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PAG-3 MAY COUPLE CELL LINEAGE CUES TO TERMINAL DIFFERENTIATION THROUGH HETERODIMERIZATION WITH UNC-3 IN DEVELOPING VA AND VB MOTOR NEURONS

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During the development of multicellular animals the potentials of blast cells are progressively restricted until differentiated cell types are formed. During this process, cells decide between survival and programmed cell death. We are interested in understanding the mechanisms that control this decision and have chosen to focus on how the pattern of programmed cell deaths in the ventral cord is determined. In the midbody of wild-type animals the six Pn.aap cells survive and differentiate to form VC motor neurons, while the Pn.aap cells in the anterior and posterior die. From two genetic screens seeking mutants abnormal in the numbers of VC motor neurons or programmed cell deaths, we found that *unc-3* and *pag-3* mutants have both extra cell corpses and extra VC motor neurons. In *pag-3* mutants these abnormalities are a consequence of a defect in neuroblast fate determination wherein the Pn.aaa neuroblasts reiterate the fate of their mothers, the Pn.aa cells, and generate extra Pn.aap-like cells. The extra Pn.aap-like cells can become VC-like motor neurons or undergo programmed cell death. Mutations in *unc-3* do not affect P cell divisions but instead affect the fates established in differentiating cells generated by those lineages. Evidence derived from analyses of the phenotype of *unc-3 pag-3* double mutants, laser ablation studies, and expression patterns of cell type-specific markers in mutants suggests that loss of *unc-3* function may result in extra cell corpses and VC motor neurons by affecting VB motor neuron differentiation.

pag-3 and *unc-3* both encode transcription factors. In developing wild-type animals antiserum recognizing PAG-3 first detected PAG-3 in the Pn.aa neuroblasts, the anterior daughters of which are abnormal in *pag-3* mutants. Expression was not detected in the Pn.ap cells, which are also neuroblasts, indicating that PAG-3 expression is activated by cell lineage cues specific to the Pn.aa neuroblasts. PAG-3 was also present in all cells generated by Pn.aa, including the VA, VB and VC motor neurons, suggesting that PAG-3 may function during differentiation of these cells. An antiserum recognizing UNC-3 first detected protein in differentiating motor neurons. More specifically, UNC-3 and PAG-3 were coexpressed in the VA and VB but not in the VC motor neurons. We found that UNC-3 and PAG-3 can heterodimerize *in vitro*. We propose that PAG-3 expression is activated by Pn.aa-lineage-specific cues to determine neuroblast fate and that PAG-3 may then serve a second function in a complex with UNC-3 in the differentiating VA and VB motor neurons generated by Pn.aa. A *Drosophila* counterpart of PAG-3, Senseless, is required for the development of peripheral nervous system neurons. Expression of Senseless and the proneural proteins, which, like UNC-3, are HLH transcription factors, are interdependent. Vertebrate counterparts of UNC-3 are widely expressed in the developing nervous system and are coexpressed with vertebrate counterparts of PAG-3 in some tissues. We suggest that we have discovered a universal mechanism whereby PAG-3 counterparts determine blast cell fates in specific lineages and then act during terminal differentiation in cells derived from those blast cells in part through heterodimerization with HLH transcription factors.