Acknowledgements
We thank Rafi Kopan and Meera Saxena for providing mNotch1 plasmids. AGP is an HHMI predoctoral fellow. JK is an investigator of the Howard Hughes Medical Institute.

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A ubiquitous family of putative gap junction molecules

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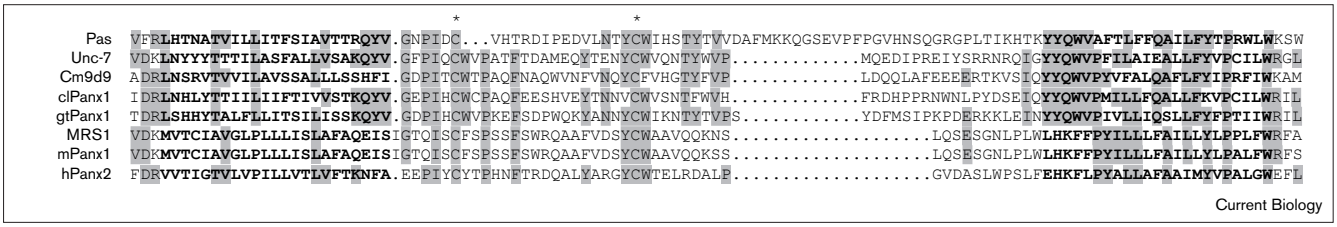
Gap junctions are one of the most common forms of intercellular communication. They are composed of membrane proteins that form a channel permeable for ions and small molecules connecting the cytoplasm of adjacent cells. Although gap junctions provide similar functions in all multicellular organisms, vertebrates and invertebrates are believed to use unrelated proteins for this purpose [1–3]. The family of gap junction molecules called connexins is well-characterized in vertebrates, but no homologs of these proteins have been found in invertebrates [1–5]. In turn, only gap junction molecules with no sequence homology to connexins have been identified so far in insects and nematodes [3–7]. It was suggested that these are specific invertebrate gap junction proteins, and they were thus named innexins (invertebrate analog of connexins) [3]. Here, we demonstrate the presence of innexin homologs in different taxonomic groups, including vertebrates.

Using PCR with degenerate primers, we cloned sequences homologous to innexins from mollusc central nervous system and flatworm whole-animal cDNA (Figure 1). This finding is important because it refutes the hypothesis that innexin proteins could represent a specific feature of recently postulated Ecdysozoa clade ('moulting animals', including among others arthropods and nematodes but not molluscs and flatworms) [3,8]. Moreover, a database search using BLAST [9] for homology matches to the new mollusc and flatworm sequences revealed similarity to two human proteins: MRS1, function unknown, predicted from cDNA sequence submitted by G.B. Bolger and M.R. Steele (GenBank accession number AF093239) and a novel protein similar to MRS1 recently predicted from chromosome 22 DNA sequence (hPanx2 in Figure 1, accession number AL022328). A PSI-BLAST search [9] unambiguously detected the same two proteins even when seeded by one of the original innexin sequences, the Unc-7 gap junction protein from *Caenorhabditis elegans*: with an *E*-value inclusion threshold of 0.01, the two human homologs were detected with expectation (*E*) values of 10^{-5} at the first iteration. In reciprocal searches initiated by the human homologs, the *C. elegans* innexins were detected with *E* values of 10^{-9} in the second iteration.

It can be argued that the presence of four (compositionally biased) transmembrane domains is a possible source of error while searching for homologous sequences. Theoretically, seeding BLAST searches with transmembrane region containing sequences may result in retrieval of similar membrane proteins that are, nevertheless, not homologous. However, in the case of innexins and related vertebrate sequences, because of the presence of a relatively well-conserved region containing two conserved cysteine residues just carboxy-terminal to the first transmembrane sequence, there is sufficient similarity outside the transmembrane regions to indicate homology: a PSI-BLAST search seeded by the hPanx2 sequence of the first putative extracellular loop flanked by only four amino acids from adjacent transmembrane regions with the *E*-value inclusion threshold of 0.05 revealed similarity to Unc-7 with *E* values of 10^{-12} in the second iteration.

Several sequences homologous to innexins were also detected among

Figure 1



The region of the highest similarity shared by predicted amino acid sequences of all putative gap junction pannexin family proteins include first two transmembrane domains and the stretch between them. An alignment of the insect (Pas, L13306) and nematode (unc-7, Z19122 and cm9d9, U59213) innexins (see [6]) is supplemented with protein sequences from the mollusc *Clione limacina* (clPanx1, AF207818), the flatworm *Girardia tigrina* (gtPanx1, AF207819), mouse (mPanx1, AF207817) and two from human (MRS1, AF093239; and hPanx2, AL022328). Predicted transmembrane domains are in bold. Invariant cysteines are indicated with an asterisk. Residues conserved in four or more family members are shaded.

human, mice and chicken expressed sequence tags (ESTs). Using this information, we cloned murine cDNA encoding a hypothetical 'innexin' molecule from a fetal brain sample. Proteins predicted from vertebrate sequences display statistically significant similarity to invertebrate innexins, including conservation of invariant cysteines and the locations of the four putative transmembrane domains [3–6]. Thus, the list of animal phyla with identified innexin family members extends to Platyhelminthes, Nematoda, Arthropoda, Mollusca and even Chordata, which makes the 'innexin' name inappropriate. Given the apparent ubiquitous distribution of this protein family in the animal kingdom we suggest that they should be called pannexins (from the Latin pan — all, throughout and nexus — connection, bond).

The identification of this new type of human putative gap junction proteins may be significant for medicine. A sequenced tagged site (STS, GenBank accession number G43027, alias stSG3927) [10] identical to the fragment of the human pannexin MRS1 places this gene nearby the centromeric border of the q21 band on chromosome 11, between genes encoding melatonin receptor 1B (locus MTNR1B) and vitamin D3 receptor-interacting protein DRIP80 (locus WI-15663). This region has been suggested to be the site of gene(s) predisposing to several major mental disorders including schizophrenia and a rare form of Charcot–Marie–Tooth disease (CMT4B) [11,12]. It is interesting to note that the more common X-linked form of the latter disease (CMTX) is caused by deficiency in a gap junction protein of the connexin family (Cx32) [1,2], suggesting that the pannexin MRS1 could be a good candidate for CMT4B.

Supplementary material

Supplementary methodological material is available at http://current-biology.com/supmat/supmatin.htm.

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