Annotated Bibliography
2003

He L, Lu X-Y, Jolly AF, Eldridge AG, Watson SJ, Jackson PK, Barsh GS, Gunn TM. Spongiform degeneration in mahoganoid mutant mice. *Science* (2003) 299: 710-12.

Mahoganoid mice carry a mutation that is functionally associated in some way with attractin; both mahoganoid (*md*, *Mgrn1*) and *Atrn* lie in the same genetic epistasis group. Accordingly, mutations at both alleles suppress agouti pigmentation function and also suppress obesity induced by ectopic expression of agouti in the central nervous system. Nevertheless, as reported by Phan *et al.* (2002), the mutant alleles of *md* that they examined did not develop neurodegeneration, in contrast to attractin mutations where all except $Atrn^{mgL}$ result in hypomyelination and vacuolation. In this report, the authors not only independently confirm that the product of the md gene (now known as *Mgrn1*) is an E3 ubiquitin ligase but further show that one mahoganoid allele (*Mgrn1-md-nc*; non-agouti curly) develops spongiform vacuolation, although at a much later age than that observed in *Atrn*-mutant mice.

Two questions follow. First, is mahogunin a functional E3 ubiquitin ligase? Second, how are mahogunin and attractin functionally related? These are the same questions that arise after review of the Phan *et al.* article. This report answers the first query. Mahogunin certainly has the ability to ubiquitinate itself. Autoubiquitination, however, provides no insight into substrates *in vivo*. Levels of attractin protein in the brain of *md* mice are comparable to those of wild-type thus mahogunin does not target attractin for proteasomal degradation. Furthermore, there is no evidence of accumulation of protein aggregates. It is suggested that mahogunin lies downstream of attractin since attractin transgenes cannot rescue the md pigmentation phenotype, but the *md* mice already have normal attractin levels so this proposal is still debatable. Given the histological similarity of prion-related spongiform vacuolation and *Atrn*- and *Mgrn1*-related neurodegeneration, together with the identification of Parkin as an E3 ubiquitin ligase, it is proposed that accumulation of an as-yet-unidentified substrate may underlie the spongiform vacuolations in general.

Muto Y, Sato K. Pivotal role of attractin in cell survival under oxidative stress in the zitter rat brain with genetic spongiform encephalopathy (2003) *Brain Res Mol Brain Res* 111: 111-22.

This report grows out of the extensive studies of neurodegeneration in the zitter rat before it was shown that the mutation affected the attractin locus. Earlier studies had focused upon biochemical mechanisms by which neuronal survival might be enhanced, and whether such mechanisms may be aberrant in the zitter rat. Drawing on studies of the late-onset human neurodegenerations where reactive oxygen species (ROS) may play a role in neuronal death, they had previously shown that hydrogen-peroxide–producing superoxide dismutase was elevated in the zitter brain and, concomitantly, peroxide-removing catalase was decreased with the effect increasing with age. A

rise in ROS is followed by a "stress response" increase in extracellular signal-regulated kinase (ERK) activity so the authors decided to extend their previous studies in zitter rats by examining the relation of ROS to ERK. On the basis that the genetic aberration is uniformly penetrant through all nucleated cell types in the body, the authors established primary fibroblasts from zitter rats (Fz cells) and Wistar tremor control (WTC) rats (Fw cells) and established zitter cells transfected with membrane attractin (Fz/Atrn.). Transfection with Atrn reverses to control levels the accumulation of intracellular ROS seen in the Fz cells and this correlates with the capacity to activate (phosphorylate) ERK. Since total levels of ERK are similar between cells, attractin plays a role in regulating the activation of ERK. The issue then becomes whether the effect is direct or indirect. Is lack of attractin affecting ERK activation directly, or is it affecting intracellular localization of kinase activities? Further, is it possible that lack of attractin is generally detrimental for the cell, leading to a "stress" response, and that there are secondary effects that additionally prevent ERK activation, a two-hit knockout for the cell accelerating cell death? Nothing about attractin's structure suggests a direct involvement in cell signaling, and these intriguing results definitely warrant further investigation.

Friedrich D, Kuhn-Wache K, Hoffman T, Demuth H-U. Isolation and characterization of attractin-2. *Adv. Exp. Med. Biol.* (2003) 524: 109-113.

This report describes an optimized technique for purification of human serum attractin using dipeptidyl peptidase IV (DPP4) activity as the criterion for fractionation. Using Blue sepharose (Cibacron Blue) affinity chromatography, strong cation ion exchange, preparative isoelectric focussing followed by preparative PAGE and a final clean-up step using strong anion exchange, the authors were able to isolate 3 mg of DPP4-active protein from 755 ml of plasma that was characterized as 175kDa secreted attractin with a 1300-fold enrichment of specific activity relative to the starting material. Most importantly, the end material appeared to have been purified to homogeneity with no detectable contamination and no reactivity with anti-CD26 antibodies but good reactivity with commercially available anti-attractin antibodies.

Using reversible inhibitors, the inhibitor profile was remarkably similar to that of CD26/DPP4. Finally, the mature N-terminal was located at position 84, leading to the conclusion that the originally-proposed catalytic serine is part of an extremely long signal peptide and is unlikely to be functional. This purification confirms in all respects the properties of the original purification in 1995 described above. Since both the earlier and present purifications relied on DPP4 activity to characterize successful isolation, this once again raises the question of the relation of attractin's DPP4 activity to that of CD26, and how to explain the results of the DeMeester group reported in 2000 where >90% of the circulating DPP4 was associated with adenosine deaminase (ADA)-binding CD26 (Link). One insight that might help elucidate this matter is the consistent observation that the specific activity of purified attractin is about ten-fold less than that of highly-purified CD26, while the serum physical presence of attractin is at least ten-fold greater than that of CD26. A small

amount of CD26 makes a lot of itself in the circulation but purification procedures based on physical properties will always favor the more highly represented protein.

Malik R, Busek P, Mares V, Sevcik J, Kleibl Z, Sedo A. Dipeptidyl peptidase-IV activity and/or structure homologues (DASH) in transformed neuroectodermal cells. *Adv. Exp. Med. Biol.* (2003) 524: 95-102.

In this report, the authors elaborate upon previous results describing non-CD26-associated DPPIV activity in neuroectodermal-derived cell lines. The enzyme activity for each of the cell lines could be described in terms of relative representation of three size-resolved DPPIV peaks: Peak 1 (between 440-669 kDa), Peak 2 (140-300 kDa) and Peak 3 (~140 kDa) where Peaks 1 and 2 have a pH optimum of pH7.4 while Peak 3 has a pH optimum of pH5.5. Peak 1 is proposed to be attractin or a complex containing attractin which is absent in some cell lines (U373, T98G, SK-MEL-28) and well-represented in others (U87, U138 and Hs683). Each of the peak activities differ significantly in their sensitivity to a panel of specific and not-so-specific DPPIV enzyme inhibitors. As an intriguing aside, the authors state that introduction of attractin-specific siRNA results in inhibition of cell proliferation and decreased survival while siRNA targeting DPPIV, DPP8, DPP9, FAPa and QPP had only a slight effect on cell growth.

Haqq AM, Rene P, Kishi T, Khong K, Lee CE, Liu H, Friedman JM, Elmquist, JK, Cone RD. Characterization of a novel binding partner of the Melanocortin-4 Receptor: Attractin-like protein (ALP) *Biochem. J.* (2003) 376: 595-605.

Reports of the relative resistance of mahogany mice to diet-induced obesity fueled the belief that the *mg* gene product may play a role in control of energy homeostasis. The identification of the *mg* gene product as membrane attractin raised the possibility that not only was membrane attractin regulating agouti-mediated antagonism of the Mc1R but it may also function in Agrp regulation of the Mc4R. These early ideas were supported by the presence of attractin transcript throughout the central nervous system. This notion, however, was sidelined by a report that attractin ectodomain could interact with agouti but not with Agrp as well as by several reports describing a progressive neurodegeneration represented initially by tremor that may have raised the basal metabolic rate. Thus, attractin probably plays little role in regulating signaling downstream of the McR4, a process itself that is relatively poorly understood. Although G-protein-binding to GPCR may occur at any of the 3 intracellular cytoplasmic loops as well as C-terminal cytoplasmic tail, this latter domain exhibits the most variability and may mediate receptor-specific functions including oligomerization.

Accordingly, this report describes use of the cytoplasmic tail of Mc4R as bait in a yeast two-hybrid screening and identification of one clone out of 30 potential interacting mouse-derived clones that may be of significant interest. This Mc4R-interacting partner is homologous to the human kiaa0534 transcript that resembles attractin, and the group here names it attractin-like protein (ALP). Kiaa0534 was initially compared with mahogany by the Moore group at Millennium in 1999, where they failed to identify any signaling motifs but did note high conservation of a membrane-proximal -MASRPFA- sequence shared between mahogany and attractin. In fact, this sequence and two other domains are shared in attractin homologs throughout metazoans. Nevertheless, deletion mutant analysis and reciprocal pulldown assays confirmed the interaction of Mc4R residues 303-313 with ALP/kiaa0534 homolog residues 1280-1317. This latter ALP sequence bridges the region of most sequence diversity between the cytoplasmic tails of ALP and attractin and falls between conserved domains 1 and 2 of the Atrn cytoplasmic tail, confirming that the domain interaction was ALP-specific and not shared with attractin (the Mc4R bait does not pull down Atrn cytoplasmic tail in the yeast two-hybrid assay).

The paper then goes on to demonstrate significant anatomical co-localization of ALP/kiaa0534 homolog and Mc4R, hinting at related functionality. Several pressing questions arise. The first is always in the context of yeast two-hybrid assays - can the interaction be demonstrated *in situ*, even using recombinant constructs expressed in mammalian cells followed by co-immunoprecipitation, rather than using purified proteins in an exogenous assay? Second, given the restricted expression of Mc4R but the ubiquitous expression of ALP, is there an alternative GPCR with a cytoplasmic tail similar to that of Mc4R that functions in tissues outside of the CNS? Additionally, it would then be predicted that ALP-knockouts, if viable, might have defective downstream signaling from the Mc4R, providing a unique tool for this poorly-understood but much discussed pathway.

And finally, as a small postscript, how does the affinity of this interaction *in vitro* compare with the 29 other Mc4R-interacting partners identified in this study that are not presnte or addresd in t article?