Critique on Boc is a receptor for sonic hedgehog in the guidance of commissural axons

Scientists discovered that Shh could function as a diffusible attractant similar to Netrin-1, but its binding receptor was unknown. Cdon and Boc were very good candidate receptors in terms of their protein structures, expression period, mutant phenotypes and properties of homologue proteins in *Drosophila*. In this paper, the authors examined the two candidates and found that Boc was expressed by commissural neurons and disruption of *Boc* in mouse resulted in the misguidance of commissural axons. In addition, Boc could bind specifically to Shh and RNAi knockdown of Boc prevented the rat commissural axons from turning towards an ectopic source of Shh in vitro. Therefore, the authors concluded that Boc was a receptor for Shh in the guidance of commissural axons.

First, the authors examined the expression pattern of Cdon and Boc in E11.5 mouse spinal cord. They examined RNA expression by in situ hybridization in normal mice and reporter-gene expression in *Boc* and *Cdon* mutant mice. They found that *Cdon* and *Boc* were expressed in dorsal ventricular zone and *Boc* extended more ventrally and laterally. The overlap of expression sites of *Boc* and *Robo3/Rig-1* revealed that *Boc* expression encompasses the region where commissural interneurons initiated their differentiation. In addition, PLAP reporter driven by Boc promoter labeled the axons projecting to the floor plate and was co-expressed with TAG1, which also suggested that *Boc* mRNA was expressed in differentiating commissural neurons. Moreover, they labeled Boc protein expression in cell bodies, axons and growth cones of dissociated commissural neurons in vitro using antibodies against Boc.

Then, they tested the commissural axon guidance in *Cdon* and *Boc* homozygous mutant mice E11.5 embryos. *Cdon* mutant mice had normal axon projections, while $Boc^{-/-}$ mice had similar phenotype with the mice after conditional removal of the Shh signaling mediator Smo in the commissural neurons, the axons of which were dispersed and invaded the ventral spinal cord with ectopic projections extending over the motor columns. This suggested that Boc might in the same pathway as Smo to guide commissural axons in response to Shh.

In addition, they examined whether Boc could bind directly to Shh. First, they expressed Boc-GFP and a variety of control proteins in COS cells, assaying them for binding to Shh and the results showed that Boc could bind to Shh specifically. Then quantitative assessment of the affinity of these interactions using Shh-AP revealed a K_d similar to that between netrin-1 and DCC. Besides, purified N-Shh bound to the affinity-purified ectodomains of Boc with the same affinity as to COS cells expressing Boc, suggesting the interaction between Shh and Boc is direct. Furthermore, deletion analysis implied that Shh-binding activity of Boc was attributed to the third FNIII domain.

Moreover, they tested the function of Boc in Shh-mediated axon turning in vitro. The siRNA against rat and mouse Boc was electroporated into dorsal region of rat spinal cord explants, which later co-cultured with COS cells expressing Shh or netrin-1. Commissural axons from explants transfected with siRNA against Boc responded normal to netrin-1 source, but did not turn towards Shh source. In addition, co-electroporation of human Boc rescued normal Shh-mediated attraction.

Collectively, I think the results convincingly demonstrate that Boc is required as a receptor for commissural axons to respond to the chemoattractive effect of Shh. It is a pity that the paper can not test the Boc protein expression on commissural axons in vivo and in the last commissural axon turning assay, it can not examine directly on explants from *Boc* mutant mice that previously used. Besides, it might be more convincing if they can show more merging pictures of $Boc^{-/-}$ with co-expression PLAP reporter and TAG1 as shown in Fig2 and supplementary Fig2f. Because although they did statistic data on TAG1/total area in 10 sections, no statistic data were shown to prove that in all examined $TAG1^{+}$ axons were supposed to express Boc mRNA. In

addition, it might be more convincing to directly calculate the proportion of axons that did not reach the floor plate instead of proportion of area. However, I guess it is very difficult.

The paper opened several interesting questions. For instances, how Boc mediates the Shh signaling pathway during axon guidance? What is the role of Smo in this pathway, if any? Besides, since we now know that netrin-1 and Shh are responsible for attractive effect in spinal cord during development, are two of them sufficient? To answer this question, we could make mice with double knockout Netrin-1 and Boc to see whether the embryo spinal cord still have commissural axon projection toward floor plate. We could also RNAi knockdown one gene expression in mutant mice with another gene KO and use the in vitro axon turning assay, if it could be successful in mutant mouse explants. Last, since Shh contributes both to cell fate specification and axon guidance, are these two roles related to each other? For instance, is that possible that the binding of Shh on Boc also induces the signaling pathway that further strengthens the original cell fate? Or is that possible that Shh's effect on cell fate specification meanwhile inhibits the expression of Boc, which block the guidance effect of Shh on them?