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The globin genes of the tammar wallaby

A thesis submitted for the Degree of Doctor of Philosophy

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Abstract

As a result of the ancient evolutionary separation between monotremes, marsupials, and eutherians, comparative molecular studies on these three groups of mammals can provide valuable information about the evolution of gene families. In the case of the globin gene families, which are the focus of this thesis, data on the molecular and chromosomal organisation of the α- and β-globin genes in marsupials, allows the timing and nature of the gene duplications that occurred during the evolution of the gene families, to be traced back to the base of the mammalian lineage (Cooper and Hope 1993; Koop and Goodman 1988). Furthermore, because marsupials represent the closest living ancestor of eutherians, they provide an appropriate outgroup for use in phylogenetic analyses of eutherian macromolecular sequences.

In the study reported in this thesis, a PCR-based approach was used to isolate the β-like globin genes that are present in the tammar wallaby, *Macropus eugenii*, including the gene that encodes the ω-globin chain. Three β-like globin genes (β-, ε-, ω-) that had previously been described at the protein level in the tammar wallaby (Holland and Gooley 1987) were characterised. ω-globin orthologues were also identified in a wide range of marsupial species, and in one of these species, the dunnart (*Sminthopsis crassicaudata*), the complete DNA sequence of the ω-globin gene was determined. Southern analysis in the dunnart and *in situ* hybridisation in the tammar wallaby, provided evidence for the unexpected conclusion that ω-globin is not part of the β-globin gene cluster in these species. RT-PCR studies using RNA isolated from a new-born dunnarts confirmed that ω-globin is expressed in this species. Therefore, this is the first report of an "orphaned" β-like globin gene that is expressed in a vertebrate.

A tammar wallaby genomic library was screened with a ω-globin specific probe in order to isolate λ-clones containing the genomic region that surrounds ω-globin. Characterisation of clones that hybridized to the probe revealed that ω-globin is located downstream from two α-like globin genes. The first of these genes, α-globin, was shown through conceptual translation to encode the major α-like globin chain found in adult tammar wallabies (Accession No. P81043). The second of these genes, θ-globin, was shown by phylogenetic analysis to be orthologous to the eutherian θ-globin genes, thus representing the first described marsupial θ-globin gene. Therefore, the 3′ end of the α-globin gene cluster in

marsupials has the same arrangement of genes, 5' α-θ 3' as that of eutherians. This observation provides evidence that ω-globin is part of the α-globin gene cluster in the tammar wallaby, an arrangement that has not been observed for any other mammalian or avian β-like globin gene.

Phylogenetic analyses using the marsupial β-globin sequences isolated in this study provide evidence that the while ε- and β-globin genes of marsupials are orthologous to the early- and late-expressed globin genes of other mammals, the tammar wallaby and dunnart ω-globin genes are orthologous to avian β-like globin genes. The grouping of a mammalian β-like globin gene (ω-globin) in a clade containing avian β-like globin genes is an arrangement that requires the current model for the evolution of globin genes to be modified.

A new model for the evolution of β-like globin genes is put forward. A major feature of the model is that ω-globin was produced by an ancient gene duplication that occurred before the divergence of avians and mammals from a common ancestor. The model accounts for the observation that marsupial ω-globin is orthologous to the β-like globins of birds, rather than to those of other mammals.

A detailed sequence analysis of the DNA surrounding tammar ω-globin was carried out, with a number of potential promoter and enhancer-like elements being identified. The promoter of ω-globin was shown to have features that are characteristic of the promoters of the early-expressed β-like globin genes of avians. Sequence alignments were used to show that two regions (~6 kb and ~2 kb) upstream from tammar ω-globin have a similar arrangement of elements to those found in the locus control regions (LCRs) known to act as enhancers of β-like globin genes.