Annotated Bibliography
1998-2000

1998

Duke-Cohan JS, Gu J, McLaughlin DF, Xu Y, Freeman GJ, Schlossman SF. Attractin (DPPT-L), a member of the CUB family of cell adhesion and guidance proteins, is secreted by activated human T lymphocytes and modulates immune cell interactions. *Proc Natl Acad Sci U S A* (1998) 95: 11336-41.

Publication of the cDNA sequence for the large soluble serum protein, gp175/DPPT-L. Appear to be two mRNA species (~9kb and 4.5 kb) that are differentially expressed in hematopoietic tissues. Both the purified protein and recombinant protein mediate clustering together of T cells and monocytes. Localizes to intracellular vesicles that are released at the membrane - proposed that the transient membrane expression is the result of the exocytosis process. The DPPIV activity, however, of the highly purified recombinant protein is significantly less than that of the natural form. The protein is renamed attractin to represent it's clustering properties and to differentiate it from CD26/DPPIV. It is also postulated that the potentiation of clustering of T cells and monocytes may account for the enhanced responses to recall antigens described above, rather than attractin acting as a component of a signal-transducing pathway.

Nagase T, Ishikawa K, Miyajima N, Tanaka A, Kotani H, Nomura N, Ohara O. Prediction of the coding sequences of unidentified human genes. IX. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro. *DNA Res* (1998) 5: 31-9.

Identification of a long cDNA from brain (KIAA0548) that will subsequently turn out to code for the membrane proximal extracellular domain, transmembrane and cytoplasmic domains of a membrane form of human attractin. Simultaneous identification of a separate cDNA (KIAA0534) that codes for a sequence that looks similar to KIAA0548. Radiation hybrid mapping suggests KIAA0548 maps to human chromosome 20 and KIAA0534 to chromosome 10.

Dinulescu DM, Fan W, Boston BA, McCall K, Lamoreux ML, Moore KJ, Montagno J, Cone RD. Mahogany (mg) stimulates feeding and increases basal metabolic rate independent of its suppression of agouti. Proc Natl Acad Sci U S A (1998) 95: 12707-12.

Until this paper, the prevalent notion was that the suppression in mg/mg mice of the agouti antagonism of alpha-MSH signaling through the Mc1R with effects upon pigmentation may also extend to the hyperphagic/ obesity-inducing effects of ectopically-expressed agouti upon the Mc4R in the brain. This report demonstrates that mg could increase basal metabolic rate and hyperphagia and block obesity independent of suppression of ectopic agouti.

1999

Nagle DL, McGrail SH, Vitale J, Woolf EA, Dussault BJ Jr, DiRocco L, Holmgren L, Montagno J, Bork P, Huszar D, Fairchild-Huntress V, Ge P, Keilty J, Ebeling C, Baldini L, Gilchrist J, Burn P, Carlson GA, Moore KJ. The mahogany protein is a receptor involved in suppression of obesity. *Nature* (1999) 398:148-52.

Gunn TM, Miller KA, He L, Hyman RW, Davis RW, Azarani A, Schlossman SF, Duke-Cohan JS, Barsh GS. The mouse mahogany locus encodes a transmembrane form of human attractin. *Nature* (1999) 398: 152-6.

Simultaneous publication in Nature of back-to-back articles on the positional cloning of mouse mahogany. In the first article, the mahogany mutation is shown to suppress dietary-induced obesity independent of Mc4R. Sequence comparison of the contig and mouse loci upstream and downstream of the mg locus map mg to mouse chromosome 2 syntenic with human 20p13. Thirty coding exons generating at least one alternative splice form are proposed. The deposited 3' end downstream of the putative conserved signaling motif (-MASRPFA-) is incorrect due to a frame shift but otherwise predicts a transmembrane protein with a large extracellular domain replete with motifs associated with cellular adhesion/communication. Evidence is cited as a footnote for two mouse secreted forms and sequencing reveals that the mg3j mutation results from a 5 bp deletion that introduces a stop codon in exon 15 after the region coding for the C-type lectin-like domain. Propose that attractin/mahogany may aid in presentation of antagonists to the melanocortin receptors or else sequesters ligand away from cognate receptor. In the second article, a similar positional cloning approach is taken and the mutations resulting in the mg and mg^{L} alleles are shown to be intronic insertions between exons coding for the cytoplasmic domain. For these latter two alleles, large mRNA species can be identified (in contrast to mg3j where mRNA is essentially absent) suggesting that a transmembrane molecule without cytoplasmic signaling function might be produced. As might be predicted by the functional association with melanocortin receptors and agouti-type molecules, strong mRNA signals are generated from brain (hypothalamus, in particular) and melanocytes. A sequence comparison with soluble human attractin clearly shows that attractin is the soluble ortholog of the mouse transmembrane mahogany gene product. Probing of human mRNA with a mouse cytoplasmic tail probe implies that the upper 9kb band probably encodes a human transmembrane form of attractin.

Jackson IJ. The mahogany mouse mutation: further links between pigmentation, obesity and the immune system. *Trends Genet* (1999) 15: 29-31.

In a succinct review, Jackson formally ties the knot connecting attractin to pigmentation, regulation of energy metabolism and immunity drawing on the functional regulation of alpha-MSH-melanocortin receptor interactions in mice to propose that attractin may be performing a similar function in the immune system. This is certainly not unfounded: pioneering work by Lipton and Catania demonstrate that alpha-MSH administered directly into the Central Nervous System or locally is a powerful immunosuppressant, probably working through induction of the anti-inflammatory IL-12 interleukin.

Lu XY, Gunn TM, Shieh KR, Barsh GS, Akil H, Watson SJ. Distribution of Mahogany/Attractin mRNA in the rat central nervous system. *FEBS Lett* (1999) 462: 101-7.

Hybridization *in situ* reveals that attractin mRNA is widely expressed throughout the brain but is not uniformly distributed, implying it is not simply a "housekeeping" gene. There is particularly strong expression in the hypothalamus, where attractin was specifically localized in discrete nucleii implying a regulatory role in behavioral and metabolic responses.

2000

Tang W, Gunn TM, McLaughlin DF, Barsh GS, Schlossman SF, Duke-Cohan JS. Secreted and membrane attractin result from alternative splicing of the human *ATRN* gene. *Proc Natl Acad Sci U S A* (2000) 97: 6025-30.

Confirmation from assembled contigs and sequencing that, as a consequence of alternate splicing, the human attractin locus can code both for the soluble attractin and for a transmembrane form almost identical with that of mouse. In fact, the transmembrane domain and cytoplasmic tail of mouse and human (represented by KIAA0548 mentioned above - 1998) are identical - reinforcing the notion of a conserved intracellular interaction - either downstream signaling, cytoskeletal interaction, or vesicle docking. The BAC clones are mapped to 20p13 confirming the assignation above. The production of the soluble form is the result of insertion of a characteristically primate LINE-1 retrotransposon element after exon 24 introducing a new exon 25 encoding a short peptide (confirmed by tryptic peptide sequencing in the 1998 PNAS paper above), a stop codon, short 3' UTR and polyadenylation site. To date there has not yet been confirmation of a circulating soluble form of attractin in any order other than primates, although development of species-specific antibodies may reveal such forms and, if found, they are unlikely to have arisen from the identical genomic event that has occurred in primates.

Dinulescu DM, Cone RD. Agouti and agouti-related protein: analogies and contrasts. *J Biol Chem* (2000) 275: 6695-8.

Several models are proposed for regulation of agouti/agouti-related peptide/a-MSH interactions with melanocortin receptors. In addition to those mentioned above, receptor desensitization through interaction with attractin is raised as a possibility. A more intriguing proposal, based on the similarity of the mahogany and mahoganoid (md) phenotypes (the latter does not map to the attractin locus), is that attractin/mahogany and mahoganoid form an extracellular interaction to maintain cell contact (synapse?).

Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, Ribel U, Watanabe T, Drucker DJ, Wagtmann N. Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci U S A* (2000) 97: 6874-9.

The underlying reason as to why pharmaceutical companies were taking an interest in circulating CD26 – inhibition of its DPPIV activity may lead to therapies enhancing the bioactivity of glucagon-like peptides that potentiate/prolong the extracellular half-life of insulin.

Durinx C, Lambeir AM, Bosmans E, Falmagne JB, Berghmans R, Haemers A, Scharpe S, De Meester I. Molecular characterization of dipeptidyl peptidase activity in serum: soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. *Eur J Biochem* (2000) 267: 5608-13.

Although modulation of neuropeptide function by digestion of N-terminal dipeptides would be an attractive mechanism for attractin function, and despite CD26 $^{-/-}$ knockout mice having a strong residual circulating DPPIV activity, it seems unlikely that the majority of circulating DPPIV activity in humans can be attributed to attractin since more than 90% of the activity binds to adenosine deaminase - CD26 is an adenosine deaminase binding protein and attractin is not. Nevertheless, in glioma cell lines devoid of apparent CD26 activity, up to 25% of the DPPIV activity can be attributed to attractin (see Malik *et al.*, 2001 in the next period file).