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Clarin-1 protein expression in photoreceptors

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Recently our group published a paper utilizing a newly developed anti-clarin-1 antibody to establish expression patterns in mouse cochlea and retina (Zallocchi et al., 2009). Just after our paper was published, another paper was paper was published that asserted *Clrn1* mRNA may not be expressed in photoreceptors (Geller et al., 2009). Given this conflict with our data, we felt compelled to provide the evidence below that shows clarin-1 immunostaining is not observed in a clarin-1 knockout mouse (the clarin-1 knockout mouse was generously provided to us by John Flannery, UC Berkeley, and described in Geller et al., 2009). This new evidence clearly demonstrates the specificity of our antibodies against clarin-1 protein, and thus proves expression of clarin-1 protein in photoreceptors. Establishing this point is of importance to allow studies aimed at defining clarin-1 function in photoreceptors to go forward without controversy. Figure S1 shows that the immunostaining reported in our paper (Zallocchi et al., 2009) for both cochlea and retina is indeed specific, since it is not detected in the clarin-1 knockout mouse. The western blot data for retina is also confirmed as specific, since the 30 kDa band is not observed in the extracts from clarin-1 knockout mouse retina.

While the study described by Geller et al. (2009) provided compelling evidence for expression of *Clrn1* mRNA in inner nuclear layer cells, with strong supporting evidence for Muller cell expression, the study did not provide substantive evidence for absence of clarin-1 expression in photoreceptors. Since a working anti-clarin 1 antibody was not available to these investigators, all of the data provided was based on mRNA analysis. The study provided no data for Clarin-1 protein expression. The *in situ* data showed *Clrn1* mRNA is detectable in the INL only up to postnatal day 12, while RT-PCR shows *Clrn1* mRNA was still abundant in adult retinas. Thus, the *in situ* hybridization analysis was not sensitive enough to rule out expression in photoreceptors. Likewise, while the studies employing genetic ablation of photoreceptors provide convincing evidence for presence of *Clrn1* mRNA in the INL, they do not rule out expression of *Clrn1* mRNA in photoreceptors, which might be present at lower levels that are effectively “masked” in this assay. Surprisingly, our data shows Clarin-1 protein is not detected in Muller cells, suggesting that the abundance of Clarin-1 mRNA in these cells is not translated or that the protein is not stable in these cells. Therefore, it appears that photoreceptors are the retinal cells of interest for dissecting the function of clarin-1 in this organ.

Supplementary Material

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References

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