Variant Breakpoint Positions on Chromosome 22 in Ph′-Positive Chronic Myelogenous Leukemias

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As a consequence of the molecular events associated with the presence of a Philadelphia (Ph′) chromosome, two different types of abnormal c-*abl* proteins, termed P210 and P190 according to their molecular weight, have been found in Ph′ positive leukemic cells [1, 2]. P210, expressed in almost all chronic myelogenous leukemias (CML) and in approximately half of the Ph′ positive acute lymphoblastic leukemias (Ph′$^+$ ALL), is codified by a hybrid gene arising from a rearrangement between the 5′ of c-*abl* oncogene and a restricted region called ("m-*bcr*" – major breakpoint cluster region) of a gene on chromosome 22, also denominated *bcr* [3–5]. By contrast, P190, present in the remaining half of the Ph′$^+$ ALLs, derives from a rearrangement involving the same two genes, but with a different breakpoint on chromosome 22, mapping more 5′ and within the first large intron of the *bcr* gene [6, 7]. The relationship between the two types of *bcr*/c-*abl* rearrangement leading to P210 or P190 protein production and to the acute or chronic leukemic phenotype of the Ph′$^+$ cells, is still matter of debate.

In order to further elucidate this problem, we have investigated the breakpoint position on chromosome 22 in a large series of Ph′$^+$ CML patients, both in chronic and in the blast phase of the disease. We studied 102 Ph′$^+$ CML patients: 79 were in chronic, 6 in accelerated, and 17 (9 lymphoid, 8 myeloid) in blast phase. Using a set of restriction endonucleases (*Bgl*II, *Bam*HI, *Eco*RI, *Bcl*I, *Kpn*I and *Hind*III) and probes corresponding to different parts of the *bcr* gene (see Fig. 1), in 96 out of the 102 cases classical rearrangements were shown to occur within the m-*bcr* region of the *bcr* gene. However, in seven cases of this group, m-*bcr* rearrangements were detected only with a probe (probe B in Fig. 1) corresponding to *bcr* sequences remaining on chromosome 22 after the t(9;22) translocation, whereas they were not detected with a probe (probe A in Fig. 1) corresponding to *bcr* sequences moving to chromosome 9. This finding is mainly due to small deletions of sequences corresponding to probe A occurring during the translocation to chromosome 9, as we never observed in our cases the loss of the entire chromosome 9q$^+$.

The most surprising result, however, was that six cases in chronic phase showed breakpoints mapping outside the m-*bcr* area: two presented breakpoints located approximaely 12 and 10 kb upstream (positions 1 and 2 in Fig. 1) and one about 20 kb downstream to the m-*bcr* region (position 3 in Fig. 1), whereas three Ph′$^+$ CML patients were apparently lacking any rearrangement of the *bcr* gene detectable with the presently available probes, which allow exploration of the entire coding part of the gene with the exception of the first large intron. The latter finding is similar to that in the majority of the Ph′$^+$ ALLs expressing P190. Although protein expression cannot easily be assessed in CML chronic phase, a Northern analysis of one of these cases showed the presence of c-*abl* transcripts of 7 and 6 kb but no hybrid 8.5-kc *bcr*/c-*abl* messages. Taken together all these findings are compatible with the presence either of a rearrange-

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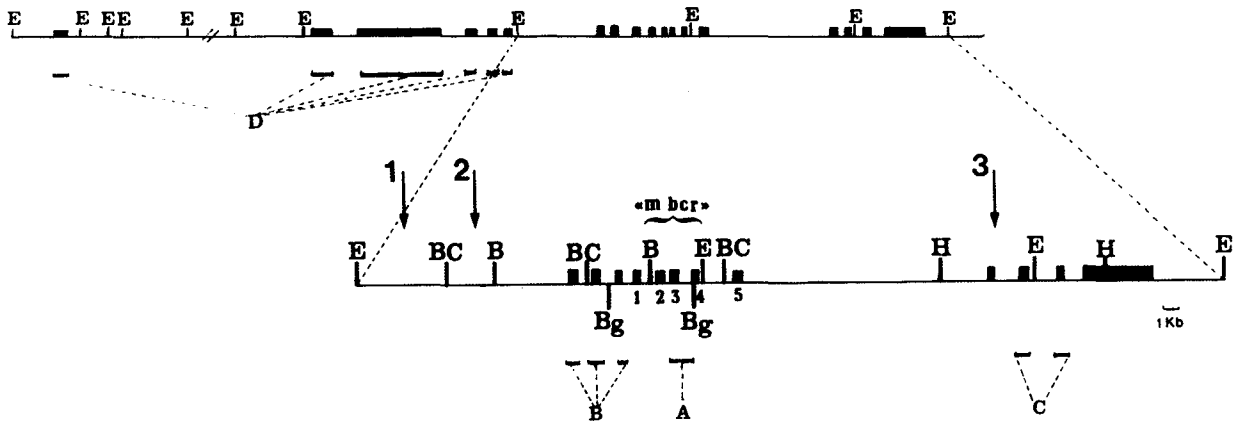

Fig. 1. Schematic map of the *bcr* gene on chromosome 22. *Black boxes* indicate exons. *A, B, C, D* represent the four probes used in this study. The major breakpoint cluster region where rearrangements usually take place in CML is marked as m-*bcr*. *Numbered arrows* point to three different variant breakpoint positions found in CML patients (see text). Abbreviations for restriction endonucleases: *E, Eco*RI; *B, Bam*HI; *BC, Bcl*I; *H, Hind*III; *Bg, Bgl*II

ment on chromosome 22 similar to that of Ph'$^+$ *bcr*- ALLs or of a rearrangement occurring outside the *bcr* gene.

Although further studies are needed to characterize completely these variant cases, they, as others recently described [8], show that a variability of the breakpoint position on chromosome 22 is present also in a minority (5%–6%) of the cases in chronic phase. At this regard it is interesting to note that the clinical presentation and disease course in patients showing variant breakpoint positions on chromosome 22 do not seem to differ from those in patients with the common type of *bcr* rearrangement. This point raises important questions concerning the relationship between the type of *bcr-abl* rearrangement, the type of abnormal *abl* protein expressed, and the clinical presentation of the disease. At present, no simple models can be proposed, but further correlations between clinical and molecular data will help to solve this problem as well as to clarify the role of the c-*abl* proto-oncogene in human leukemogenesis.

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