of apoptosis as different isoforms of some mediators have opposing influences on this pathway [45–47]. This study is the first to examine the mechanism underlying the production of a cancer-

associated splice form. Whether the production of a particular splice form in malignant cells is an acquired phenotype that provides a malignant clone with a particular advantage remains to be demonstrated. In the case of *BCR/ABL* it is easy to envisage that the production of the more highly transforming p190 fusion would confer a selective advantage on a subclone that expressed this splice form. In line with this, our previous study found that co-expressing CML patients had higher white-blood-cell and blast-cell counts than did patients who expressed the p210-encoding transcript [24].

The exon-skipping phenotype conferred by the juxtaposition of *ABL* exon 2 with upstream *BCR* exons detected in CML patient samples, and modelled by our minigene constructs, is unusual. Most cases of exon-skipping that have been studied to date have dealt with the variable inclusion of a particular exon containing or flanked by elements which regulated its inclusion [48–50]. In this case the required element lies in an exon that is included and affects the variable inclusion of upstream exons. This exon-skipping could be viewed as a competition for splicing to *BCR* exon 1. This exonic element may prove to be a splicing enhancer that plays a role in the physiological splicing of the c-*ABL* transcript where alternative first exons are utilized [21]. The putative *trans*-acting factor that interacts with *ABL* exon 2 may play a role in the regulation of exon joining rather than exon definition.

The genomic structure of the *BCR/ABL* fusion gene consists of two large introns, intron 1 and the gap between the last *BCR* exon and *ABL* exon 2 (Figure 6). We propose a model in which the internal *BCR* exons are spliced together prior to the removal of these two large introns. In this model the exclusion of these two large 'exons' would lead to the production of the p190 transcript; an event that could be promoted by the interaction of some *trans*-acting factor with *ABL* exon 2, either by slowing the joining of *ABL* exon 2 to the proximal *BCR* exon and/or by promoting the interaction of the *BCR* exon 1-bound splicing factors with *ABL* exon 2-bound splicing factors (Figure 6). In the

context of our minigene transcripts these intron sizes are much reduced, which may explain why the alternatively spliced forms detected do not exactly reflect those seen in patient samples, as we detect forms that lack *ABL* exon 2 as well as the expected *BCR1/ABL2* alternatively spliced form. This model may serve to detect those factors that play a role in the regulation of exon joining, rather than exon definition. Also, the use of large minigene constructs like those in these studies may facilitate our understanding of the mechanisms by which splicing may be regulated over longer distances.

In this study we have developed a model for the alternative splicing of the *BCR/ABL* fusion transcript previously detected in CML patient samples [22–24]. These studies determined that this exon-skipping is induced by the juxtaposition of *ABL* exon 2 with upstream *BCR* exons in the context of a fusion transcript. In addition, this alternative splicing is cell-type-dependent and may require the interaction of a hypothetical *trans*-acting factor with a *cis*-element present within *ABL* exon 2. We do not know what role these elements have in regulating the splicing of *c-ABL*, where exon 2 is joined to either of two upstream exons, 1a or 1b [21]. Further analysis of this model will help to elucidate the mechanism underlying co-expression of p190- and p210-encoding transcripts in CML.

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