Some remarks on the use of TaqI to detect highly repetitive DNA sequences in human chromosomes

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In the attempt to conclude investigation of the action of restriction endonucleases on eukaryote chromosomes, we carried out a series of experiments digesting *in situ* human metaphase chromosomes with AluI/TaqI followed by Giemsa staining. We focused on the centromeric regions of chromosomes1, 2 and 16 and noted that those areas appeared as intensely stained blocks after AluI digestion, but were dramatically reduced in size or completely destroyed after subsequent TaqI treatment. These results permitted us to draw some conclusions on the highly repetitive DNA composition of these regions, in terms of alphoid and classical satellite DNAs.

Key words: Restriction enzymes- DNA - repetitive DNAs.

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Restriction endonucleases (Res) are molecular tools capable of localising *in situ* repetitive sequences by specific attack, followed by removal/non-removal of DNA blocks from certain regions of eukaryote chromosomes (Mezzanotte *et al.,* 1983; Gosalvéz *et al.,* 1997). Although most questions relative to the molecular mechanism(s) producing the cytological effects observed after RE digestion *in situ* have been clarified, some doubts remain. For instance, still open to discussion is the cause of the dramatic increase in hybridisation labelling areas when fluorescence *in situ* hybridisation (FISH) is carried out on human chromosomes using alphoid DNAs as probes in preparations predigested with either AluI or TaqI (Nieddu *et al.,* 1999). In this connection, it has been hypothesized that this increase in fluorescence may be due to i) the reorganisation produced in centromeric heterochromatin by RE action which, in turn, would render accessible sequences impervious to hybridisation in standard conditions or ii) the presence in paracentromeric heterochromatin of certain human chromosomes (1, 9, 16) of alphoid sequences, in addition to classical satellite DNAs (Nieddu *et al.,* 1999). This contrasts with the belief that the former is found only in centromeres and the latter only in paracentromeric areas (Tyler-Smith & Willard, 1993). The last point to be clarified, ever-present when dealing with this subject, is what role, if any, specific proteins associated with specific DNA sequences, for instance specific centromeric proteic components (Tanaka *et al.,* 2005), play in determining these results. We report and discuss the results of a series of experiments designed to answer these questions.

Materials and Methods

Human metaphase chromosomes were obtained from peripheral blood lymphocyte cultures by standard procedure. Preparations were air-dried for

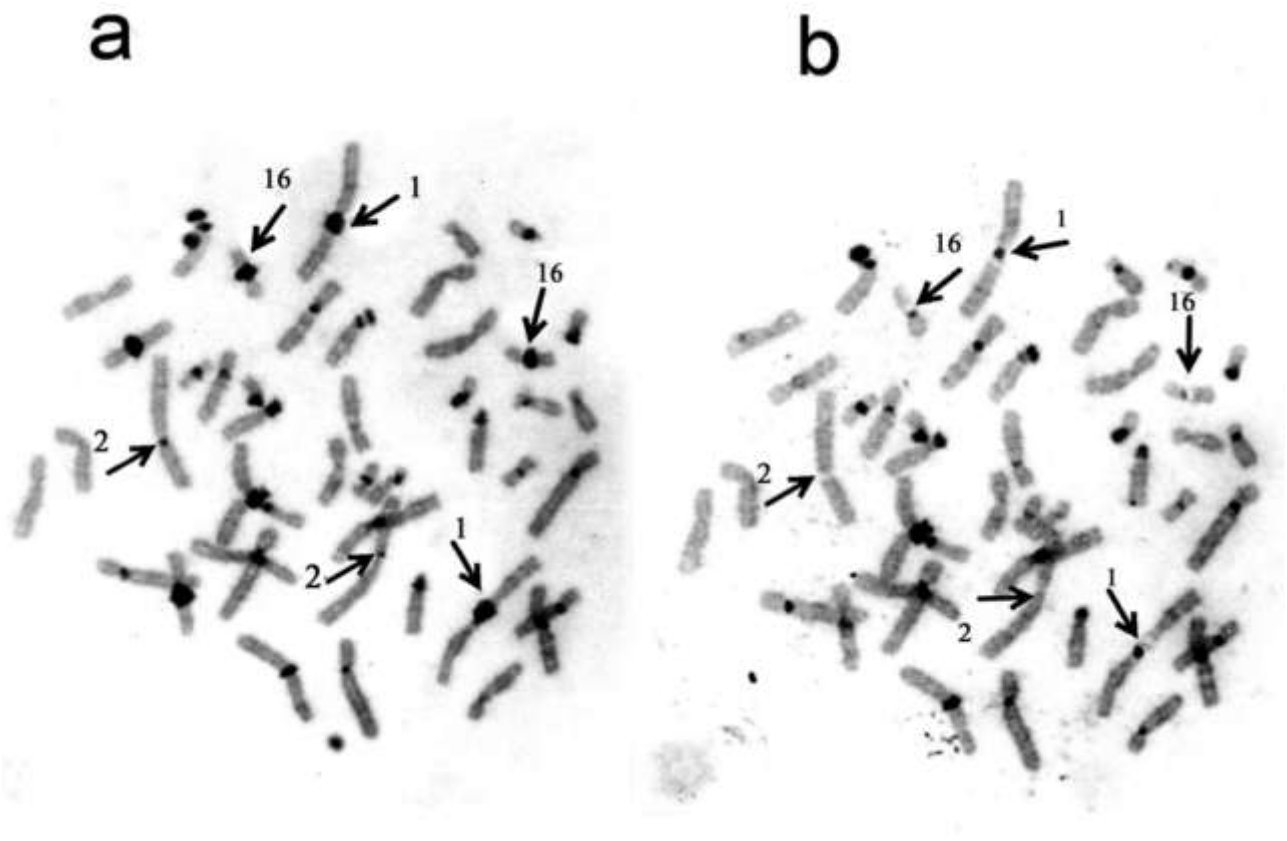

Figure 1. Human metaphase chromosomes stained with Giemsa after digestion *in situ* with AluI (a) and subsequent digestion with TaqI (b). Note heterochromatic centromeric blocks on chromosomes 1, 2 and 16 (see arrows), which are partially (chromosomes 1 and 16) or totally digested (chromosome 2) after TaqI treatment.

24 h and subsequently digested *in situ* with 30 U of AluI (Invitrogen) for 16 – 18 h a 37°C according to Mezzanotte *et al.* (1983). Slides were stained with 4% Giemsa (Sigma) in deionised water.

After microscopic observation, slides were destained in methylic alcol: acetic acid 3:1.

Subsequently, the same slides were digested *in situ* with 30 U of TaqI (Invitrogen) and treated as previously described. Before digestion *in situ* with RE , chromosomes were pretreated on some slides with proteinasi K (50 ng/mL) (Sigma) in Tris HCl 20 mM pH 7.4, Ca Cl 2 2 mM for 2-3 min at room temperature. Chromosomes were then dehydrated with an alcohol series (60%, 80%, 100%). For reasons of brevity, we focused our attention on chromosomes 1, 2 and 16, although our observations with Giemsa suggest that results relative to these chromosomes may be extended to others whose heterochromatic areas possess similar characteristics.

Results and Discussion

AluI digestion of human chromosomes followed by simple Giemsa staining produces the typical C-like bands previously described (Mezzanotte *et al.,* 1983). Double digestion *in situ* using AluI followed by TaqI shows a conspicuous gap produced in the constitutive hetechromatin regions of a number of chromosomes, including the paracentromeric area of chromosomes 1 and 16, while some dot-like centromeric structures are still present (Figure 1 a, b). In other words, the thick, AluI-induced band, similar to the classical C-band (Sumner, 1972), is reduced to a small dot plus a clearcut paracentromeric gap after subsequent TaqI digestion. Moreover, the thin AuI C-like band present in chromosome 2 is completely removed, and a sort of small gap is evident in the centromeric area of the same chromosome when TaqI follows AluI digestion. When proteinase K treatment is carried out on fixed, standard chromosomes, a type of G-like banding appears (Sumner *et al.,* 1971). In chro-

mosomes pretreated with proteinase K, RE digestion does not reveal changes, unlike what we observed in chromosomes digested only with Res. In this connection, it is important to stress that we used proteinase K at a concentration which permitted us to visualise a chromosome structure similar, even though often more swollen, to that observed in standard fixed chromosomes. This increase in proteinase K concentration produced dramatic morphological alteration of chromosomes which, as a consequence, could not be used in subsequent experiments. It is known that two main classes of highly repetitive DNA sequences are located in the constitutive heterochromatin of human chromosomes, representing about 13% of total genomic human DNA (Tyler-Smith & Willard, 1993): i) alphoid DNAs, variants of a basic repetitive sequence unit composed of 170 bp and ii) three so-called *classical* satellite DNAs, isolated by CsCl gradients and indicated as satellites I, II and III (Prosser *et al.,* 1986). The centromeric heterochromatic area of human chromosomes 1 and 16 contains specific alphoid DNAs and massive quantities of classical satellite II DNA (Tagarro, 1993), but only small amounts of classical satellite III DNA in chromosome 1 (Nieddu *et al.,* 2003). On the other hand, TaqI cuts classical satellite II very efficiently (Frommer *et al.,* 1982)), showing limited activity on alphoid DNA (Nieddu *et al.,* 1999). These considerations would account for the gap observed in the paracentromeric areas of chromosomes 1 and 16, as well as for the Giemsa-positive spot found in the centromeric region of the same chromosome after AluI/TaqI double digestion, due to the fact that the AluI C-like band, except for its centromeric component, is destroyed. As already shown by the persistence of positive signal in FISH experiments using specific alphoid DNA probes (Nieddu *et al.,* 1999), this component would represent alphoid DNA and, above all, demonstrates that this DNA fraction is localised exclusively in the centromere, excluding the possibility that it is scattered in the paracentromeric area of chromosomes such as 1 and 16, as previously postulated (Buno, 1997). On the other hand, taking into account that human classical satellite II is extensively digested by TaqI (Frommer *et al.,* 1982), our data would indicate that the centromere of chromosome 2, in addition to its specific alphoid DNA, also contains classical satellite II DNA that is not attacked by AluI, thus producing a C-like band, which is cleaved and removed by TaqI. In this case, it is interesting to note that in spite of the gap observed in the chromosome 2 centromere after TaqI digestion, FISH carried out using specific alphoid DNA as a probe shows positive hybridisation signal in this area. This observation allows us to hypothesize that, among the factors responsible for the production of C-like bands, one should consider above all the presence of classical satellite DNAs and not alphoid DNAs. Lastly, the fact that proteinase K pre-treatment does not affect our results would suggest that, at least in this case, the proteic component, associated with specific DNA fractions (Tanaka *et al.,* 2005) does not play any role in determining the activity of these Res in fixed eukaryote chromosomes.

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