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Short sequence report

Expression and phylogenetic analysis of the ninth complement component (C9) in rainbow trout

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The ninth component of complement (C9) is a single-chain glycoprotein that is involved in the formation of the membrane attack complex (MAC) on the surface of target cells [1]. The MAC is assembled by sequential binding of C5b with C6, C7, and C8 and incorporation of a variant number (6–16) of the C9 molecules into the nascent complex [2]. Once assembled on the target surface it forms transmembrane channels causing membrane damage and cytolysis [3]. The terminal complement components C6, C7, C8α, C8β, and C9 (TCC) are structurally related proteins, differing in size and complexity. They may have emerged through a series of duplications of an ancestral gene and differ in their modular composition, the most complex being C6 and the simplest, C9. Which came first evolutionarily, is still under debate. In brief, two theories have been proposed. The first one supports the notion that the ancestral gene had a complex modular composition [4]. A series of duplications of an ancestral C6/C7-like gene, in combination with a tendency to lose modules, resulted in successive complement proteins with decreasing modular complexity [5]. The second one, on the other hand, supports the hypothesis that C9 emerged from the original duplication of the common ancestral gene to perforin and TCC [6]. According to this hypothesis C8, C7, and C6 should have successively emerged from C9 through later gene duplication events. In teleosts, the MAC complex has been microscopically observed as small pores in the cell surface [7]. The C9 component has been characterised in Japanese flounder (*Paralichthys olivaceus*) and pufferfish (*Fugu rubripes*) [8,9], while sequences of C9 from zebrafish (*Danio rerio*), and tetraodon (*Tetraodon nigroviridis*) are deposited in databases. Previous reports have presented a partial primary sequence, as well as the domain structure and the function of the rainbow trout (*Oncorhynchus mykiss*) C9 [10,11].

To clone the full-length cDNA of the C9 gene from rainbow trout, specific oligonucleotides were designed based on the partial sequence of trout C9 (GeneBank: X05474), These primers were subsequently applied to PCR reactions, in combination with reverse and forward λgt11 primers, using as template phage DNA from trout liver cDNA library [12]. The full-length trout C9 cDNA of 2104 bp, with an open reading frame of 601 aa and a putative signal secretion peptide (1–23 aa), was obtained. The deduced amino acid sequence of trout C9 was aligned with those of various

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species, using the Clustal W program [13]. Trout C9 shows the highest identity score 66 and 61% with pufferfish and Japanese flounder C9, respectively, followed by human and African frog counterparts with 38 and 39% identity, respectively (Fig. 1). The identity score with the other trout TCC proteins ranges between 26 and 28%. All the characteristic modular domains of the terminal complement components (TSP1, LDLa, MACPF, and EGF) are present in trout C9, and in contrast to the mammalian counterparts, trout C9 contains an additional carboxy terminal TSP1 domain [11]. Trout C9 contains two C-mannosylation motifs (WXXWXXW), located in the TSP1 domains, four potential *N*-glycosylation sites (Asn-X-Ser/Thr), in contrast to the two sites of the mammalian counterparts, and all the cystein residues show high conservation (data not shown).

Phylogenetic tree (Fig. 1) was constructed based on the deduced amino acid sequences of TCC polypeptides using the neighbour-joining (NJ) algorithm with the Tree Top program, and bootstrap 100 [14]. Recent data from the cephalochordate *Branchiostoma belcheri* and from the decoded genome of the urochordate *Ciona intestinalis*, have shown

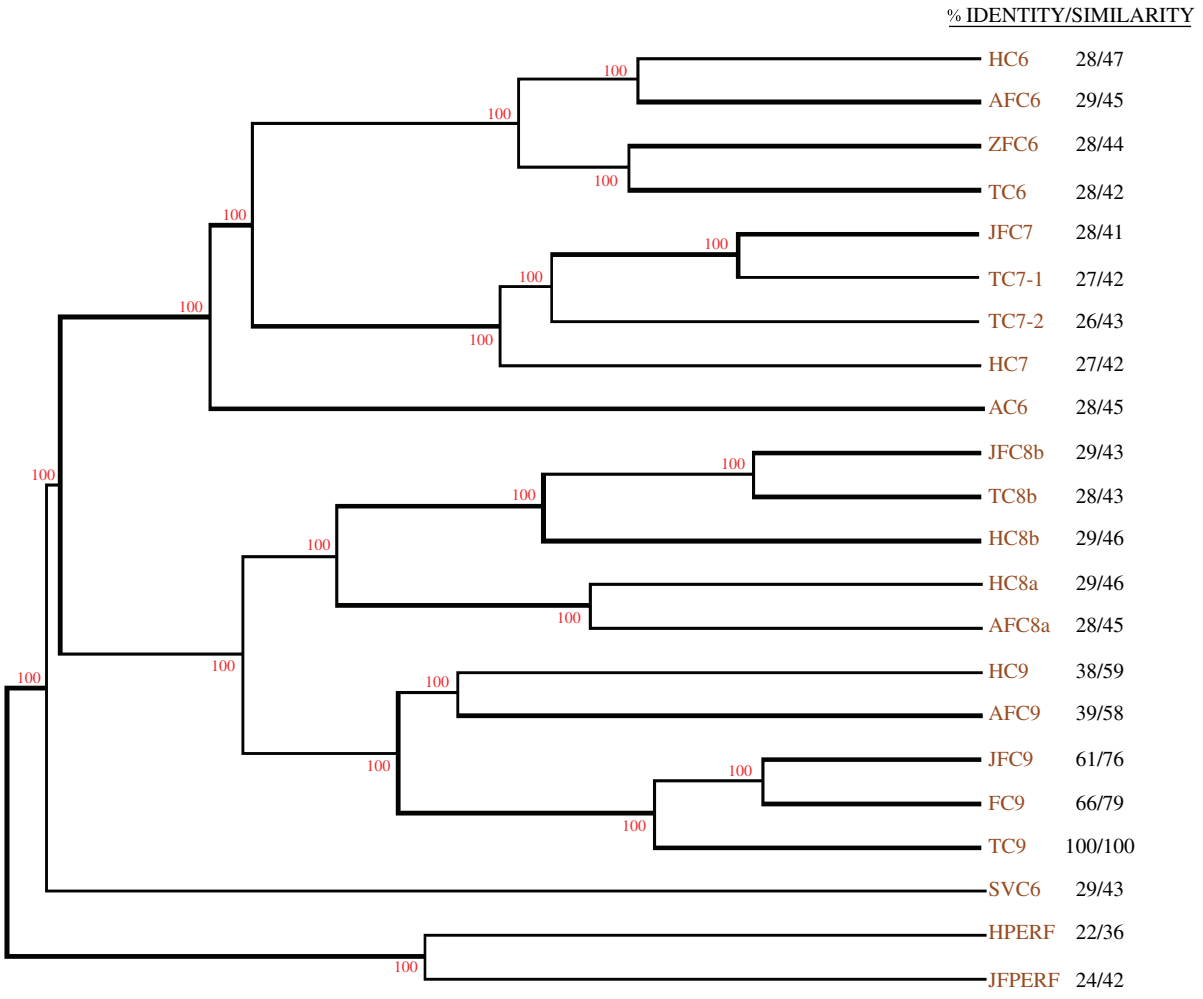

Fig. 1. Phylogenetic tree of the terminal complement components C6, C7, C8β, C8α and C9 from various species. TC7-2 (trout C7-2, CAF22025), TC7-1 (trout C7-1, CAD92841), HC7 (human C7, NP_000578), JFC7 (Japanese flounder C7, BAA88899), AC6 (amphioxus C6-like, BAB47147), SVC6 (sea vase C6-like, *Ciona* 131279), HC6 (human C6, AAH35723), TC6 (trout C6, AJ622903), ZFC6 (zebrafish C6, NP_956932), AFC6 (African frog C6, AAH42265), HC8b (human C8β, NP_000057), TC8b (trout C8β, AAL16647), JFC8b (Japanese flounder C8β, BAA86877), HC8a (human C8α, NM000562), AFC8a (African frog C8α, NM001005445), TC9 (trout C9, CAA29037), HC9 (human C9, NP_001728), AFC9 (African frog C9, AAH54952), JFC9 (Japanese flounder C9, BAA86878), FC9 (fugu C9, AAC60288). The HPERF (human perforin, NP_005032) and JFPERF (Japanese flounder perforin, BAC76420) were used as outgroup. The relationships among the various components were analysed by the neighbour-joining method. Numbers on the branches are bootstrap values.