Reviewer’s report

Title: Palm is expressed in both developing and adult mouse lens and retina

Version: 2 Date: 22 March 2005

Reviewer: Lyn D Beazley

Reviewer’s report:

General

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The manuscript describes the expression of a prenyl-palmitoyl anchored membrane protein in the developing mouse eye and in dissociated retinal cultures from embryonic day (E) 7 chicks. The experiments are well carried out with well described results, however the purpose of the experiments is not clear and the experiments appear to be randomly brought together with little synthesis. The authors have several reasons for examining Palm expression, but the results of the experiments fail to provide answers to the questions they ask.

Their reasons for examining Palm expression are as follows and I point out some inconsistencies:

Palm is downstream of the transcription factor Pax6. The authors state in the introduction that Pax6 is crucial for eye development, and that Palm has a Pax6 binding site. However, Palm is primarily expressed in the brain and they wish to examine its expression in the retina and lens. They show correlation of expression in the retina at most developmental ages but do not provide any functional evidence for regulation of Palm by Pax6. Furthermore, it is well known that Pax 6 is expressed in a dorso-ventral gradient yet this aspect is not considered.

Palm was shown to be upregulated in a microarray study of Pax6 overexpressing mice. However, the present study does not significantly advance our understanding of the relationship between Pax6 and Palm.

To examine the subcellular localisation. Palm is mostly a cytoplasmic protein (discussion, p12) however, the authors find it in the membrane. This aspect should be further discussed.

To compare Palm expression with two other members of the paralemmin family using rt-PCR. The justification of this experiment is given in the discussion: to examine the possibility of overlapping and/or antagonistic function of the family members, however the experiment would not allow any conclusions to be drawn and the authors do not discuss the question further. Rt-PCR is performed on dissected tissue and cannot demonstrate co-localisation. Because mRNA levels are measured, it can even less show overlapping or antagonistic function. Furthermore, the analysis of the rtPCR results is not well explained. Did the authors perform any melt curve analysis to check the specificity of their amplification? Did they sequence the product they were amplifying? How would the presence of splice variants affect their results and how do they know how which ones they are quantifying? It would be interesting to relate the splice variant expression with the subcellular localisation. An increase in the expression of splice variants with the palmitylation/prenylation site may explain the abundance of membrane-associated protein they observe. Such an analysis would also bring the discussion of splice variants into context. Also, how did they perform the comparison of Palm family members to the housekeeping genes? The standard procedure is to measure deltaCt
values but there is no indication of this method. I do not understand how the authors quantified by “primer efficiency” particularly when they say that primer efficiency was the same for all targets (p8). When they say “in triplicate three times” do they mean nine times?

The discussion of Palm-AKAP fusion products is interesting but does not appear to be relevant to the study. Again, in depth rt-PCR analysis could shed some light on this issue.

Minor point to improve manuscript:
Page 3, para 2, line 3 from retinal progenitor….
Page 5, para 2, line 7, give PBS in full the first time it is mentioned
Page 6 para 3, pH of PBS?
Page 10, para 2, final line (and elsewhere) abbreviations should be rationalised
Page 11, para 2, lines2-3 (and elsewhere) In vivo in italics
Page 12, para 1, line 1, explain specificity of RT-97
Page 12, para 2, final line include retina in list?
Page 15, para 2, line 2, data are

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)

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What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:

I declare I have no competing interests