Critique on Drosophila Neuroblasts Sequentially Express Transcription Factors which Specify the Temporal Identity of Their Neuronal Progeny

Although spatial patterning is well studied, but we know little about the mechanism underlying the sequential generation of different cell types. Utilizing various molecular markers and immunostaining in Drosophila, this paper showed that neural precursors sequentially express the transcription factors Hunchback(Hb)->Kruppel(Kr)->Pdm->Castor(Cas). Besides, the authors of this paper demonstrated that Hb and Kr were necessary and sufficient for first-born and second-born cell fates respectively. Furthermore, their functions were observed in several lineages and were independent of cell type involved.

First of all, the authors identified Kr as a new deep layer transcription factor and showed the expression pattern of the four transcription factors. They found that Hb, Kr, Pdm and Cas were sequentially and transiently expressed in neuroblasts. Then they demonstrated that these transient expressed transcription factors maintained for a period of time in their neural progeny. They characterized three model neuronblast to prove that, including an early forming neuroblast(7-1) and two late-forming neuroblasts(7-3 and 2-4). Moreover, they found that the gene expression of these transcription factors was not correlated to a particular cell type.

Besides, they did experiments to see whether Hb and Kr were necessary and sufficient for specifying first-born and second-born cell fates, respectively. They showed that in hb^- neuroblast 7-3 lineage, the number of GMC-2 doubled at the expense of 1/1G neurons or first-born 1/1G neurons were specifically lost. In contrast, in neuroblast 7-3 that continuously expressed Hb, all neurons exhibit first-born 1/1G fates. These findings implied that Hb was indeed not only necessary but also sufficient for specifying first-born cell fates. In addition, they examined the effect of Hb on first-born motoneuron fate in multiple neuroblast lineages, including lineage 1-1 and 4-2. They found that the phenotypes of hb^- were consistent with a transformation of GMC-1 to a later-born GMC fate while continuously expressing Hb produced duplications or triplications of first-born cell fate. They also observed a reduction in the number of motoneuron projections in hb^- and an increase in the number of motoneurons in neuroblast that continuously expressing Hb. Furthermore, they also examined the effect of Hb on first-born glial cell fates. The hb^- mutants lacked first-born MM-CB glia at the midline, but have no change in the number of later-born channel glia at the midline. In contrast, misexpression of Hb in all neuroblasts resulted in extra MM-CB glia at the midline and a decrease in the number of midline channel glia. The results above suggested that Hb's regulation on first-born cell fate was independent of cell type identity.

In addition, the authors showed that Kr was necessary and sufficient for second-born cell fates. In neuroblast 7-3 lineage, Kr^- had variably effects on first-born 1/1G sibling neurons and second-born interneuron 2 was almost always missing while the third-born interneuron 3 was almost always normal. In contrast, continuously expressing Kr resulted in all but two GMC-1 neurons differentiate as GMC-2 derived interneuron 2. In 7-1 lineage, Kr mutants frequently missed one of the U3/U4 motoneurons while continuously expressing Kr leaded to extra Eve+ neurons with all differentiating as U3 or U4 motoneurons except the normal pair of early-born U1/U2 motoneurons.

Last, the authors examined the regulatory interaction between Hb, Kr, Pdm and Cas expression by overexpressing each transcription factor and observing whether gene expression of the other had an increase or decrease. They showed that each gene can activate the next gene in the pathway and repress the "next plus one" gene.

Generally speaking, I think the paper is fairly convincing in demonstrating the effect of Hb and Kr. However, it has some weak points. First, the paper does not provide enough evidences to support the whole sequential

Hb->Kr-> Pdm->Cas expression in neuroblasts. Besides, some figures seemed not so clear. For instances, in fig1b, the cell pattern seems a mosaic and can hardly tell the layer. So it might be difficult to tell which cell groups change the gene expression during different time period. In fig5I, I can not see the “striking increase” in the number of motoneurons. Last, the finding that regulation of Hb and Kr is independent of cell type identity might not be so surprising because at the start of differentiation, those cells might still not have cell type identity.

The paper leaves many interesting questions. For instance, as the paper mentioned, the four transcription factors examined were not necessary for driving sequential gene expression. Then what is the driving mechanism underlying this sequential expression? Besides, the temporal gene expression pattern in neuroblasts is very similar to the spatial gene expression pattern at cellular blastoderm. Is there any relation between these two pattern?