How to detect dying neurons in the central nervous system?

The central nervous system (CNS) changes dynamically throughout an animal life. In the fruit fly, *Drosophila melanogaster*, asymmetric mitosis of the neuroblasts during embryonic development produces about 10,000 differentiated neurons that form a juvenile (larva) CNS. These neurons are specified to perform larva-specific functions during larval growth period. However, during metamorphic development, they follow mainly two fates; remodeling and apoptosis. In the case of the former, a large number of larval neurons persist throughout pupal development, but they are structurally as well as functionally modified to redefine their roles in adult CNS. In contrast, some other larval neurons are not required for the adult CNS, thus are genetically programmed to die during metamorphosis. We coined a new term, *metamorphoptosis*, referring to the programmed cell death (PCD) associated with metamorphosis in animals. Interestingly, these dying neurons are highly heterogenous with respect to their timings of death, the mechanisms underlying death signaling, and the types of neuro-signaling molecules they carry.

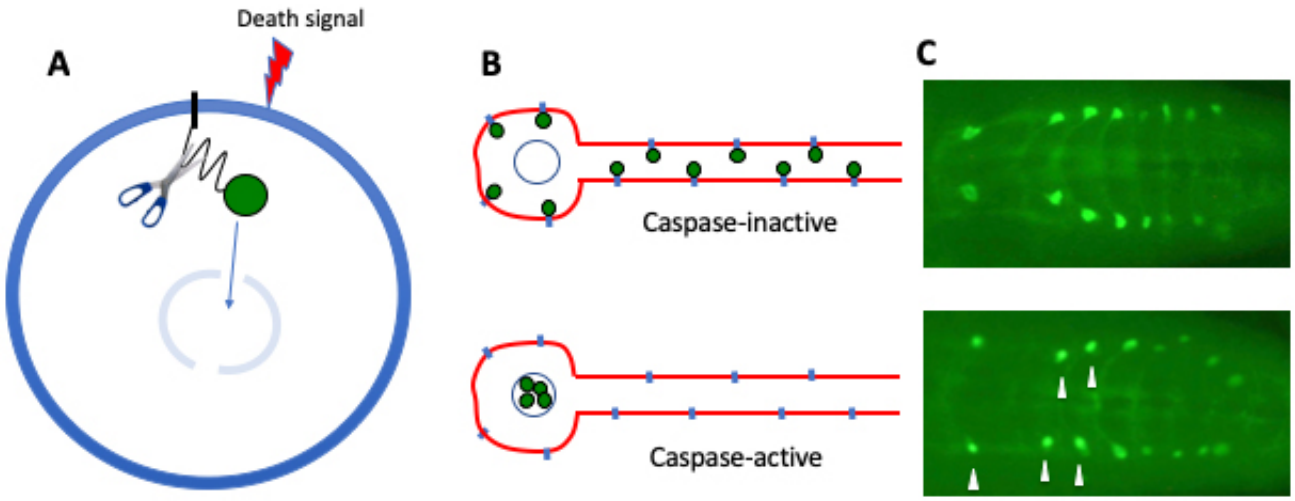

Fig. 1. (A) A schematic diagram of Casor. In response to death signals, activated caspases (scissors) cleave the membrane-bound substrates, resulting in the nuclear GFP.
(B, top) A neuron before caspase activation. The membrane-bound Casor is distributed to neuronal membrane. (B, bottom) A neuron after caspase activation. Because of the nuclear GFP, only nucleus contains GFP. (C) Actual images of before (top) and after (bottom) of caspase activation in neurons. Arrowheads indicate neurons with nuclear GFP signals.

So far, two major metamorphic stages of the neuronal metamorphoptosis are; one shortly after the onset of metamorphosis, and the other shortly after adult emergence. The neurons in the former case must have performed functions specific to larval stage, and then eliminated immediately after their roles are done. In the latter case, these neurons are likely to play important roles in pupal development and adult ecdysis. In addition to these two groups, there are other neurons that do not fall into these criteria, as they undergo cell death in the middle of pupal development. To further comprehend the significance of apoptosis of doomed neurons, it is important to understand the physiological and neurological roles played by these neurons. The molecular cell death mechanisms in Drosophila have been well understood. The key molecular executioners are enzymes called caspases that degrade various cellular substrates, including poly (ADP-ribose) polymerase (PARP). In live cells, caspase activities are suppressed by Drosophila inhibitor of apoptotic protein 1 (DIAP1). In response to death signals, transcriptional activation of cell death inducers, reaper, hid, grim, and sickle, antagonize DIAP1, leading to the degradation of DIAP1. As a consequence, caspases are activated and execute cells.

Despite such well-established cell death mechanisms, as mentioned earlier, initiation of apoptosis of doomed neurons in the CNS does not take place uniformly. Hence, it is difficult to predict when, where, what types of neurons are eliminated in the CNS during metamorphosis. To help this issue, we developed a green fluorescent protein (GFP)-based biosensor, named Casor, which was designed to detect neurons containing active caspases *in vivo*. The Casor is an engineered caspase substrate, and composed of mCD8 transmembrane domain fused with caspase cleavage site derived from PARP, and nuclear GFP. When Casor is expressed in cells, it is targeted to the neuronal cell membrane. However, when these neurons receive death signals, activated caspases cleave PARP, thereby releasing nuclear GFP which subsequently translocates to the nucleus. The nuclear GFP signals are readily detectable under fluorescence microscope in both fixed tissues and live animals. Such simple expression and detection system enabled us to identify caspase-active neurons in the metamorphosing CNS, as we demonstrated in two previously known groups of doomed neurons. Using this method, we were able to determine the exact developmental timing of the death of neurons and their neuronal identities in the CNS during metamorphosis.

Jae Park
University of Tennessee, USA

Publication

Identifying and monitoring neurons that undergo metamorphosis-regulated cell death

(metamorphoptosis) by a neuron-specific caspase sensor (Casor) in Drosophila melanogaster.
Lee G, Kim J, Kim Y, Yoo S, Park JH

Apoptosis. 2018 Jan