Lack of imprinting of *BCR*

Sir — A recent report by Haas and colleagues[1] has suggested that genomic imprinting may be involved in the genesis of the reciprocal translocation t(9;22) (q34;q11), known as the Philadelphia chromosome (Ph). The Ph translocation joins the *BCR* and *ABL* genes producing a fusion protein driven by the *BCR* promoter. The resulting abnormal protein is believed to be involved in the malignant transformation of myeloid cells[2]. This malignancy-specific translocation is found in virtually all cases of chronic myelogenous leukemia (CML)[2]. No genetic predisposition towards development of Ph+ CML has been noted[3] and the occurrence of the translocation is thought to be sporadic. However, Haas *et al.*[1] reported chromosome heteromorphism studies in Ph+ CML patients using a microscopically visible polymorphism of the nucleolar organizing region (NOR) on chromosome 22p to assess the parent-of-origin of the translocated chromosome 22. Their data suggested that the chromosome 22 which participated in the translocation was of maternal origin in all 11 informative patients.

A possible explanation for the observation of the exclusive participation of the maternally derived chromosome 22 would be the normal parental imprinting of *BCR* such that only the maternal allele is active. Parental imprinting is a mechanism by which only one parental allele of a diploid locus is active[4]. Thus, chromosome 22 rearrangement may be random but only the translocation involving the maternal 22 with an active *BCR* promoter would functionally be able to drive trans-cription of the fusion gene and impart a selective advantage on a cell as a prelude to CML.

To test this notion of parental imprinting, we used a polymorphic CGG-repeat[5] in the 5' untranslated region of *BCR*. As this repeat is highly polymorphic and transcribed, it is possible to determine, in a heterozygote, if one or both alleles are expressed. This is accomplished by PCR using radiolabelled primers flanking the CGG-repeat to amplify cDNA template converted from RNA using reverse transcriptase. Following polyacrylamide gel electrophoresis and autoradiography, the repeat length is determined relative to single basepair size standards and the expected length of a fully sequenced allele of known repeat number[5]. One μg of RNA extracted from two normal individuals, heterozygous for CGG-repeat length, show both alleles expressed following reverse transcription and CGG-repeat PCR amplification (Fig. 1*a*, lanes 1 and 4). Control experiments with RNase (lanes 2 and 5) and without reverse transcriptase (lanes 3 and 6) are blank, verifying the origin of the amplified DNA as being RNA. Therefore, we find evidence for expression, in whole blood, of both *BCR* alleles rather than the expected monoallelic expression of an imprinted gene.

Could these results be due to a mixed population of cells each expressing a single but different allele, a situation analogous to imprinting, termed allelic exclusion[6]? Three fibroblast colonies, each derived from a single progenitor cell of a normal individual, heterozygous for *BCR* alleles with 3 and 6 trinucleotide repeats, were similarly tested for allelic expression. As was shown for whole blood, each fibroblast colony also exhibited biallelic expression (Fig. 1*b*, lanes 1, 3 and 5). Controls without reverse transcriptase (lanes 2, 4 and 6) were blank, demonstrating these results were also derived from RNA. Therefore, in both a complex mixture of cells (whole blood) as well as pure clonal populations of cells (fibroblasts), biallelic expression of *BCR* occurs.

We conclude that *BCR* is not functionally imprinted at the level of gene expression in human blood or fibroblasts. As genomic imprinting is believed to be determined early in development and imparts monoallelic gene expression upon most cells[4], our findings with two cell types, one hematopoietic in origin, are probably applicable to the excess granulocytes characteristic of CML. Therefore it is not likely that an imprinting mechanism is involved in the development of the Ph chromosome and subsequent CML.

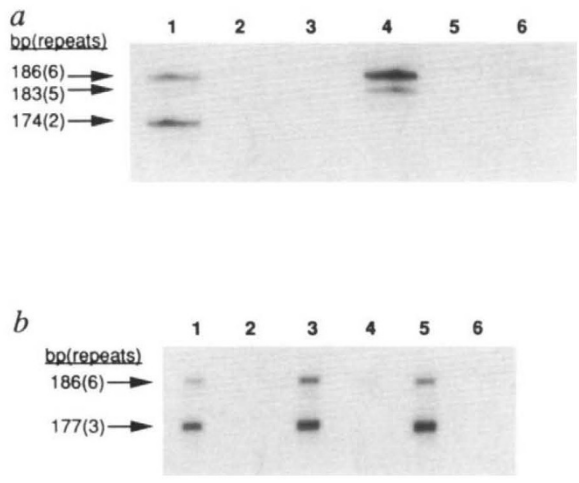

Fig. 1 Reverse transcription PCR of the *BCR* CGG-repeat. *a*, Analysis of whole blood RNA of 2 normal individuals. Lanes 1 and 4 show biallelic expression from RNA derived from each individual with the first showing alleles with 2 and 6 repeats (lane 1) and the second individual showing alleles with 5 and 6 repeats (lane 4). Lanes 2 and 5, treated with RNase and lanes 3 and 6 without reverse transcriptase are controls. *b*, Analysis of fibroblast colonies derived from single cells. Lanes 1, 3 and 4 show biallelic expression from RNA of three fibroblast colonies from an individual heterozygous for alleles of 3 and 6 repeats. Lanes 2, 4 and 6 are controls without reverse transcriptase. DNA from all three individuals exhibited the same results (data not shown). PCR primers and methods are described elsewhere[5,7].

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