Physical mapping¹ of T cell receptor loci (*TRA@*, *TRB@*, *TRD@* and *TRG@*) in the opossum (*Monodelphis domestica*)

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Rationale and significance

Comparative gene mapping between marsupials and eutherians provides insight into the evolution of the mammalian genome. The opossum (*Monodelphis domestica*) is the first marsupial to be sequenced yet surprisingly very few genes have been physically mapped in this species. The T cell-mediated immune response plays an important role in antigen recognition in the vertebrate immune system. T cells can be grouped into two subsets, $\alpha\beta$ and $\gamma\delta$, based on the expression of T cell receptor α (TRA) and β (TRB) chains, or γ (TRG) and δ (TRD) chains, respectively. Opossum orthologues of *TRA@*, *TRB@* and *TRD@* loci have been previ-

ously isolated and characterized at the cDNA level (Baker et al., 2001, 2005). Opossum *TRG@* sequences have been identified in the current *M. domestica* genome sequence assembly (Z.E.P. and R.D.M. unpublished). Here we have isolated BAC clones containing *TRA@*, *TRB@*, *TRD@* and *TRG@* and localized them to opossum chromosomes. Opossum *TRA@* and *TRD@* co-localize to 1p whereas *TRB@* and *TRG@* map to 8q and 6q respectively. The co-localization of *TRA@* and *TRD@* is consistent with the *TRD@* genes being nested within the *TRA@* locus in the opossum genome (Z.E.P. and R.D.M., unpublished), which is identical to the arrangement found in eutherians (Chien et al., 1987). These data will assist in assigning opossum genome sequence data to chromosomes.

Materials and methods

BACs containing the *TRA@*, *TRB@*, and *TRG@* 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 manufacturers' recommended protocols. Overgo sequences used were *TRA@*, 5'-CCAGCTCTGTACCACTAAGAAGCC-3' and 5'-CAAAGCCTTGCTCTGCATGTGAAC-3'; *TRB@*, 5'-GAGTGACTCCCCAAAGGTAAGT-3' and 5'-GAACTGTACTTG-GCAACGAAAGTTG-3'; and *TRG@*, 5'-GCCACCAAAGCCCATC-TTTTTC-3' and 5'-GCCC-AAGTCCTCGTGCTGGTAG-3'. A *TRD@* containing BAC was identified in the *M. domestica* VMRC-18 library by

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Fig. 1. FISH mapping of opossum BACs containing *TRA@*, *TRB@*, *TRD@* and *TRG@* to MDO1p, MDO8q, MDO1p and MDO6q, respectively.

identifying a clone containing BAC end sequence flanking the *TRD@* locus in the current *M. domestica* genome sequence assembly. All BACs were checked by internal sequencing to confirm the presence of each locus on the clone.

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).

	<i>TRA@</i>	<i>TRB@</i>	<i>TRD@</i>	<i>TRG@</i>	<i>TRG@</i>
<i>Probe name:</i>	VM6_148J14	VM6_183L13	VM18_213O11	VM6_25D15	VM6_30J16
<i>Probe type:</i>	BAC clone	BAC clone	BAC clone	BAC clone	BAC clone
<i>Insert size:</i>	157 kb	162 kb	161 kb	168 kb	157 kb
<i>Vector:</i>	pCC1BAC	pCC1BAC	pCC1BAC	pCC1BAC	pCC1BAC
<i>Proof of authenticity:</i>	sequence	sequence	sequence	sequence	sequence

Results

Mapping data:

	<i>TRA@</i> (VM6_148J14)	<i>TRB@</i> (VM6_183L13)	<i>TRD@</i> (VM18_213O11)	<i>TRG@</i> (VM6_25D15)	<i>TRG@</i> (VM6_30J16)
<i>Most precise location:</i>	1p	distal 8q	1p	distal 6q	distal 6q
<i>No. of cells examined:</i>	12	20	16	20	20
<i>Number of cells with specific signal</i> <i>(chromatids per cell):</i>	1 (0), 2 (0), 3 (1), 4 (11)	1 (0), 2 (0), 3 (1), 4 (19)	1 (0), 2 (1), 3 (0), 4 (15)	1 (0), 2 (0), 3 (1), 4 (19)	1 (0), 2 (0), 3 (0), 4 (20)

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