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A PROTEOGLYCAN BIOSYNTHETIC PATHWAY INVOLVED IN *C. elegans* EMBRYOGENESIS AND VULVAL MORPHOGENESIS AND IN HUMAN AGING DISORDERS

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Mutations in eight *sqv* (squashed vulva) genes can result in several developmental abnormalities, including defective vulval invagination and maternal-effect lethality. We report the cloning of two *sqv* genes, *sqv-2* and *sqv-6*, and discuss their roles in a molecular genetic pathway defined by the six previously cloned *sqv* genes. We also report the characterization of *sqv-5* and *sqv-7* expression. The molecular identities of the eight *sqv* genes suggest that the molecular basis for the *sqv* defects lies in the disruption of the biosynthesis of glycosaminoglycans (GAGs) of the structure (Serine residue in the protein core)-Xylose-Galactose-Galactose-Glucuronic acid-(X-Glucuronic acid)n, where X is either N-acetylgalactosamine or N-acetylglucosamine.

The biosynthesis of GAGs requires the synthesis of nucleotide sugars in the cytoplasm and the translocation of nucleotide sugars into the endoplasmic reticulum (ER) and/or Golgi, where polymerization of sugars is catalyzed by glycosyltransferases. SQV-4 is a UDP-glucose dehydrogenase, a key enzyme in UDP-Glucuronic acid synthesis. SQV-7 is an atypical multi-pass transmembrane protein that transports UDP-Glucuronic acid, UDP-N-acetylgalactosamine and UDP-Galactose from the cytoplasm into the ER and/or Golgi. Mammalian homologs of *sqv-3*, *sqv-6* and *sqv-8* encode glycosyltransferases necessary for the biosynthesis of the GAG-protein linkage region of proteoglycans: SQV-6, SQV-3 and SQV-8 are similar to Xylosyltransferase, Galactosyltransferase I, and Glucuronyltransferase I, respectively. SQV-1 is a cytoplasmic protein with weak similarities to nucleotide-sugar modifying enzymes, and SQV-2 and SQV-5 are both type II transmembrane proteins and candidate glycosyltransferases. We postulate that *sqv-1*, *sqv-2* and *sqv-5* are components of the same GAG biosynthesis pathway and that the GAGs are important for cell-cell or cell-matrix interactions in embryonic and vulval development.

Preliminary antibody staining using rabbit polyclonal antibodies against SQV-4, SQV-5 and SQV-7 shows localization of these proteins consistent with their deduced functions. SQV-4 appears to localize broadly to the cytoplasm of many tissues, including the vulva, consistent with its function in the biosynthesis of UDP-glucuronic acid in the cytoplasm. SQV-5 shows punctate cytoplasmic localization in the vulva and germline, consistent with our model that SQV-5 functions as a glycosyltransferase in the Golgi. SQV-7 also shows punctate cytoplasmic localization in the hypodermal seam and gonadal distal tip cells, consistent with its function in transporting nucleotide sugars into the Golgi. We plan to characterize the subcellular localization of SQV-5 and SQV-7 further using immuno-electron microscopy.

Mutations in the human homolog of *sqv-3* are implicated as the cause of a progeroid variant of the connective-tissue disorder Ehlers-Danlos syndrome. All eight *sqv* genes have close human counterparts, suggesting that a common pathway for modifying important cell surface and/or extracellular GAGs is present in humans and in *C. elegans*. Defects in the human counterparts of other *sqv* genes therefore may be responsible for aging disorders and connective tissue diseases.