## Original article

# Identification of common fragile sites in chromosomes of 2 species of bat (Chiroptera, Mammalia)

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**Summary** – In the karyotypes of the bat species *Molossus ater* and *M molossus*, spontaneous and bromodeoxyuridine (BrdU)- or aphidicolin (APC)-sensitive fragile sites were located. Four chromosome regions harbored APC-sensitive fragile sites: 1q9 and 8q4 in both *M ater* and *M molossus*, 3q3 in *M ater*, and 1p7 in *M molossus*. The fragile sites in 1q9 and 8q4 were also observed without induction in *M molossus*. BrdU-sensitive fragile sites were not detected. Despite observations in several other species, the fragile sites detected in *Molossus* are not coincident with the breakpoints involved in the chromosome rearrangements occurring in the evolution of 7 species of the Molossidae family.

fragile site / chromosome / bat / bromodeoxyuridine induction / aphidicolin induction

**Résumé** – **Identification de sites chromosomiques fragiles communs à 2 espèces de chauve-souris.** *L'analyse de la fragilité chromosomique spontanée ou induite par bromodéoxyuridine (BrdU) et aphidicholine (APC), réalisée sur le caryotype de 2 espèces de chauve-souris,* Molossus ater *et* M molossus, *a permis d'identifier 4 sites fragiles induits par APC: 1q9 et 8q4 chez* M ater *et* M molossus, *3q3 chez* M ater *et 1p7 chez* M molossus. *Les sites fragiles en 1q9 et 8q4 ont aussi été observés chez* M molossus *sans induction. Les sites fragiles repérés dans ces espèces ne coïncident pas avec les points de cassure impliqués dans les réarrangements chromosomiques qui ont eu lieu au cours de l'évolution de 7 espèces de la famille des Molossidae.*

site fragile / chromosome / chauve-souris / induction par bromodéoxyuridine / induction par aphidicholine

## INTRODUCTION

Fragile sites are specific points on chromosomes which are expressed as non-randomly distributed gaps and breaks when chromosomes are exposed to specific agents or culture conditions (Berger et al, 1985). The induction of fragile site expression is generally related to imbalance of deoxyribonucleotide pools during $G_1$ and S phases following thymidylate stress (Yan et al, 1988) or treatment with the thymidine analogue bromodeoxyuridine (BrdU) (Sutherland et al, 1985). Expression of fragile sites can also be induced at high frequencies by inhibitors of DNA semiconservative and repair synthesis, including aphidicolin (Glover et al, 1984), arabinofuranosyl cytosine, and arabinofuranosyl adenosine (Leonard et al, 1988).

Although the biological significance of fragile sites remains unclear, they have attracted attention since the rare fragile site in Xq27.3 and a type of X-linked mental retardation in humans were associated (Sutherland and Hecht, 1985). Furthermore, several findings have correlated fragile sites with chromosomal rearrangements in cancer (Le Beau, 1986; De Braekeleer, 1987; Miró et al, 1987), infertility in humans (Schlegelberger et al, 1989), breakpoints involved in chromosomal evolution of primates (Miró et al, 1987), and preservation of syntenic groups in mammals (Djalali et al, 1987; Threadgill and Womack, 1989).

More than 100 fragile sites have been identified in human chromosomes, all classified by their band location, gene symbol, population frequencies, and mode of induction (Miró et al, 1987; Hecht et al, 1990). BrdU-sensitive fragile sites have also been described in Chinese hamsters (Hsu and Sommers, 1961; Lin et al, 1984), cactus mice (Schneider et al, 1980), cattle (Di Berardino et al, 1983), and reindeer (Gripenberg et al, 1991). BrdU- and/or folate-sensitive fragile sites were recently reported in the horse karyotype (Rønne, 1992). Aphidicolin (APC)-sensitive fragile sites have been detected in the chromosomes of mice (Djalali et al, 1987; Elder and Robinson, 1989; McAllister and Greenbaum, 1991), rats (Robinson and Elder, 1987), dogs (Wurster-Hill et al, 1988; Stone et al, 1991a, 1991b), pigs (Riggs and Chrisman, 1991), and rabbits (Poulsen and Rønne, 1991). Folate-sensitive fragile sites were detected in the Persian vole (Djalali et al, 1985), the mouse (Sanz et al, 1986), cattle (Uchida et al, 1986), and in the Indian mole rat (Tewari et al, 1987).

To determine the potential phylogenetic implications of chromosomal fragility in the evolution of bats, common BrdU- and APC-sensitive fragile sites in the karyotype of 2 species of the family Molossidae (Chiroptera, Mammalia) were examined.

## MATERIALS AND METHODS

Primary cultures of fibroblasts were derived from explants of ears from a total of 9 animals of the species Molossus ater and 8 from Molossus molossus. The cultures were established and maintained in Eagles' minimum essential medium (MEM) supplemented with 20% fetal calf serum, L-glutamine, penicillin and streptomycin. BrdU (20 µM) and APC (0.02 µM) were added to cultures 26 h before harvest. In order to avoid the photolysis of DNA containing BrdU, the culture flasks were kept in the dark and covered with aluminium foil after BrdU was added. Each experiment

was performed with concurrent control cultures. Colchicine ($4 \times 10^{-4}$M) was added to the cultures 30 min before harvest. Cells were exposed to 0.8% sodium citrate for 30 min, fixed in methanol/acetic acid 3:1, dropped onto wet slides, and air-dried. Slides were homogeneously stained with 2% Giemsa and around 100 metaphases from coded slides of treated and untreated cultures of each animal were scored for breaks, gaps, and rearrangements. After identification of the lesion, the slides were destained and GTG banding (G-band after trypsin and giemsa treatment) was used to identify the exact localization of the aberrations.

To determine the presence of a fragile site, 2 criteria were considered: (i) the occurrence of at least 2% lesions at a given chromosome region in cells submitted to a certain culture condition in at least 2 animals of the same species; and (ii) the homozygous expression of a lesion. A chi-squared analysis of the distribution of anomalies was performed to determined whether their frequencies were equally distributed in treatments and controls.

## RESULTS

The diploid number of chromosomes in *M ater* and *M molossus* is $2n = 48$ and their karyotypes have similar morphology and G-band pattern (fig 1). The frequencies of spontaneous, BrdU- and APC-induced lesions in bat chromosomes are given in table I. These lesions manifested themselves as either nonstaining gaps, chromatid or chromosome breaks, or deletions.

**Table I.** Frequency of aberrations observed in bromodeoxyuridine (BrdU) and aphidicolin (APC) treatments and controls.

| Species | | BrdU | | APC | |
|---|---|---|---|---|---|
| | | Treated | Untreated | Treated | Untreated |
| *M ater* | No of specimens | 5 | 5 | 4 | 4 |
| | No of metaphases scored | 500 | 489 | 381 | 376 |
| | No of abnormal metaphases (%) | 18 (3.6) | 3 (0.6) | 35 (9.2) | 16 (4.2) |
| | No of aberrations | 20 | 3 | 50 | 22 |
| *M molossus* | No of specimens | 5 | 5 | 5 | 5 |
| | No of metaphases scored | 400 | 365 | 453 | 500 |
| | No of abnormal metaphases (%) | 19 (4.8) | 14 (3.8) | 52 (11.5) | 24 (4.8) |
| | No of aberrations | 19 | 18 | 75 | 29 |

The number of aberrations in BrdU-treated and untreated (control) cultures of *M ater* and *M molossus* was low, but BrdU-treated cells were significantly more damaged than controls ($\chi^2 = 8.9$; 1 df; $P < 0.05$). Only 3.6% of the cells in the BrdU-treated cultures and 0.6% of cells in the control cultures showed chromosome lesions in *M ater*, with a total of 20 and 3 events, respectively. In *M molossus* 3.8% of the cells in the control culture and 4.8% of BrdU-treated cells showed chromosome lesions, with a total of 18 and 19 events, respectively. The location of these gaps
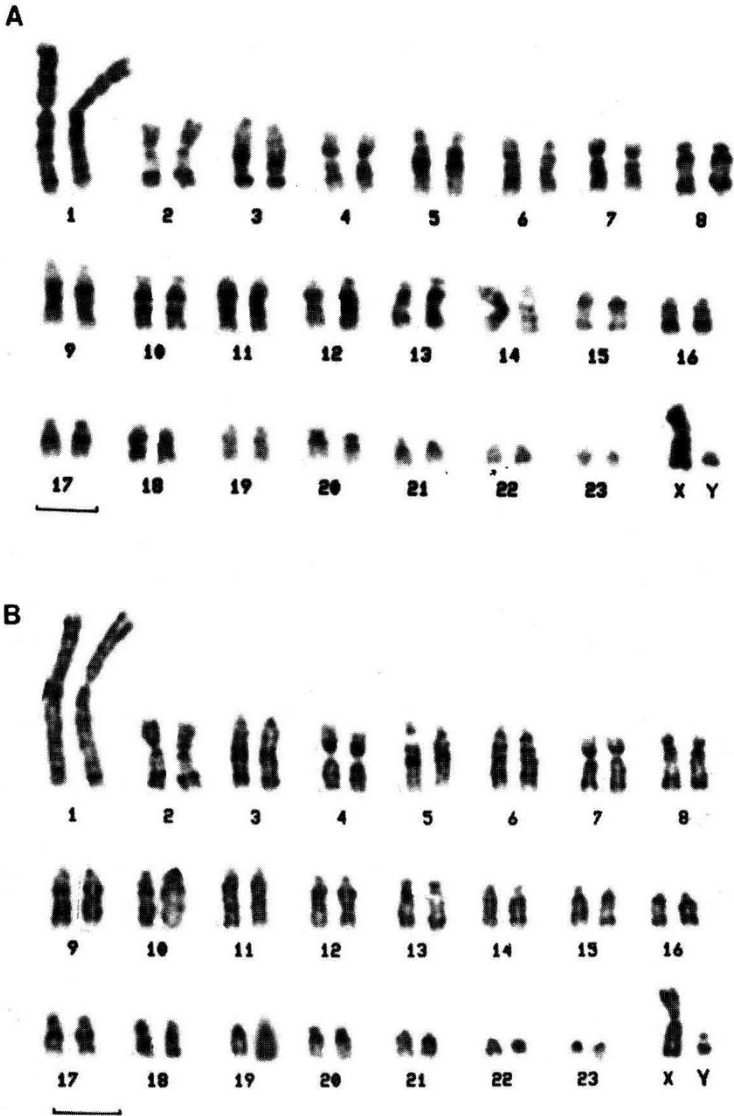
**Fig 1.** Karyotypes with G-patterns of A *Molossus ater* and B *M molossus*. Bar represents 5 µm.

and breaks was variable but they occurred in the euchromatic chromosome arms. Chromatid gap was the most frequent event.

Four chromosome bands exhibited lesions in at least 2% of the cells in the BrdU-treated cultures: 1q5 and 1q9 in *M ater*; 1q13 and 8q4 in *M molossus*. *M molossus* also exhibited lesions in 1p7 in the control cultures. Nevertheless, none of these

bands were considered to harbor fragile sites since the aberrations were not observed in the homozygous conditions or in more than one animal of the same species.

The APC treatment was more effective in the induction of chromosomal aberrations than BrdU: 9.2% of the cells presented a total of 50 anomalies in *M ater*; 11.5% of the cells exhibited a total of 75 aberrations in *M molossus* (table I). More than one lesion or the homozygous expression of a given aberration occurred in a number of cells. In these tests, the most frequent type of aberration was the chromosome gap. The chi-squared analysis detected significantly more damaged chromosomes in the APC-treated than in the control cultures ($\chi^2 = 20.0$; 1 df; $P < 0.001$).

Fourteen regions in the euchromatic arms in which such lesions occurred were identified in at least 2% of the cells: 1p7, 1q5, 1q9, 1q13, 3q3, 4q3-4, 5q8, 7q3-4, 8q4, 8q5-6, 10q3-4, 20q2 and Xq4-6 in the APC-treated cultures and 1q13-15 in the control cultures (fig 2A). However, only 4 of these 14 regions fulfilled the criteria to be qualified as harboring fragile sites (fig 2B): 1q9 and 8q4 in both *M ater* and *M molossus*, 1p7 in *M Molossus*, and 3q3 in *M ater*. The fragile sites in 1q9 and 8q4 were also observed without induction in *M molossus*. The highest expression rate (8%) was achieved by 8q4. Furthermore, an interindividual variation in the frequencies of expression of the fragile sites was observed in all of the 4 bands, as well as an interspecific variation observed in 1q9 and 8q4.

It is important to emphasize that the 5 bands referred to above as presenting lesions in the test with BrdU (1p7, 1q5, 1q9, 1q13 and 8q4) are included in the 14 identified in APC treatment and 3 (1p7, 1q9, and 8q4) are included in the 4 that harbored fragile sites.

## DISCUSSION

The mechanisms of expression of the BrdU-sensitive fragile site are not totally understood. The chronology of the events after exposure to this chemical indicates that it acts during the late S-phase and affects late replicating regions (Sutherland *et al*, 1984, 1985). An increased frequency of gaps and breaks in the chromosomes of *M ater* and *M molossus* was observed when the thymidine analogue BrdU was incorporated. However, the frequencies and conditions in which these alterations were expressed did not fit the criteria for qualification of the affected region as harboring fragile sites. These findings may be related to the period of exposure to BrdU (26 h). Although exposure to BrdU for 18–26 h has been used for experiments with human lymphocytes and several mammalian fibroblasts (Schneider *et al*, 1980; Lin *et al*, 1984; Fundia and Larripa, 1989), the highest expression of common BrdU-sensitive fragile sites in human lymphocytes was achieved after 4–12 h of treatment (Sutherland *et al*, 1984, 1985). Furthermore, fragile sites have been identified in both lymphocyte and fibroblast cultures, but the cells in the latter appear refractory to their expression (Robinson and Elder, 1987). Hence, the lower frequency in the expression of the fragile sites in bat fibroblasts may be due to the specific refractivity of this cell type as well as to a susceptibility of lymphocytes.

The chromosome aberrations observed in BrdU-treated cells in the present study consisted mainly of chromatid gaps, which is similar to the findings of Lin *et al* (1984) in the hamster genome. Reviewing the genetic toxicology of BrdU, Morris
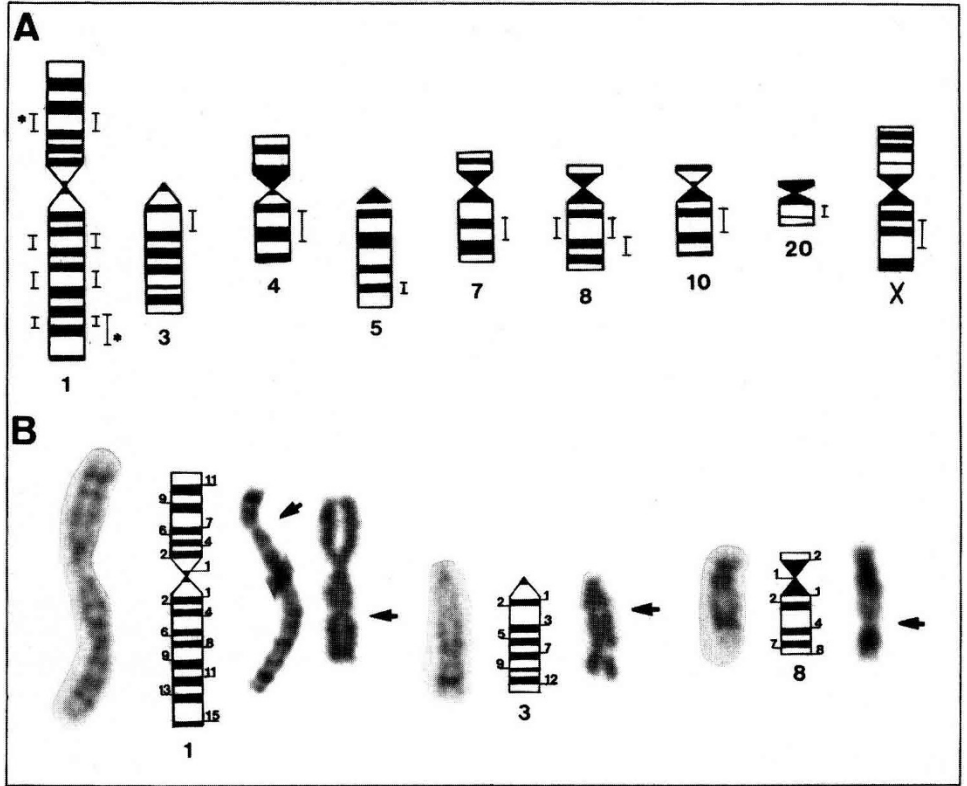
**Fig 2. A.** Idiogram of the chromosomes illustrating the bands that exhibited lesions in the BrdU-treatment (left) and APC-treatment (right); * represents lesions detected in control cultures. **B.** APC-sensitive fragile sites in 1p7, 1q9, 3q3, and 8q4. Normal chromosomes on left. Arrows indicate fragile sites.

(1991) also confirmed that the aberrations induced by this chemical were primarily of the chromatid type and included gaps, breaks and interchanges.

APC, a diterpenoid mycotoxin that inhibits alpha DNA polymerase associated with DNA replication, induces gaps and breaks at common fragile sites in human chromosomes (Glover *et al*, 1984), either as chromosome or chromatid aberrations (Murano *et al*, 1989). The most frequent type of aberration exhibited by APC-treated cells in this study was the chromosome gap. The results may reflect the number of cycles a cell had completed after the introduction of APC into cultures, and/or even the efficiency of the repair mechanisms.

It is interesting to note that the fragility observed in the Xq4-6 was displayed by only 1 animal of the species *M ater*, and so this region was not qualified as harboring a fragile site in this work. Corresponding X-fragility has been observed in several distantly related mammalian species including humans, horses, rats, rabbits, pigs, dogs, and cattle (Rønne *et al*, 1993). The putative Xq4-6 fragility observed in this

study may then correspond to the Xq22 fragility observed in humans, horses, and rats (Rønne *et al*, 1993).

Since the species present complete homology in their karyotypes, the interspecific variation was surprising. Conservation of 5-azacytidine-sensitive fragile sites was described in primates (Schmid *et al*, 1985), as well as fragility in bands shared by horses and humans (Rønne, 1992). Beyond the interspecific and interindividual variations observed in the number of regions harboring fragile sites, individual variation in the frequency of cells expressing the fragile sites was also observed among positive specimens, as previously reported, for instance, in rabbits (Poulsen and Rønne, 1991) and humans (Craig-Holmes *et al*, 1987). Variation in the molecular nature of the fragile sites could explain variation in expressivity, as exemplified by the human fragile site in Xq27.3. A highly polymorphic CGG repeat was discovered within the gene FMR-1 mapped in this region and a somatic mosaicism was well documented, indicating mitotic instability of alleles (Fu *et al*, 1991). Large expansions of the repeated region (250–4 000 repeats) are probably more easily detected by cytogenetic analysis than small expansions (52–200 repeats).

Despite the observed association between the fragile sites and the breakpoints involved in chromosomal rearrangements in several animal species (Djalali *et al*, 1985; Miró *et al*, 1987; Riggs and Chrisman, 1991), our results did not show any coincidence between the detected bands harboring fragile sites in the species of *Molossus* and the breakpoints involved in chromosomal rearrangements occurring in the evolution of 7 species of the family Molossidae (Morielle-Versute, 1992). However a more detailed study is necessary to verify the complete relationship between these 2 phenomena in bats.

## ACKNOWLEDGMENTS

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