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Localization of the properdin factor complement locus *Pfc* to band A3 on the mouse X chromosome

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Summary

The locus for properdin (properdin factor complement, *Pfc*), a plasma glycoprotein, has been mapped to band A3 of the mouse X chromosome by *in situ* hybridization to metaphase spreads containing an X;2 Robertsonian translocation. The X-linkage of the locus has also been confirmed by analysis of *Mus musculus* x *Mus spretus* interspecific crosses. The XA3 localization for *Pfc* places it in the chromosomal segment conserved between man and mouse which is known to contain at least six other homologous loci (*Cybb*, *Otc*, *Syn-1 Maoa*, *Araf*, *Timp*).

1. Introduction

Properdin is a plasma glycoprotein which greatly enhances the complement-mediated clearance and cytolytic systems in both natural and acquired resistance to infection (Thompson, 1987). It stabilizes the $C\overline{3b_nB}b$ enzyme complex of the alternative pathway of the complement system, which in turn activates components C3 and C5 resulting in opsonization of foreign material and assembly of the membrane attack complex on target cells. In man, deficiency of properdin renders the patient particularly prone to death from meningococcal infection (Sjoholm *et al.* 1982). Since all the deficient patients reported so far have been male and, on average, approximately half of the normal level of properdin is present in the serum of obligate female carriers, it has been concluded that the deficiency is linked to the X chromosome. Furthermore, both the deficiency locus (BFD) and the properdin structural locus (PFC) have been mapped to the proximal region of the human X chromosome short arm (Goonewardena *et al.* 1988; Goundis *et al.* 1989). Comparative mapping of X chromosome loci in man and mouse facilitates the identification of candidate mouse homologues for human genetic disorders and contributes to the existing knowledge of the conservation of the chromosomal segments between the two species (Amar *et al.* 1988; Searle *et al.* 1989). In order to add to this data, we have positioned the properdin (*Pfc*) locus on the mouse X chromosome by *in situ* hybridization and also confirmed the X-linkage through the analysis of restriction fragment length polymorphisms (RFLP) in the F1 offspring of interspecific crosses.

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2. Materials and Methods

The cloning of the mouse properdin cDNA into the vector pAT153 has been previously described (Goundis & Reid, 1988; Goundis, 1988). For the *in situ* hybridization studies, the complete cDNA ($m\bar{P}$) encoding the mouse properdin locus was purified free from vector sequences and labelled by nick-translation with ^{3}H-dCTP (Amersham International) to a specific activity of $> 10^8$ dpm/μg. For filter hybridization experiments, $m\bar{P}$ or a 830 bp *Bam*HI–*Cla*I fragment ($m\bar{P}1$) from the 3′ section of the cDNA, was labelled to a specific activity of 10^9 dpm/μg by the random primer technique (Feinberg & Vogelstein, 1983) using an Amersham multiprime kit.

Air-dried mitotic chromosome preparations were obtained from lipopolysaccharide stimulated cultures prepared from the spleens of mice homozygous for the Rb(X.2)2Ad Robertsonian translocation (Adler *et al.* 1989). In these mice, both X chromosomes are marked as being involved in the two metacentrics in an otherwise all acrocentric karyotype. Slides were probed as previously described (Lyon *et al.* 1986) with the addition that G-bands were induced after slide

development by a brief incubation in 2xSSC at 60 °C followed by Giemsa staining (Adolph *et al.* 1987).

Mice were obtained from stocks maintained at the MRC Radiobiology Unit and standard breeding methods used to produce F1 offspring. DNA was prepared from mouse samples (*Mus musculus* (C3H/HeHx101/H hybrids), *Mus spretus* and their offspring) as previously described (Laval & Boyd, 1989) and digested with *Taq*I (Gibco BRL). Restriction digests were transferred to Zeta-probe (Biorad) or Gene Screen Plus (Dupont) nylon membranes according to the manufacturer's instructions. Post hybridization wash stringency was 0·5xSSC at 63 °C.

3. Results

The probe for properdin showed a specific signal on the X chromosome after *in situ* hybridization to Rb (X.2) 2Ad metaphases. Of the 116 grains scored in 100 mitotic cells, 37 were observed over the two X chromosomes, whereas, if the grains were randomly distributed over all chromosomes, only 7·2 grains would be expected (XX = 6·18 % of the genome). This assignment of properdin to the mouse X chromosome by *in situ* hybridization was confirmed by Southern blot analysis of the *M. musculus* and *M. spretus* stocks and the F1 progeny of both sexes. As can be seen from Fig. 1, probe mP̄1 detects two bands (1·6, 3·1 kb) in *Taq*I digests of DNA prepared from *M. musculus*

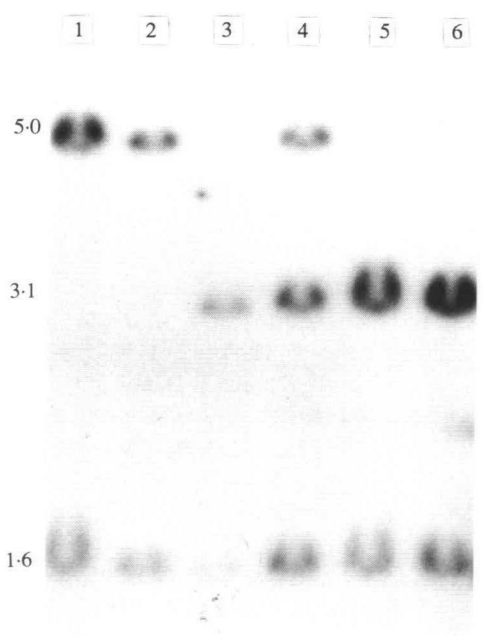

Fig. 1. Autoradiographic bands observed after hybridization of mP̄1 to *Taq*I digests of DNA prepared from *M. musculus*, *M. spretus* and their F1 progeny. Track (1) *M. spretus* male (2) *M. spretus* female (3) *M. musculus*/*M. spretus* F1 male (4) *M. musculus*/*M. spretus* F1 female (5) *M. musculus* male (6) *M. musculus* female. *M. musculus* bands are at 1·6 kb and 3·1 kb; *M. spretus* bands are 1·6 kb and 5·0 kb. Less DNA was inadvertently loaded into tracks 2 and 3 and therefore the bands are correspondingly fainter.

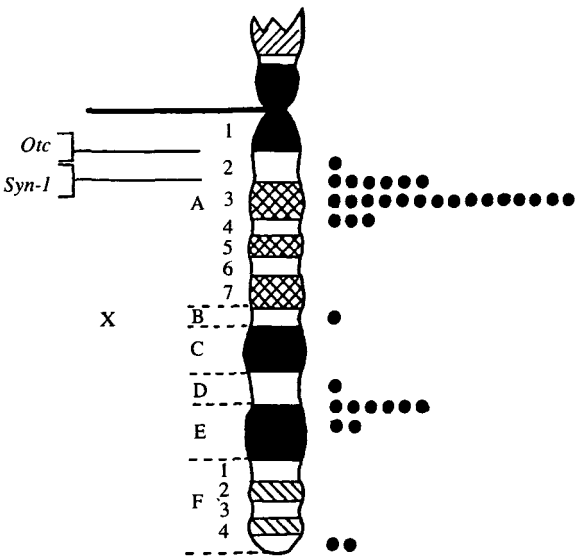

Fig. 2. Grain distribution over the X chromosome after hybridization with the properdin probe. The positions of two other loci, *Otc* and *Syn*-1, in this region which have been mapped by *in situ* hybridization are also shown. The idiogram of the X chromosome is taken from Genetic Variants and Strains of the Laboratory Mouse (1981).

male and female mice (C3H/HeH, 101/H and their F1 hybrid 3H1). Two bands are also observed when *M. spretus* DNA is probed with mP̄1; one of the same size (1·6 kb) as seen in the *M. musculus* sample and another at 5·0 kb. Both male and female F1 hybrid progeny inherit a *M. musculus* X chromosome from their mothers and hence the 3·1 kb band. However, only the F1 females inherit a *M. spretus* X chromosome from their fathers and therefore the 5·0 kb band is present in F1 females and not F1 males. This pattern of inheritance is strongly indicative of X-linkage.

We were also able to position the properdin locus on the mouse X chromosome from the *in situ* hybridization experiments. Of the total of 37 grains scored over the X chromosome, 25 were over bands XA2–XA4 with the centre of the distribution over XA3 (Fig. 2). Nine grains were also observed over bands XD–XE. As we can find no evidence for a second locus from filter hybridization studies employing the complete cDNA (mP̄) or fragments generated from it (e.g. mP̄1) as probes at a wash stringency of 0·5xSSC at 63 °C, this signal is unlikely to be due to a highly homologous locus and a more plausible explanation is that it is an artefact. However, we cannot eliminate the possibility that this minor peak of grains represents a weakly cross-hybridizing sequence at this position on the mouse X chromosome.

4. Discussion

The localization of properdin (*Pfc*) to mouse band XA3 places it in a conserved segment which stretches proximally from above *Hprt* to the *Cybb* locus in the mouse and from above the centromere to CYBB in man (Amar *et al.* 1988); Searle *et al.* 1989; see Fig. 3). This finding is consistent with the location predicted

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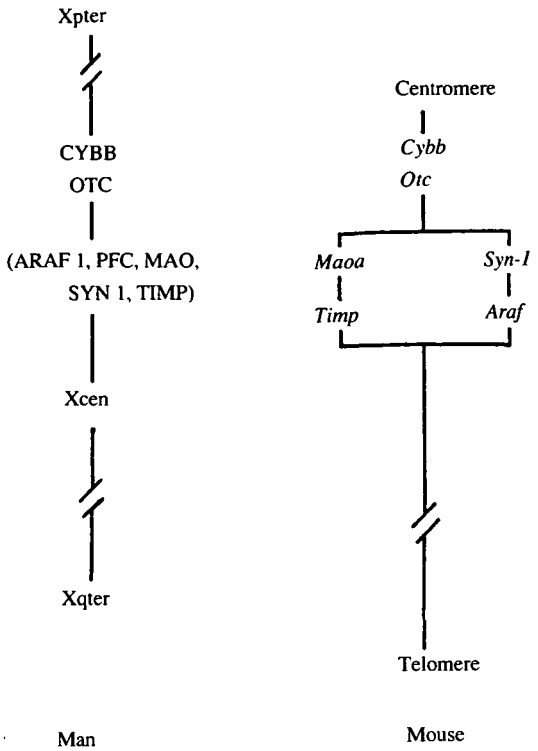

Fig. 3. Known orders in man and mouse for loci in the X chromosomal conserved segment containing the properdin locus. In the mouse, *Maoa–Timp* and *Syn*-1–*Araf* are known to lie distal to *Otc*, but their inter-relationship is unknown and they are therefore depicted as two parallel maps. CYBB, *Cybb*: chronic granulomatous disease, cytochrome b-245, beta polypeptide; OTC, *Otc*: ornithine transcarbamylase; SYN1, *Syn*-1: synapsin 1; ARAF1, *Araf*: Raf related oncogene; MAOA, *Maoa*: monoamine oxidase A, TIMP, *Timp*: tissue inhibitor of metalloproteinases. Only one locus is indicated for monoamine oxidase as evidence for two tandem loci in the mouse has only been presented as a published abstract (Derry *et al.* 1989).

from the localization of the human locus to Xp11.4 (Goundis *et al.* 1989). The assignment of mouse properdin to XA3 suggests that it lies very close to the neural phosphoprotein, *Syn*-1, which has been mapped to XA1–XA4 by *in situ* hybridization with a peak grain distribution in the bands XA2–XA3 (Yang-Feng *et al.* 1986). *Syn*-1 has been placed distal to *Cybb* and proximal to *Araf* through analysis of interspecific backcross animals (Amar *et al.* 1988). However, the relative order of *Syn*-1 and *Araf* with respect to two other homologous loci, *Maoa* and *Timp*, which have been genetically mapped with respect to *Otc* and *Cybb* (Derry *et al.* 1989), is not known in either species. In man, the relative order of the homologous loci, ARAF1, MAOA, PFC, SYN1, TIMP, has yet to be defined, although all appear to lie proximal to OTC from physical mapping data (Mandel *et al.* 1989). The Xp11.4 localization for PFC suggests that it lies distal to the MAOA locus which has been mapped to Xp11.23–Xp11.4 by *in situ* hybridization (Goundis *et al.* 1989; Levy *et al.* 1989); however, this order awaits confirmation from translocation mapping studies. We are currently mapping *Pfc* with respect to *Maoa* and *Otc* in interspecific backcrosses to order them in the mouse and thereby predict locus order in man.

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