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Dendritic translocation of climbing fibers: a new face of old phenomenon

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Abstract

During postnatal development of the cerebellum, the number of climbing fibers that innervate individual Purkinje cells decreases from many to one. This is one of the most characterized models of activity-dependent refinement of synaptic circuitry in the mammalian brain. As surplus climbing fibers are eliminated, subcellular location of climbing fiber terminals moves from the soma to the dendrites of Purkinje cells. The role of this dendritic translocation in climbing fiber elimination has been under debate for a long time, but recent studies have significantly changed the view. Traditionally, dendritic translocation was considered neither sufficient nor necessary for climbing fiber elimination, but experimental evidence obtained over the past 5 years indicates crucial roles of dendritic translocation for selectively maintaining one fiber while removing all the others. Here, I provide an overview of: (i) several key findings that have contributed for developing the traditional and current views of dendritic translocation, (ii) how our novel experimental approach supports the current view, and (iii) a remaining question that is yet to be answered.

Keywords

Development; Synapse elimination; Climbing fiber; Purkinje cell; dendritic translocation; in vivo microscopy

Main text

Climbing fibers in the cerebellar cortex are the terminal axonal arbors of inferior olivary neurons in the medulla oblongata, which provide excitatory synaptic drive on to Purkinje cells. In mature cerebellum, each olivary axon divides into 6-7 collaterals each of which forms a climbing fiber terminal arbor that innervates a single Purkinje cell [1]. This one-to-one relationship between climbing fiber and Purkinje cell is not yet established at birth. Each Purkinje cell is initially innervated by multiple climbing fibers, but activity-dependent competition during early postnatal life eliminates all but one climbing fiber by the end of the 3rd postnatal week [2, 3]. Similar forms of activity-dependent refinement of synaptic connectivity, generally called synapse elimination, are widely seen in the developing

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nervous system [4, 5]. Since a genetic program only defines a rough connectivity between groups of pre- and postsynaptic neurons in the mammalian nervous system, activity-dependent processes such as synapse elimination are required to establish a finer, more precise connectivity to form functional synaptic circuitry.

Nascent climbing fiber-Purkinje cell synapses are formed on Purkinje cell soma, but the synapses gradually move from the soma to the dendrites of Purkinje cells after the 1st postnatal week [6, 7]. The timing of this dendritic translocation coincides with that of synapse elimination [3, 8], thus it is highly reasonable to ask what roles dendritic translocation plays in synapse elimination (Fig. 1). A prevailing idea until recently was that dendritic translocation and synapse elimination were independent of each other. This idea was based largely upon the experimental observation of climbing fiber synapse elimination in several spontaneous mutant mice that show abnormal cerebellar development. In spontaneous mutant strains *staggerer* and *hyperspiny Purkinje cell (hpc)*, dendritic translocation does not occur [8]. Climbing fiber synapse elimination is also impaired in *staggerer* mice [9, 10], but in *hpc* mice, the single climbing fiber innervation of Purkinje cell is successfully established [11]. Therefore, it appears that there is no correlation between dendritic translocation and synapse elimination.

This argument was made more than 20 years ago [8]. Comparing different cerebellar mutant mouse strains is a clever experimental approach and the conclusion seems reasonable. Although biological processes in mutant animals may significantly differ from those occurring under normal circumstances, the phenotype of *hpc* mice; synapse elimination completes without dendritic translocation, lets us think that synapse elimination and dendritic translocation are independent of each other. Until recently, there had not been any experimental evidence that directly challenged this idea.

But, in 2009, Hashimoto et al. showed that only one climbing fiber can undergo dendritic translocation and proposed a novel model of synapse elimination [7]. According to their model, the innervation fields of winning climbing fiber and its losing competitors are first segregated between dendrites and soma of Purkinje cell and then subsequent, non-selective removal of somatic synapses establishes single climbing fiber innervation. This model implies that dendritic translocation plays a crucial role in synapse elimination, which challenges the traditional view of dendritic translocation mentioned above. Furthermore, their finding also challenges a previous study showing that multiple climbing fibers can undergo dendritic translocation in the cerebellum of normal mice [12]. Therefore, almost 40 years after the initial discovery of climbing fiber synapse elimination, our understanding of seemingly very simple and fundamental aspects of this process became controversial. What is the role of dendritic translocation in synapse elimination, and how many climbing fibers can actually undergo dendritic translocation?

To solve these controversies, we employed *in vivo* time-lapse microscopy for the first time to study competitive synapse elimination in the mammalian brain [13]. *In vivo* time-lapse microscopy allows repetitive imaging of same cells for a period of days to weeks in intact animals, thus it is arguably the most direct and straightforward approach to study dynamic cellular changes. In fact, application of *in vivo* time-lapse microscopy has significantly

advanced our understanding of synapse elimination at the neuromuscular junctions. However, it had been difficult to apply this technique to the central nervous system.

We have successfully developed a new method for labeling two competing climbing fibers (i.e., innervating the same Purkinje cell) with different colored fluorophores and repeatedly imaged the same pair of competing fibers *in vivo* during the late stage of synapse elimination. We have found that: (i) climbing fiber terminals are stabilized on dendritic surface, (ii) only one climbing fiber can translocate to the dendrites whereas its competitors are restricted around the soma, (iii) the climbing fiber that begins translocation almost always becomes the winner, and (iv) selective photo-ablation of the winning (i.e., translocating) fiber allows its losing competitor to become a new winner [13]. These results confirm the study by Hashimoto et al. (2009) and indicate that, under normal circumstances, dendritic translocation is a key cellular event that determines the winner during synapse elimination. Although single climbing fiber innervation is established without dendritic translocation in *hpc* mice [8, 11], it is possible to assume that synapse elimination proceeds with significantly different mechanism in this mutant strain.

Recently, Kano and co-workers have identified several key molecules that mediate the removal of somatic climbing fiber synapses at the final stage of synapse elimination [14, 15]. These findings support the model that they previously proposed: after the only one climbing fiber has translocated to the dendrite, all remaining somatic synapses are non-selectively removed to complete synapse elimination. However, it is currently unknown why only one climbing fiber can translocate to the dendrites while its competitor cannot. One possibility is that all losing climbing fibers are sufficiently weakened both functionally and structurally before dendritic translocation begins [7]. Therefore these losing fibers have already lost their ability to translocate to the dendrites when their winning competitor begins translocation. An alternative possibility is that the first climbing fiber that begins translocation somehow actively prevents its competitors to translocate. Our selective photo-ablation experiment supports this possibility. However, it should be noted that photo-ablation of translocating fiber is a highly unphysiological event, and thus, our result does not conclusively mean that losing fibers retain their ability to translocate to the dendrites under normal circumstances. At present, it remains elusive whether or not Purkinje cells have a specific mechanism that allows a translocating fiber to confine its competitors around the perisomatic region. One of the important directions in the future study is to address this point and reveal how the lone dendritic translocation is achieved.

Acknowledgments

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Figure 1.

Three different models of how dendritic translocation proceeds during climbing fiber elimination. For the simplicity of the figure, only two climbing fibers (CFs) that compete for the same Purkinje cell (PC) are shown. In model 1, multiple CFs translocate to the dendrite. In model 2, only one CF translocates to the dendrites at a time, but the one that first translocates does not necessarily become the winner. Both of these models suggest that dendritic translocation is not a winner-specific event and multiple CFs remain compete during translocation. In model 3, only one CF translocates to the dendrites and the one that first translocates always becomes the winner. If this is the case, it is suggested that: (i) the competition is practically over before the onset of dendritic translocation and only the predetermined winner can translocate, or (ii) dendritic translocation determines the winner by providing irreversible competitive vigor to the CF that first translocates.