Poxvirus Genetic Recombination during Natural Virus Transmission

By PAUL D. GERSHON, R. PAUL KITCHING, JEF M. HAMMOND AND DONALD N. BLACK*

AFRC Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey GU24 0NF, U.K.

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SUMMARY

We have compared detailed physical maps of the genomes of four capripoxvirus isolates, representing four capripoxvirus genome types. The comparisons strongly suggest that the progenitor of one of these isolates arose by genetic recombination between members of two of the other three types.

Genetic recombination between the genomes of orthopoxviruses, detected by selection or screening in laboratory animals or cell culture, is well documented (Ball, 1987; Bedson & Dumbell, 1964a, b; Chernos et al., 1985, 1987; Fenner & Comben, 1958; Sam & Dumbell, 1981; Woodroofe & Fenner, 1960, and references therein). Genetic recombination has also been demonstrated within the genus leporipoxvirus, with 'malignant rabbit fibroma virus' (MRV) being derived by genetic recombination between two other leporipoxviruses, i.e. myxoma virus, the causative agent of myxomatosis, and Shope fibroma virus (SFV) (Strayer et al., 1983a, b; Upton & McFadden, 1986). MRV was initially isolated from an extract of a tumour taken from a rabbit inoculated with an uncloned stock of SFV (Strayer et al., 1983a). It could thus have arisen in wild rabbits infected with both SFV and myxoma virus, or during passage of SFV stock in cells or laboratory rabbits latently infected with myxoma virus (Williams et al., 1972). We have been unable to find published examples of poxvirus recombinants which have clearly arisen during natural virus transmission. Here we present strong evidence that the common ancestor of a group of newly isolated capripoxviruses arose by genetic recombination between members of two other types of capripoxvirus.

Capripoxvirus genomes are closely related, with even the most divergent genomes possessing greater than 95-8% nucleotide sequence homology (Gershon & Black, 1988). To date we have analysed the genomes of 26 capripoxvirus isolates by restriction enzyme digestion (Black et al.,

![SalI Site Map](image)

![AvaI Site Map](image)

![PstI Site Map](image)

Fig. 1. *PstI*, *AvaI* and *SalI* site maps of YG-1 DNA. Maps were determined by standard and field inversion gel electrophoresis of complete, double and partial genomic restriction digests and blot hybridizations, using cloned and uncloned probes as described previously (Gershon & Black, 1987, 1988).
1986, unpublished data) and on this basis have divided them into four types. Type 1 isolates were found to come only from sheep; type 2 isolates were derived only from goats; type 3 isolates were derived from sheep, goats and cattle but were restricted to Africa; type 4 isolates were derived from either sheep or goats in Africa or the Middle East. Previous phylogenetic analysis based on HindIII site maps of the genomes of representatives of these types suggested that the genome of the type 4 isolate Yemen goat-1 (YG-1) may possess regions characteristic of the genomes of type 2 and type 3 isolates (Gershon & Black, 1988). We have further investigated this possibility by determining the PstI, AvaI and SalI site maps of YG-1 DNA, and comparing detailed physical maps of the genomes of four isolates, India sheep-1 (INS-1), Iraq goat-1 (IrG-1), Kenya cattle-1 (KC-1) and YG-1, representative of each of types 1 to 4 respectively.

The genomes were compared in groups of three such that each group comprised representatives of three of the four genome types. For each group of three, the HindIII site maps of the genomes (Gershon & Black, 1988) were aligned with one another by the insertion of pads. Pads were added (Gershon & Knowles, 1988) at the positions of known deletions and in regions where fragment length differences of greater than 5% were observed (Gershon & Black, 1988). PstI, AvaI and SalI sites (Gershon & Black, 1988; Fig. 1) were then added to the aligned HindIII site maps, preserving the relative positions of all of the sites as determined by double digestion of genomes and cloned genomic fragments. The restriction sites which are conserved between all three members of each group were then removed, leaving skeleton maps showing only 'non-conserved' sites. These 'non-conserved' sites are either present on one genome and absent from the other two, or present on two and absent from the other one. Thus, within a group of three, pairs of genomes share either the presence or the absence of each 'non-conserved' site. A genome which has arisen by recombination between two distinguishable parental genomes should possess some subgenomic regions homologous to one parent and others homologous to the other. These regions should be identifiable by the shared presence and absence of 'non-conserved' sites. If a genome has arisen by recombination alone, the parents should not share site presences or absences to the exclusion of the putative recombinant.

Fig. 2 shows the four possible three-way combinations of the four genome types identified here. Within each triplet shared site presences and absences are connected with vertical and bowed lines. A clear fit to the above model of a recombinant genome occurs only in the comparison of KC-1, IrG-1 and YG-1 (Fig. 2a). Fig. 2(a) strongly indicates that regions 0 to 30,
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52 to 92 and 134 to 145 (numbers representing kb from the left-hand terminus) of YG-1 DNA are derived from IrG-1 DNA, and that regions 35 to 47 and 112 to 126 of YG-1 DNA are derived from KC-1 DNA. We suggest that point mutations have caused the loss of a HindIII site and the gain of an AvaI site in the region 15 to 18 of IrG-1 DNA, the loss of a HindIII site in the region 70 to 72 of YG-1 DNA, and the gain of a SalI site in the region 105 to 108 of KC-1 DNA. The origin of regions 30 to 35, 47 to 52, 92 to 112 and 126 to 134 cannot be identified from the present data, due to their lack of non-conserved HindIII, PstI, AvaI and SalI sites. We suggest from these data that the type 4 isolates, represented here by YG-1, arose by genetic recombination between type 2 and type 3 isolates, represented by IrG-1 and KC-1 respectively.

Three of the four isolates studied here are field isolates of capripoxvirus. As such, they have been derived directly from infected animals and have been propagated in cultured secondary lamb testis cells in this laboratory only for the minimum time necessary to achieve virus growth sufficient for DNA isolation. The fourth isolate, IrG-1, is a vaccine strain and has thus undergone a considerable amount of laboratory passage. We have, however, shown that the HindIII, PstI, AvaI and SalI site maps of its genome are indistinguishable from those of the genome of the field isolate India goat-1 (Gershon & Black, 1988, unpublished data). The premise that YG-1 did not arise during laboratory passage is further strengthened by the geographically and temporally distinct isolation of other capripoxviruses whose genome digest patterns are identical to those of YG-1 DNA (P. N. Bhat & D. N. Black, unpublished data).

That genetic recombination between poxvirus genomes has occurred in nature, in the absence of laboratory screening or selection, highlights a possible hazard which could arise from the use of vaccines based on poxviruses. Genetic recombination between such a poxvirus vaccine and a naturally occurring poxvirus could generate a novel virus with altered pathogenic properties. The generation by genetic recombination of a poxvirus with a modified pathogenicity has previously been demonstrated within the genus leporipoxvirus in which the recombinant MRV derives its pathogenic properties from both of the parents, SFV and myxoma virus. Early during infection MRV induces fibromas which are indistinguishable from those of SFV, though at later times the tumours generalize and metastasize with an invasive profile similar to that of myxoma (Strayer et al., 1983a, b).

In the event of recombination between a vaccine and a naturally occurring poxvirus, any hazard from increased pathogenicity would be exacerbated if the new virus were highly transmissible. In this respect, particularly broad host range properties are attributable to the orthopoxviruses, including vaccinia virus (Baxby et al., 1986). The transmission of orthopoxviruses between a number of hosts has been clearly demonstrated (Jayo et al., 1986; Lal & Singh, 1977; Pilaski et al., 1986), may be more widespread than is presently realized (Baxby et al., 1986), and could be accentuated by the use of baits for the oral distribution of vaccines based on vaccinia virus (Blancou et al., 1986). In addition, there is strong evidence that orthopoxviruses can be transmitted from animals to man (Eggerink et al., 1986; Palca, 1988; Pether et al., 1986). Field trials of vaccines based on genetically engineered vaccinia virus (Blancou et al., 1986; Grigera, 1986; Koprowski et al., 1987; Palca 1986a, b, 1988; Zagury et al., 1987, 1988) should throw some light on the likelihood of orthopoxvirus genetic recombination occurring during vaccination programmes.

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REFERENCES


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