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Expression of the Novel Basic Helix-Loop-Helix Gene dHAND in Neural Crest Derivatives and Extraembryonic Membranes during Mouse Development

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Expression of HAND genes in sympathetic adrenal lineage suggests that HAND genes may regulate Mash-1 independent neuronal genes[1-2]. HAND genes are also expressed in other cell types, *e.g.* Cardiac cells, trophoblasts, and decidua, suggesting that HAND genes are not cell fate determination factors. It is unclear how HAND genes function specifically in different types of cells[3]. Combinational actions of HANDs with other cell-lineage specific transcription factor may determine each cell fate and differentiation processes[4]. Identifying the transcription target genes of HANDs and Mash-1 will be important to elucidate the function of these bHLH factors in SNS factors in SNS development.

Molecular genetic techniques allow one to create mutant mice, in which a particular gene is manipulated. The gene of interest may be over-expressed by inserting multiple copies of transgene; or its expression may be eliminated be gene targeting; or the gene may be mutated so that a functionally altered protein is translated. To define dHAND gene function during embryogenesis, we will use gene target techniques to disrupt the gene in embryonic stem cell (ES) cells. Productions of chimeric mice and germline transmission of the mutated allele permit us to generate homozygous mutant mice lacking a functional gene. Characterization of dHAND expressed pattern and its hierarchy relationship with Mash-1 and dHAND in the mice. We will extend previous thin section *in situ* hybridization studies by conducting whole mount *in situ* hybridization in mouseembryos using dHAND probe. Whether dHAND and Mash-1 are expressed in the same neuron or distinct neuronal types are not clear. If the expression of dHAND precedes that of Mash-1, detect dHAND in the Mash-1 mutant mice and the result will suggest that dHAND is upstream of Mash-1.

The basic helix-loop-helix (bHLH) transcription factor dHAND is expressed in a number of

neural crest derived lineages including the sympathetic nervous system (SNS) during early development in mice. To determine the role of dHAND in SNS cells on sympathetic neurons, P19 wild type cells and constitutively expressing dHAND P19 cells (P19-dH) were aggregated with or without retinoic acid (RA). Unlike P19 cells, when P19-dH cells were differentiation by aggregation and replaced, they formed neurons which that expressed the pan-neuronal marker -tubulin III and neuro-filaments independent of treatment with RA. dHAND activates expression of Mash-1 independent of RA. Sympathetic specific marker eHAND and dHAND as well as other sympathetic neuron-expressed markers peripheral, trypsin hydroxylase, Phox 2a, Phox 2b were induced in P19 (dH) cells in the presence or absence of RA but not in RA-treated P19 wild type (WT) cells. These data suggest that dHAND functions as a determination gene for sympathetic neuronal fate and that dHAND can auto-activate and cross-activate a cascade of the bHLH transcription factors either directly or indirectly, which in turn activate downstream genes leading to differentiation of sympathetic neurons.