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A new role for the extracellular calcium-sensing receptor demonstrated by using CCK-eGFP BAC mice

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ONE OF THE THINGS THAT MAKE studying gastrointestinal processes both exciting and fun is the application of new techniques to address a problem where understanding has been constrained by previous techniques. Two complementary papers (10, 19) in this issue illustrate such an application. The new technique is the use of bacterial artificial chromosome (BAC) transgenic mice. The problem is nutrient sensing by enteroendocrine cells. Widely used in neuroscience research (5), the BAC mice allow either targeted gene deletion or overexpression (20). Many transgenic BAC mice express regulatory sequences together with enhanced green fluorescent protein (eGFP), which also allows identification of specific cell types. The eGFP allows these cells to be recovered using FACS for further analysis. Such BAC transgenic mice may then be mated with knockout or reporter mice to further define the presence of either a receptor or a pathway activated by the receptor.

The satiating effects of dietary protein may be due in part to cholecystokinin (CCK) secretion from duodenal I cells. Although aromatic amino acids have been understood to be mediators of CCK secretion from these cells, the technical difficulties of recovering sufficient amounts of homogeneous I cells from fetal or adult intestine are character building. The use of cell lines (i.e., STC-1) that are tumorigenic but endocrine derived is informative but equivocal. Consequently, little is understood about the cellular mechanism by which amino acids affect CCK secretion. The extracellular calcium-sensing receptor (CaSR), originally cloned from the parathyroid, but found in distinct regions of the gastrointestinal system, may be activated by aromatic amino acids, consistent with the CaSR being an amino acid sensor (1, 2). Because the CaSR knockout is perinatal lethal, it was "rescued" by taking parathyroid hormone (PTH) out of the system through breeding with $CaSR^{+/-}PTH^{+/-}$ heterozygotes. The resulting $CaSR^{-/-}PTH^{-/-}$ are sustained with 2% Ca^{2+} in their drinking water (8, 9). Analyses using heterologous CaSR expressing cells have shown differences in intracellular Ca^{2+} oscillation frequency (13, 14) generated by aromatic amino acids compared with other ligands, but direct in vivo evidence from enteroendocrine cells has been lacking. Indeed, we still don't understand the physiological importance of circulating amino acids in regulating the CaSR's activity outside the gastrointestinal tract (3).

The study by Liou et al. (10) from the laboratories of Raybould and Wank is as impressive as it is elegant. They used FACS to isolate and enrich duodenal I cells from the CCK-eGFP BAC transgenic mice and demonstrated that the CaSR was present on these cells. Expression of other putative mediators of amino acid sensing, such as PepT1 or the umami taste receptor, were also assessed. Liou et al. show that these cells generated intracellular Ca^{2+} ($[Ca^{2+}]_i$) changes that were CaSR dependent, stereoselective for L-phenylalanine (L-Phe), and responsive to type II calcimimetic cincalcet. Increases in extracellular Ca^{2+} ($[Ca^{2+}]_o$) did not stimulate CCK secretion from eGFP I cells, but L-Phe stimulation of CCK required "physiological" levels of calcium, consistent with how L-amino acids allosterically activate the CaSR. Liou et al. also ablated the CaSR by mating the CCK-eGFP mice with CaSR/PTH double homozygous knockout mice. Interestingly, they found that $[Ca^{2+}]_o$ stimulations and both L-Phe and cincalcet caused a decrease in CCK secretion compared with basal secretion. Particularly cogent are their comments reviewing CaSR-mediated G_q coupling with L-Phe stimulating $G_{12/13}$ and the cation channel TRPC1. They suggest that the splice variant of the CaSR, which lacks exon 5 and is known to be present in the CaSR/PTH double homozygous knockout mice (12), may explain the effect of cincalcet reducing CCK secretion below basal levels, but clearly more work needs to be done. The same authors recently used CCK-eGFP BAC mice to demonstrate a role for GPR40 to mediate long-chain fatty acid-induced secretion of CCK (11).

Equally impressive and elegant is the study by Wang et al. (19) from the laboratory of Liddle. Wang et al. also used the CCK-eGFP BAC mice and isolated mucosal cells with FACS and demonstrated the CaSR's expression on them. They also measured changes in $[Ca^{2+}]_i$ and showed that both L-Phe and tryptophan caused transient increases that were prevented by the CaSR inhibitor Calhex 231. Both amino acids stimulated CCK release, which was prevented by this inhibitor. Whole cell patch-clamp technique demonstrated the CCK-eGFP cells had a predominantly outwardly rectifying K^+ current; this was inhibited by addition of L-Phe resulting in the depolarization required for CCK secretion from these cells. Both this paper (19) and the study from Wank and colleagues (10) extend our understanding of CaSR in the gastrointestinal system and illustrate the complexity of nutrient sensing in enteroendocrine cells.

We will see greater use of eGFP BAC [usually generated from the GENSAT Brain Atlas Project (5) and obtained from Mutant Mouse Regional Resource Centers] mice to address problems that have heretofore been technically challenging. For example, a SOX9eGFP mouse, also generated from GENSAT, will allow further definition of intestinal epithelial stem cell niche determinants (6), and a gastrin eGFP BAC mouse has recently been characterized (18). It is important to note that BAC mice generated by the GENSAT project are on a CD1 background and consequently must be backcrossed on the same background as the investigators' transgenic or knockout mice to

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permit quantitative comparisons. In general, demonstrating that FACS isolation itself has not influenced the function of the isolated cells is required. Some understanding about whether BAC reporters faithfully recapitulate expression of the endogenous gene may also be worthwhile. Although the dramatic and exciting studies using the NF-κB reporter mice deserve citation (4, 7), eGFP tags on specific gastrointestinal cell types such as the stem cell marker Lrg5 (Jackson Laboratories) allow experimental designs that previously were only dreamed about (16, 17). For example, gene microarray analysis of Lrg5-eGFP cells showed Ror2 (the coreceptor for Wnt5a) but not CaSR, yet CaSR expression is stimulated by Wnt5a (13; unpublished observations). Understanding Wnt coreceptor plasticity, signaling, and Wnt family molecule plasticity in the normal and diseased intestine, as well as nutrient sensing in enteroendocrine cells, will be facilitated by eGFP BAC reporter mice.

GRANTS

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DISCLOSURES

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