Physical mapping¹ of immunoglobulin loci *IGH@*, *IGK@*, and *IGL@* in the opossum (*Monodelphis domestica*)

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Manuscript received 7 November 2005; accepted in revised form for publication by M. Schmid, 1 February 2006.

¹ To our knowledge this is the first time these genes have been mapped in *Monodelphis domestica*.

Rationale and significance

Comparisons between marsupial and eutherian chromosomes provide valuable insight into the evolution of the mammalian genome (O'Brien et al., 1999). The grey short-tailed opossum (*Monodelphis domestica*) is the first marsupial to have its genome completely sequenced. Unfortunately very few opossum genes have yet been physically mapped. Here we report the chromosome location of important marker genes.

The adaptive immune system of vertebrates has evolved to recognize and respond to an exceptionally diverse range of antigens. Immunoglobulins (Ig) act as one of these mediators of antigen recognition. Ig molecules are comprised of two identical heavy chains in association with two identical light chains, each possessing variable and constant regions. In marsupials, as for eutherian mammals, Ig heavy chains are encoded by a single locus (*IGH@*) in the genome, and two light chain isotypes, Igk and Igl, are encoded at separate unlinked

loci (*IGK@* and *IGL@*, respectively). Opossum Ig heavy and light chain genes have been previously characterized, predominantly at the cDNA level (Aveskog and Hellman, 1998; Lucero et al., 1998; Miller et al., 1998, 1999). We isolated BAC clones containing opossum *IGH@*, *IGK@* and *IGL@* and localized them to opossum chromosomes. *IGH@* and *IGK@* map to proximal and distal 1q respectively whereas *IGL@* is located on distal 3q. These data will assist in anchoring opossum sequence contigs to chromosomes.

Materials and methods

BACs containing the *IGH@*, *IGK@*, and *IGL@* loci were identified in the *M. domestica* VMRC-6 BAC library (Childrens' Hospital Oakland Research Institute, Oakland CA, USA) by probing library filters using overgos, following the manufacturer's recommended protocols. Overgo sequences used were: *IGH@*, 5'-GACAAGCTACTTCCCTGAGC-CAGT-3' and 5'-ATTCCATGTCACGTGTCACCTGGCTC-3' specific for the IgG constant region; *IGK@*, 5'-CACGGGGATCAGCACCAGTGT-GAC-3' and 5'-CTTGCTGTCCTGTTCTGTACACT-3' specific for the Igk constant region; and *IGL@*, 5'-CCAAGAAATGGCTCCCTGT-GTCCA-3' and 5'-CTGGTGAGCACCCCACTGGACACA-3' specific for the Igl constant region. Internal sequencing was used to confirm the presence of each locus on the BACs. In addition, BAC end sequence was obtained from all clones. These sequences were then identified in the current *M. domestica* genome sequence assembly (GenBank Accession No. AAFR00000000.2), to confirm their location flanking each of the Ig loci.

Metaphase chromosomes from a male *M. domestica* fibroblast cell line were prepared as previously described (Carvalho-Silva et al., 2004). FISH was carried out on the chromosome spreads following the method described by Alsop et al. (2005).

Supported by the National Science Foundation (award MCB-0234930 to R.D.M.).

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Fig. 1. FISH mapping of opossum BACs containing *IGH@*, *IGK@* and *IGL@* to proximal 1q, distal 1q, and distal 3q respectively.

Results

Mapping data:

	<i>IGH@</i>	<i>IGK@</i>	<i>IGL@</i>
<i>Probe name:</i>	VM6_21H10	VM6_298C16	VM6_53G20
<i>Probe type:</i>	BAC clone	BAC clone	BAC clone
<i>Insert size:</i>	159 kb	152 kb	133 kb
<i>Vector:</i>	pCC1BAC vector	pCC1BAC vector	pCC1BAC vector
<i>Proof of authenticity:</i>	sequence	sequence	sequence

	<i>IGH@</i> (VM6_21H10)	<i>IGK@</i> (VM6_298C16)	<i>IGL@</i> (VM6_53G20)
<i>Most precise location:</i>	proximal 1q	distal 1q	distal 3q
<i>No. of cells examined:</i>	17	20	16
<i>No. of cells with specific signal:</i>	1 (0), 2 (2), 3 (3), 4 (12) chromatids per cell	1 (0), 2 (5), 3 (0), 4 (15) chromatids per cell	1 (0), 2 (4), 3 (0), 4 (12) chromatids per cell

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