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Characterisation of *Acropora* AmTPR1, *Drosophila* Dpit47 and mouse TTC4 – a tetratricopeptide (TPR) gene family involved in development and cell proliferation



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In June 2009**



Thesis submitted in fulfilment of the requirements of the degree of Doctor of Philosophy in the School of Pharmacy and Molecular Sciences at James Cook University of North Queensland

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Lucija Tomljenovic

ABSTRACT

The tetratricopeptide repeat (TPR) is a protein-protein interaction motif present in a variety of functionally unrelated proteins. Recently, the first TPR gene was cloned from the coral *Acropora millepora*-AmTPR1. AmTPR1 has high similarity to human TTC4 and *Drosophila* Dpit47, genes implicated in tumorigenesis and cell proliferation. Using a comparative genomics approach, this thesis characterises the AmTPR1/Dpit47/TTC4 gene family in an attempt to understand the evolution of function.

Semi-quantitative PCR analysis indicates that AmTPR1 is expressed at low levels throughout the early development of *Acropora*. The AmTPR1 transcript was generally distributed in the early embryo, but by the end of gastrulation, transcripts were specifically associated with a subset of transectodermal cells. Treatment of *Acropora* embryos with the glycogen synthase kinase-3 (GSK-3)-specific inhibitor alsterpaullone resulted in increased expression of AmTPR1, suggesting a role of canonical Wnt/ β -catenin signalling in regulating AmTPR1 expression. Consistent with this, several sequences showing 100 % homology to the core TCF/LEF-binding consensus sequence, were identified in the putative promoter regions of AmTPR1 and its closest relatives *Hydra* HmTPR1 and *Nematostella* NvTPR1 genes. Compared to AmTPR1, *Drosophila* Dpit47 showed different expression characteristics. Dpit47 transcripts could not be detected in early development of *Drosophila* (stages 6-11), while in late embryos (stages 13-16), strong and specific expression of Dpit47 was observed in the central nervous system. Furthermore, in contrast to AmTPR1, the expression of Dpit47 is likely to be regulated by Myb/E2F/DREF- and not by Wnt/ β -catenin – dependent transcriptional regulation. Despite having different expression patterns, yeast 2-hybrid analysis indicates that both AmTPR1 and Dpit47 interact with Hsp90 and DNA polymerase α , suggesting the possibility of functional conservation.

The expression profile of mouse TTC4 differed from both AmTPR1 and Dpit47. In mouse neuroblastoma N2A cells, treatment with the GSK-3 inhibitors kenpaullone and LiCl had no effect on TTC4 expression. Instead, the expression of TTC4 in N2A cells was downregulated in response to depolarizing stimuli, such as 85 mM KCl. Addition of 2.3 mM Ca^{2+} exacerbated the extent of depolarization-induced downregulation of TTC4,

indicating that TTC4 expression was Ca^{2+} -dependent. However, the mechanism of Ca^{2+} -dependent regulation of TTC4 expression under depolarizing conditions did not require extracellular Ca^{2+} influx through L-type Ca^{2+} channels or N-methyl-D-aspartate (NMDA) - receptor channels as treatment of N2A cells with nifedipine (L-type Ca^{2+} channel blocker), and NMDA did not affect the extent of TTC4 downregulation in response to 85 mM KCl. Instead, treatment with 20 mM tetraethylammonium chloride (TEA) greatly exacerbated the extent of TTC4 downregulation in response to 85 mM KCl in N2A cells. The K^+ channel opener mallotoxin and the mitogen bradykinin were both able to attenuate the effect of TEA on TTC4 expression under depolarizing stimuli, indicating that TTC4 expression was dependent on K^+ channel activity. Consistent with the involvement of Ca^{2+} in regulating TTC4 expression, four nuclear factor of activated T cells (NFAT) binding sequences were found in the 2 kb 5' region of the mouse TTC4 gene. Finally, TTC4 expression was higher in proliferating than in quiescent N2A cells and the greatest extent of upregulation was observed at the G1/S transition, suggesting TTC4 transcription was cell-cycle dependent.

In summary, despite lineage-specific differences in the expression patterns and regulatory characteristics, *Acropora* AmTPR1, *Drosophila* Dpit47 and mouse TTC4 each appear to function as developmental genes involved in the regulation of proliferation coupled to the cell cycle.

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In the end, if I had forgotten anyone, please forgive me, this is the last section of my thesis that I am writing, it is just after midnight and I just want to finish it, go home and sleep ☺

Just before I go, thank God for coffee, science research would not have come as far without it !!!

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