RESPONSE TO COMMENTS BY JEFF AXELROD RE OUR "DISPATCH"

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We discuss the points raised by Jeff Axelrod one by one. His comments are in bold, our answers below each in plain text (see doi:10.1016/j.cub.2008.09.002)

1. That our mathematical model [1] is challenged by overexpression assays showing Pk and Dsh are not needed in sending and receiving cells. This was predicted by the model (Equation S2), which posits that Fz and Vang can mutually recruit each other, and that Pk and Dsh are required for amplification of recruitment. Amplification occurs in wild type but is unnecessary in the overexpression assays. This was the stated rationale for our assays in Chen et al. [2].

In the Amonlirdviman model [1] great importance was given to Dsh and Pk — no less than 9 of 10 reactions include Pk or Dsh. Therefore the model is challenged because, in pk^{-} and dsh^{-} mutant flies, the asymmetric localisation of "core" proteins does not occur [3, 4], and the model predicted that, without the normal feedback amplification responsible for this localisation, polarity should not propagate. Yet it does. Our challenge comes not only from "overexpression assays", such as overexpressing fz^{+} in pk^{-} and dsh^{-} flies [5] but also from fz^{-} clones that repolarise nearby cells, even in flies lacking Pk [6, 7]. Note these are the same kinds of clones, in which various "core" PCP genes (*fz, pk, dsh, Vang*) were removed or overexpressed, that were used by Axelrod and colleagues to build their model [1]. The parameters for their feedback/amplification model were selected so that the output of the simulation gave similar results to those observed with these clones in vivo.

2. That our model is challenged by the observation that fz mutant cells can be repolarized. This is predicted by similar reasoning: Vang in a mutant cell can be recruited by Fz in an adjacent cell. Strutt and Warrington [8] showed that proximal (Vang) and distal (Fz) complexes independently contribute to morphological polarization. Notably, our model, but not all others, also accommodates the finding that vang mutant cells can be repolarized [5].

In the Amonlirdviman model [1] the polarity of a cell was determined by the accumulation of Dsh where there is high Fz activity and thus, if a cell does not have Fz, it should not localize Dsh and therefore it should not be polarised. Yet, we showed that fz^{-} cells can be repolarised [6].The recent evidence of Strutt and Warrington that asymmetric activity of either Vang or Fz can polarise cells [8] helps explain our find-

ing, but does not resolve the conflict between our result and Amonlirdviman model.

3. That our earlier model [1, 3] is invalidated by inclusion of Fmi. While our earlier biological model incorporated Fmi [3], because it was insufficiently understood, it was assumed to serve a permissive bridging function (Supplementary materials) and therefore not mathematically modeled in Amonlirdviman et al. [1]. Nonetheless, the model was a correct abstraction (see [9] for additional evidence) that can now be further refined.

All the extant mathematical models by Axelrod and colleagues do not "incorporate" Fmi [1, 10]. In fact, Fmi was ignored by Axelrod and colleagues for 4 years until it surfaced in their 2008 paper [2], in spite of our earlier demonstration of the function of Fmi in polarity propagation [6] and the two subsequent papers that built on that discovery [11, 12]. This earlier work was either not properly credited or not credited at all in Chen et al. [2]. Our 2004 model [6] not only "incorporates" Fmi, our experimental evidence puts Fmi at the centre, just as the "new", non-mathematical model does [2] —showing that the 2008 model is not new. A passing sentence in supplemental materials which acknowledges the existence of Fmi as a possible passive conduit for Fz-Dsh/Vang-Pk interactions [1] cannot be used to claim any priority for a model in which the central role of Pk and Dsh has been discarded and the central function of Fmi established.

4. That Chen et al [2] reached a conclusion similar to Lawrence in prior studies: "namely that Fmi is needed in both sending and receiving cells." This was the starting point for our experiments as clearly delineated in the introduction to Chen et al. Our aim was to understand whether Fmi is passive or instructive in the signaling process, and if instructive, to understand how asymmetry is achieved. While various ideas had been proposed, these questions were not answered by existing data.

This "starting point" of Chen et al. [2] is not "clearly delineated" in their introduction. One part of their results section is even entitled 'Fmi is required on both sides of intercellular boundaries' but it is not made clear that we showed this in 2004 [6]. What was proposed in our 2004 paper [6] is that Fmi-Fmi bridges function asymmetrically in communicating Fz-based information between cells (exactly the title of [2]). The question of how far Fmi is passive or instructive is answered neither by Chen et al, [2], nor by the other papers discussed in our Dispatch.

5. Our data showing that FzΔCRD can at least partially rescue polarity signaling [2] are positive data, while the other experiments with similar but not identical constructs showed negative or ambiguous results. We believe positive data are stronger than negative data. The Fmi overexpression data show definitively that Fz-Vang contact is not required for signaling.

This is a matter of different opinions as we discuss in the Dispatch. In Chen et al. [13] it was reported that FzΔCRD rescues very weakly and all the subsequent work has corroborated this, but not explained what it means. As for positive data being intrinsically better; in our opinion, the quality of evidence is more important than

whether the data is positive or negative. As for overexpressing Fmi in the absence of Vang and Fz we agree that this shows that the Fz-Vang contact is dispensable, but the model in Chen et al [2] has Fmi and Fmi-Vang being equivalent and this proposition raises new problems.

6. That Wu and Mlodzik's interaction between Fz and Vang in in vitro assays [14] is inconsistent with our model. Our model does not exclude such an interaction. However, our experiments show PCP signaling can occur in its absence.

We discuss these matters in the Dispatch but we do not write that Wu and Mlodzik' Fz/Vang interaction [14] is inconsistent with Chen et al's interpretation [2]. We do point out that this interaction between Fz and Vang could be within one cell, ie in cis. Chen et al do [2] argue that any interaction between Fz and Vang is "unlikely".

7. In a key assay in Chen et al. [2], we show that Fmi functions instructively by overexpressing it in clones that lack Vang, Fz or both. It is claimed this result is invalidated because Wu and Mlodzik's genotypes produced double mutant clones with considerable non-autonomy. However our genotypes produced clones with negligible non-autonomy similar to *fmi*$^{-}$ clones (we can only speculate about the reason for the difference), and therefore our Fmi overexpression result is valid. Moreover, we do not rely only on this result, since in a separate, and unambiguous, case Fmi overexpression in a Vang mutant clone reverses the polarity of neighbors [2].

Chen et al. [2], and Strutt and Strutt's results [5] are compromised by the use of an inadequate fz^{+} rescuing transgene. Which means that they are, in effect, making $Vang^{-}$ fz^{-} clones in a hypomorphic *fz* mutant background. We think that the results of adding *UAS.fmi* to such a complex genetic situation cannot be usefully interpreted. Moreover there is no way around Wu and Mlodzik's results with a true rescuing transgene [14], especially as, unlike the other papers, Wu and Mlodzik utilise null alleles of both *Vang* and *fz*. Our 2004 findings [6] fit perfectly with the result that $Vang^{-}$ fz^{-} clones repolarise similarly to fz^{-} clones and argue that this polarisation must depend on Fmi, meaning that Fmi can send a message about the (null) state of Fz activity from cells that are devoid of both Fz and Vang.

Also, the fact that Wu and Mlodzik's true $Vang^{-}$ fz^{-} clones reverse polarity in the absence of Fmi [14] obviously and seriously undermines the significance of the result of over-expressing Fmi in $Vang^{-}$ fz^{-} clones. In this case Jeff is preferring a negative result (he prefers to believe that $Vang^{-}$ fz^{-} do not repolarise) to the positive one (as reported by Wu and Mlodzik and in agreement with our 2004 model).

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