Supplemental Results

Reassessment of HLH-12 assignment to the Achaete-Scute family: During the course of these studies, we noted that there are unresolved issues regarding the assignment of HLH-12 to the Achaete-Scute family of bHLH transcription factors. This assignment was based on low bootstrap support (LEDENT and VERVOORT 2001). We performed two simple BLASTP searches using either the entire HLH-12 protein sequence or its bHLH domain alone against the NCBI non-redundant protein sequence database. We then repeated each search (entire protein and bHLH domain alone) individually against the genomes of Homo sapiens, Mus musculus, Rattus norvegicus, Gallus gallus, Xenopus laevis, Danio rerio, Drosophila melanogaster. In all searches we found that the highest-scoring hits (at least the top 10 best hits) do not belong to the Achaete-Scute family of bHLHs but rather to the Twist/Atonal/Paraxis families. More importantly, we noticed that the basic domain of HLH-12 consists of the conserved motif "-R--AN-RER-R" (see Figure below), which is characteristic of the Twist/Atonal/Paraxis/MyoR/Ngn/NeuroD bHLH transcription factors, while the Acheate-Scute bHLHs contain the conserved basic motif "-----RN-RER-R" (see Figure below). The basic motif of bHLH proteins is critical for E-box binding specificity and substitution studies have revealed that changes in its composition lead to changes in the DNA binding/function of the bHLH proteins (DAVIS et al. 1990; DAVIS and WEINTRAUB 1992; KOPHENGA NAVONG et al. 2000). Further studies are required to determine if HLH-12 can indeed be considered a member of the Achaete-Scute family of bHLH transcription factors.

![Alignment of the basic regions of HLH-12 and homologous proteins from other species](image)

Figure legend: Alignment of the basic regions of: HLH-12 and homologous proteins from other species (best BLASTP hits in: Drosophila melanogaster (tap)/ NP_524124/E value=5e-06/57% of protein, Xenopus laevis (Scleraxis)/NP_001092152/E value=2e-06/45% of protein, Danio rerio
(Paraxis)/NP_571047/E value=2e-05/54% of protein, Homo sapiens
(TCF15/Paraxis)/NP_004600/E value=1e-05/42% of protein), related class II bHLH proteins in C.
elegans (HLH-8/ NP_509367.1, LIN-32/NP_508410.2, CND-1/NP_498115.1, NGN-1/
NP_500236.1, HLH-10/AAA82418, HLH-1/NP_001021893.1), members of Achaete-Scute (A-S)
family bHLH TFs (Hydra vulgaris CnASH/AAA93012, C. elegans HLH-14/ NP_495131.2,
Drosophila melanogaster Achaete/NP_476824.1, Xenopus laevis ASH3b/ NP_001079125.1, Mus
musculus Mash1/NP_032579.2,), class I bHLH protein HLH-2 (Ce E/Da)/ NP_001021581.1, and
the HLH-12(R15K) and HLH-12(R25K) mutants generated in this study. Red letters indicate
highly conserved amino acid residues in classes I and II bHLH transcription factors (TFs), blue
indicates residues conserved between the Twist/Atonal/Paraxis/MyoR/Ngn/NeuroD families of
bHLH TFs, violet indicates conserved amino acids between A-S family bHLH TFs, and green
indicates mutated amino acids (generated mutant alleles in this study). Gray digits indicate the
number of the first and last amino acid of the basic domain in each protein.

HLH-19 has similarly been put into the Achaete-Scute family though we found that
everything mentioned above for HLH-12 also applies to HLH-19 (BLASTP search results and
amino acid composition of the basic region).

LEDENT, V., and M. VEROORT, 2001 The basic helix-loop-helix protein family: comparative
DAVIS, R. L., P. F. CHENG, A. B. LASSAR and H. WEINTRAUB, 1990 The MyoD DNA binding domain
DAVIS, R. L., and H. WEINTRAUB, 1992 Acquisition of myogenic specificity by replacement of three
KOPHENGNAVONG, T., J. E. MICHNOWICZ and T. K. BLACKWELL, 2000 Establishment of distinct
MyoD, E2A, and twist DNA binding specificities by different basic region-DNA