

**BIOSYNTHESIS OF TRUNCATED N-LINKED OLIGOSACCHARIDES RESULTS
FROM NON-ORTHOLOGOUS HEXOSAMINIDASE-MEDIATED MECHANISMS
IN NEMATODES, PLANTS AND INSECTS**

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Supplementary Data

Two figures (Supplementary Figures 1 and 2) are included in this supplement.

Supplementary Figure 1: Alignment of four novel hexosaminidases from *Caenorhabditis elegans*. The putative protein sequences of HEX-2, HEX-3, HEX-4 and HEX-5 are presented with residues identical between two or more sequence highlighted in grey. The HIGADEA sequences are in bold (see also Figure 1); the putative transmembrane sequences are in italics, potential N-glycosylation sites are double-underlined and residue numbers are given at the end of each line.

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HEX-2  MFPMRCIRRRSIDFVLKGVILTTICLFLFHMTSSYPKGGISQRALDSMQKEPQTPVLVQK 60
HEX-3  MLR-GFFGRRRSAWVRLIYLCIVTIIIFLFATSQFKSTTTTHARFVPEPSDPRLHPNSPLQ 59

HEX-2  RENFEESIDVAVEQESKKENPFVNQOASTENAKPITQEVKIERPSRDNEFYKNVVIHFDL 120
HEX-3  APKAQEPAAAETTKKPSRQPHHTTAKPRTEN-VEINGEKYPKLSPDGNLIPQRRIVHLDL 118
HEX-4  MHKMSKLCFLALLSVTFMLIFVLTTPYSNDRSSYAAAYEGGIDPRKTRQFKNIIVHLDL 58
HEX-5  MCNIFQIVVWPKMLLRRTICILACIVQFATCGYQRSIVHFDL 41

HEX-2  KGAPPKVDYFLDLLRLIAKGGATGILLEWEDMFPWTGKLEQFKNTDAYSESDVDMILSEA 180
HEX-3  KGAPYKPEFFTELFFAFFNRIQATGILLEWEDMFPFKGRIRGAINKNAYSMTVEHILQEA 178
HEX-4  KGAPPRVEYLIEFFKLLSKHVDGILIEYEDMFPYSGDIEEIRRDLYSENDIRRIIQAA 118
HEX-5  KGAPPKVAYFKQLLTTISGLGATGVLEWEDMFPYQGGLSRVVKNAYTEEEVISVLEHA 101

HEX-2  TKLKLVDIPLVQTFGHLEWILKYEEMRKYRENDAYPQVLCLGNEEGVEFVREMIRQVAKK 240
HEX-3  HQKHLQIIPLVQTMGHLEWILKLEEFAHLREDTRFPQVICFSDENAWELIKEMIEEVANV 238
HEX-4  EVHNLEVIPLIQSFGHLEFVLKSKFMGLSEDLIDLNTICISDSKSIDIVKQMIEQIRRL 178
HEX-5  QQLQLEVIPLVQTLAHMEWILKTEEYSVLREDERYPMVACIGNPESLDIILDSVNQLMRI 161

HEX-2  HAKYGIPFFHIGADEAFEFVGCQESLDWIKKNGKNGRKQLLALAHLKAIAEFAKQQTGDS 300
HEX-3  HKKYGMSYFHIGADEAFQIGICNAS-ITQIKKEFTRERLMLW-HIARTAR-FVKEKYPET 295
HEX-4  HP--NSTRIHIGADEAYHVAEDQRCIERM-EKESIGKSDLKLEHIAKIGK-FARENAGFE 234
HEX-5  HSNFNTGYVHIGADEAFQVGCDADREILPVKYDNNKLRMIFDHLRMVSL-NITEEYPST 220

HEX-2  TQILAWHDMLKDFDSRLIKNLELGQIIQPVVWDYSENIITLND--YIFSA-LAENFPTMW 357
HEX-3  -QVLAWHDMLASAMESDIEDYKLTELLQPVLWNYAEDLDIYLP--RSTWMLR-NFRNVW 351
HEX-4  -TVFANDMDFKESEETIRKSKINKFIVPVVWGYRTDVTENGYFPDGLFERIFNVDFRFY 293
HEX-5  -KVLMWYDELKSAPLELIKEYNLDNLVIPPVWVYTANLNDLP--SEMWKNMSYSFKEVW 277

HEX-2  ASSAYKGANYPASASTSEVRHYETNNRNWIRTKQQERKFKNGFQGIIVTGWQRYDHLAGL 417
HEX-3  GSSAWKGADGPARYSTNANHLYLKNHESWIKQFTMVY-KDFEVVEGLIMAGWSRYDHFAVL 410
HEX-4  VASAFKGADGARQQFSNISRYLENQKSYVNLMDLHKNAAQKVDGIFVTGWSRFNHFNAL 353
HEX-5  GGSAFKGADGASRYWNRKPYILNNKEWYLQNEKYK-PQFTTFDSIIITGWQRYDHFFASL 336

HEX-2  CETLPIGTASMMLQMQIALNAPALDLEGTRQKAATLLECQ----GFNVDGVKVVSNQCK 472
HEX-3  AETIPVALPTLAMSMETMIE--GRPLAGNYPVTSELLQCTPPLDLGFTAT-----GCK 461
HEX-4  CELLPVAIPSLIVDLFYLNY--QLTEKDAWRAMKSSLECE--NRRHLRGILAESTVHGCK 409
HEX-5  CELWPTSMVSLALNLIVLTK--FHIDKESAEQVIQALNCP--QTTLDQLVAGS--DRCR 390

HEX-2  YRGFQTYLIIYQSEVPNLFARIDSELSKNHLLMGWANRYNRKYNISQNWYHREMLPFVQQL 532
HEX-3  FPGNRIYEL-INEMYQKQMLRTYRLDDYELNGWLSRVADDYSVSSHWYIDKIENMIEMH 520
HEX-4  FPGADVFEI-----IMHDWKRAVDRRIFGKPDQQSPDSEAIET 448
HEX-5  FPGYRVRDS-IRDYVQLKTFFENSTWVHNRENGWLQSSHMRISANPYIDAIGKAYERT 449

HEX-2  VGQYDDYESDLRASMKDLYFENTIDEFIYEN---LGEMSEKLHGYLEETIQLDKLRAWPK 589
HEX-3  ATPLETVADDLRFEMERIEFKDTVDEFIFTY---LGEDLEWFNRKRETIRKVSTSQTFPK 577
HEX-4  LKNLKKSLNQSILYKTDAEVFNQYLHRVHSLIAQNTRTEITN 491
HEX-5  LKKLDQLSNLSLTSFSEVFYPDVIEEFKTDYFQPFYEDLQKR--KESVDNIDTKRFYVPR 508

HEX-2  RHFPIKK 596
HEX-3  RPFIESAKSEKTCT 591
HEX-5  PWFRR 512

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Supplementary Figure 2: Incubation of an asialoagalactoglycopeptide (dabsyl-GnGn) with recombinant *Caenorhabditis* hexosaminidases. Culture supernatants of yeast transformed with *hex-1*, *hex-2*, *hex-3*, *hex-4* or *hex-5* partial cDNAs were incubated overnight with dabsyl-GnGn (an asialoagalactofibrin glycopeptide) prior to analysis of the incubations by MALDI-TOF MS. A reduction in m/z from 2062 to 1859 is compatible with the removal of one non-reducing terminal GlcNAc residue from GnGn; laser-induced degradation of the dabsyl moiety results in a loss of m/z 132 (species indicated by an asterisk). In comparison to the HPLC data with GnGn-PA as a substrate (Figure 5), these spectra validate that only HEX-2 and HEX-3 have the obvious ability to remove one GlcNAc residue from the α 1,3-antenna. Glycan structures are depicted according to the nomenclature of the Consortium for Functional Glycomics

