Hypothesis

Do longevity assurance genes containing Hox domains regulate cell development via ceramide synthesis?

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Abstract A gene family containing a longevity assurance gene (Lag1p) motif is described. Database searches revealed >40 members of this family of transmembrane proteins, two of which have recently been shown to regulate the synthesis of ceramide, a lipid second messenger involved in a variety of cellular processes. We speculate that other family members, some of which contain a Hox domain, may also be involved in the synthesis of specific ceramide pools, perhaps explaining the role of longevity assurance genes in regulating development.

Key words: Ceramide; Apoptosis; Signaling; Longevity assurance gene; Hox domain; Transcription

The past decade has seen an explosion of interest in sphingolipid biology due in part to the identification of ceramide as a key signaling molecule in cell growth, differentiation and apoptosis [1–4]. Ceramide, which consists of a long chain base (i.e. sphingosine, phytosphingosine, or sphinganine) to which a fatty acid is attached via an amide bond, is generated either via sphingomyelin hydrolysis, or de novo via ceramide synthase (sphinganine *N*-acyltransferase, EC 2.3.1.24) [5], and both of these pathways are regulated in cell signaling. Recent studies have demonstrated that de novo ceramide synthesis is regulated by members of the longevity assurance gene (*LAG1*) family [6–8]. We have now analyzed the NCBI database, revealing ~40 homologs of *LAG1*, and surprisingly in higher organisms some contain a homeobox (Hox) domain, a transcription factor involved in developmental regulation.

The *LAG1* gene was first discovered in yeast where it was shown to regulate life span [9]. It is conserved across species, and the biochemical defect caused by *LAG1* deletion in yeast could be complemented by the human homolog, *uog1*. A Lag1p motif was originally described as a region of 52 amino acids with >50% sequence similarity [10]. PSI blast analysis [11] using the Lag1p motif (Fig. 1) from yeast (gi_6321784, residues 246–297) as a query at NCBI yielded 48 hits, with 15 displaying an e-value ≤ 0.005. An additional 34 sequences with significant e-values were obtained upon second iteration and no new sequences were obtained upon subsequent iterations. Web-based SMART analysis [12] (http://smart.embl-heidelberg.de) demonstrated that all family members contained four to seven transmembrane domains. Surprisingly, the Hox domain was found in one distinct sub-class (Fig. 1; e-value < 0.05) in insects and vertebrates, but not in yeast, worms and plants. CLUSTAL X multiple sequence alignment analysis [13] demonstrated that the domain architecture is distinct for each species (Fig. 1). In some cases the Hox domain was located immediately prior to the first putative transmembrane domain, whereas in others it was located between the first and second transmembrane domains.

To date, only *uog1* and *LAG1*/*LAC1* have been analyzed with respect to their roles in de novo ceramide synthesis, and neither of these contain a Hox domain. Remarkably, the yeast genes *LAG1*/*LAC1* regulate the synthesis of ceramide containing C26-fatty acids [6,7], whereas the murine homolog, *uog1*, specifically regulates the synthesis of ceramide containing C18-fatty acids [8]. We speculate that other family members may also be involved in regulating ceramide synthesis, although comparison of the number of homologs in different species does not suggest a simple relationship between the number of homologs and the number of ceramide species. One possibility is that the lag1p motif may be involved in substrate binding and the invariant residues could participate in the catalytic reaction. Does the presence of a Hox domain suggest that the Lag1p motif acts as a ceramide sensor in some of the homologs in an analogous fashion to the sterol-sensing ability of the sterol regulatory element binding protein (SREBP) [14], in which a transcriptional activation domain is released by proteolysis when sterol levels are low? If so, the Hox domain may be involved in transcriptional regulation directly linked to ceramide levels or to rates of ceramide synthesis. As mentioned earlier, *LAG1* was first discovered as a gene involved in longevity regulation, and ceramide is a key player in cellular senescence. Experimental analysis of the role of *LAG1* family members and of the Lag1p motif in the regulation of ceramide synthesis will determine whether this novel gene family joins the growing list of genes involved in developmental regulation.

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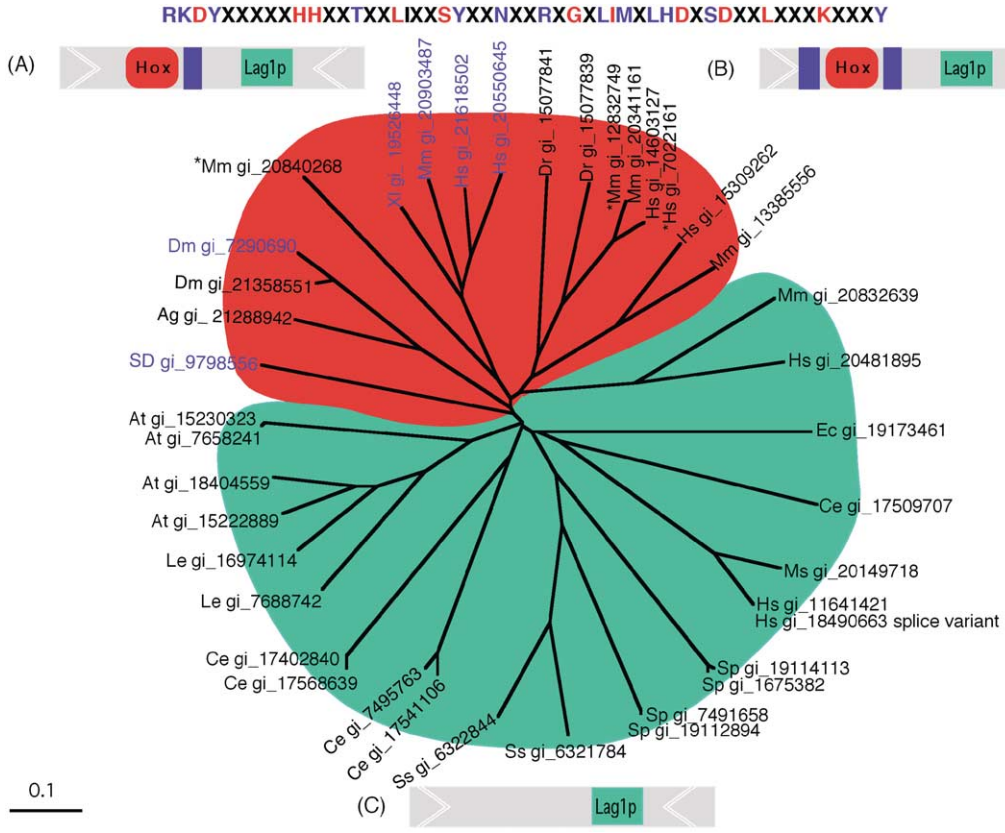

Fig. 1. Phylogenetic tree of Lag1p family members. Homologs based on PSI blast searches of the Lag1p motif (third iteration) were aligned by CLUSTAL X, a non-rooted phylogenetic tree constructed and confirmed by bootstrapping. The Lag1p motif is given at the top of the figure, with the most conserved amino acid residues in red, partially conserved residues in blue, and non-conserved residues in black. Two distinct families can be seen in the tree, indicated by red and green blocks that do or do not contain the Hox domain, respectively; however, three sequences that cluster to the Hox domain-containing homologs do not contain a Hox domain (indicated by asterisks). The Hox domain-containing Lag1p homologs are divided into two sub-families, in which (A) the Hox domain is located before the first putative transmembrane domain (Genebank identifiers shown in blue), and (B) in which the Hox domain is located between the first and second transmembrane domains; not all features are shown, as each homolog has a different length and has a different number of putative transmembrane domains. Lag1 homologs that do not contain a Hox domain are shown in the green area of the tree. Species names and Genebank identifiers are given. Key: Ag, *Anopheles gambiae*; At, *Arabidopsis thaliana*; Ce, *Caenorhabditis elegans*; Dm, *Drosophila melanogaster;* Dr, *Danio rerio*; Ec, *Encephalitozoon cuniculi*; Hs, *Homo sapiens*; Le, *Lycopersicon esculentum*; Mm, *Mus musculus*; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Sd, *Subretis domuncula*; Xl, *Xenopus laevis*.

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