plications for collagen VII gene- and protein-therapy studies planned in RDEB.


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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/307/5716/1773/DC1
Materials and Methods
Figs. S1 to S4
Table S1
References


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# Uncharged tRNA and Sensing of Amino Acid Deficiency in Mammalian Piriform Cortex


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**Recognizing a deficiency of indispensable amino acids (IAAs) for protein synthesis is vital for dietary selection in metazoans, including humans. Cells in the brain's anterior piriform cortex (APC) are sensitive to IAA deficiency, signaling diet rejection and foraging for complementary IAA sources, but the mechanism is unknown. Here we report that the mechanism for recognizing IAA-deficient foods follows the conserved general control (GC) system, wherein uncharged transfer RNA induces phosphorylation of eukaryotic initiation factor 2 (eIF2) via the GC nonderepressing 2 (GCN2) kinase. Thus, a basic mechanism of nutritional stress management functions in mammalian brain to guide food selection for survival.**


When supplies of high-quality plant protein or animal source foods are scarce, omnivores are at risk for IAA deficiency. Selection of such high-quality foods played an important role in human evolution (*1*); scarcity is a worldwide problem still common today. In disease or trauma, inadequate protein intake further compromises recovery, as found with gelatin in the 1800s (*2*). Combining vegetable protein sources with complementary IAA patterns (e.g., beans and rice) to maintain appropriate IAA balance is a longstanding cultural practice that predates knowledge of IAA and implies existence of innate detection of IAA deficiency in humans. Moreover, similar IAA complementation occurs in mammals and birds (*3–5*).

Sensing of IAA deficiency is seen in animals including invertebrates within minutes after diet introduction (*3–6*). Such sensing does not depend on olfactory, taste, or other peripheral systems (*7–9*). In the rat, the pyramidal output neurons of the APC are activated by intracellular IAA deficiency, without selective extracellular IAA receptors. These cells project to appropriate feeding control circuits (*10*). In rats and birds, lesions of the APC (*11*, *12*), abolish the rejection of IAA-deficient foods. Microinjections of 1 to 2 nmol of the limiting IAA into APC selectively increase intake of the deficient diet (*13–15*) within 20 min (*15*), without other behavioral changes. Replacement of the limiting IAA into the APC has behavioral, anatomical, and biochemical specificity in the restoration of feeding on an IAA-deficient diet (*13*, *15*). Similar injections into hypothalamic areas known to affect food intake increase deficient diet intake (*16*) or decrease feeding of a stock diet (*17*), but the behavioral/feeding effects are too late to implicate the hypothalamus in the rapid sensing that we report. Injections of nonlimiting amino acids have no effect (*13*).

In yeast, amino acid deprivation leads to accumulation of uncharged tRNA, inducing GCN2-mediated phosphorylation to form the phospho-protein eukaryotic initiation factor 2α (p-eIF2α). Subsequent cellular adaptation to amino acid deprivation is marked by decreases in global protein synthesis complemented by increased transcription of genes related to amino acid biosynthesis (*18*). For animals unable to synthesize IAAs, sensing a deficiency and neural signaling necessarily precede appropriate diet selection, which must occur before the remaining amino acids are metabolized and lost (*3*). Here we report a homologous pathway in the APC that acts 20 min after introduction of an IAA-deficient meal (*19*).

Diets devoid of an IAA deplete the APC of the limiting IAA by 56% within 21 min (*20*). We hypothesized that if uncharged tRNA provides the initial signal for IAA depletion in the APC, inhibition of tRNA charging should decrease food intake. We directly inhibited tRNA acylation in the APC using alcohol derivatives of amino acids (amino alcohols) microinjected into the APC as for IAAs in (*13*). Injections of 0.5 μl were made bilaterally over 5 min. Doses (per side) of L-threoninol, D-threoninol, or L-prolinol (each, 4 nmol) and D,L-serinol (150 nmol) were dissolved in saline. L-Leucinol (3 nmol) was dissolved in 0.5 ml ethanol and then diluted 1:100; control rats were given vehicle injections. Animals with misplaced cannulas determined by histology were omitted from the data set, but the lack of effect of L-leucinol or L-threoninol in these animals provides an additional anatomical control. Subsequent food intake of diets, threonine basal (BAS), leucine BAS, or threonine BAS–devoid of threonine (TD) (*6*), was measured at 20 min, 40 min, 1 hour, 3 hours, 6 hours, and 21 hours after food presentation (*21*) (Fig. 1). The amino alcohols, such as threoninol, are potent, reversible inhibitors of the ATP exchange reaction in activating amino acids for acylation of their respective


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tRNA synthetases (*22*). After injection of L-threoninol, threonine BAS (*6*) intake was decreased (Fig. 1A) as if the rats were eating TD. To learn if this effect is seen with another IAA, L-leucinol was injected; it decreased intake of leucine BAS similarly (Fig. 1B). The stereoisomer, D-threoninol, did not affect threonine BAS intake (Fig. 1C). As with D-threoninol, the amino alcohol derivatives of the dispensable amino acids serine and proline, D,L-serinol and L-prolinol, were without effect. These results show that inhibition of the tRNA synthetase is stereospecific and selective for the limiting IAA in this in vivo model.

To evaluate competition for tRNA acylation, rats were preloaded with threonine using a "corrected" diet (*6*). High dietary L-threonine competed with injected L-threoninol (Fig. 1D), preventing the food intake depression by the amino alcohol. The effects were reversible when the corrected diet was fed during the postinjection test period, which showed that there were no nonspecific effects of amino alcohols on neural tissue or other short-term processes. These results suggest that dietary IAA depletion is recognized at the level of uncharged tRNA in APC.

In the yeast GC response, uncharged tRNA binds to a regulatory site in GCN2 that is homologous to histidyl-tRNA synthetases, which increases p-eIF2α (*18*). Although some cellular stressors activate multiple eIF2 kinases, dietary IAA deprivation increases p-eIF2α in a strictly GCN2-dependent manner (*18*, *23*). To test the roles of GCN2 and eIF2α in mammalian brain, we used GCN2 null ($GCN2^{-/-}$; C57BL/6J background 8 to 10 generations) mice (*23*, *24*) and their BL/6J controls ($GCN2^{+/+}$). The mouse homolog, mGCN2, is highly expressed in brain (*25*), and when activated, directly phosphorylates eIF2α (*26*). Both L-histidine limitation in the presence of L-histidinol in liver and L-leucine deprivation in embryonic stem cells and mouse liver, increase p-eIF2α in $GCN2^{+/+}$ mice, with no effect in $GCN2^{-/-}$ mice (*23*, *24*). In Western blots (*19*) of APCs dissected as in (*27*), there was more p-eIF2α protein in $GCN2^{+/+}$ than in $GCN2^{-/-}$ mice (Fig. 2A). This was not due to the absence of total eIF2α protein in the APC of the $GCN2^{-/-}$ mice, as there were equal amounts of total eIF2α in APCs from both genotypes (Fig. 2B). Intake of TD was correlated ($P < 0.001$) with p-eIF2α only in the $GCN2^{+/+}$ mice (Fig. 2C). The percent of phosphoprotein to total eIF2α in APCs taken from $GCN2^{-/-}$ mice after 20 min of eating either threonine BAS or TD was the same as that seen in mice that failed to eat. Therefore, GCN2 is required for the TD effect in eIF2α phosphorylation. After prefeeding threonine BAS (*6*), $GCN2^{+/+}$ mice ate significantly less TD ($P = 0.015$), whereas $GCN2^{-/-}$ mice ate the same amounts of both threonine BAS and TD diets over 40 min (Fig. 2D). Because the $GCN2^{-/-}$ mice failed to recognize the deficient diet, we suggest that the GCN2 kinase is also essential for early recognition of IAA deficiency in the mouse.

Levels of p-eIF2α in immunoblots from rat APCs taken after L-threoninol versus saline injections were similar to those after feeding TD versus threonine BAS (*19*) (Fig. 3). Four eIF2 kinases exist in mammals; each is activated in response to specific environmental

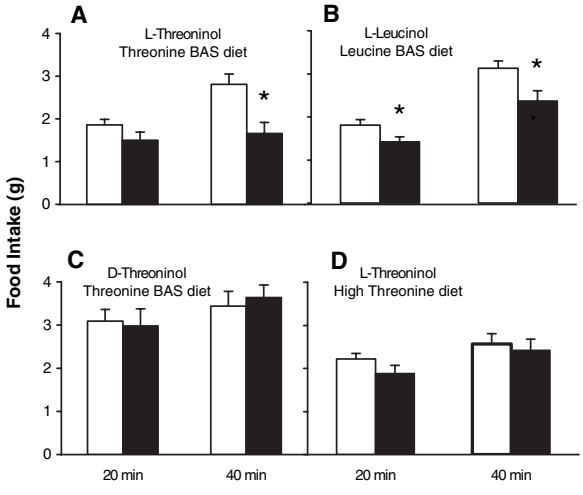


**Fig. 1.** Food intake responses after amino alcohol injection into APC of rats (*13*, *15*). Food intake seen at 40 min remained the same at 1 hour, but did not differ between groups after 3 hours in any experiment. Controls, vehicle-injected groups, are indicated by white bars; experimental groups are represented by black bars. Values are means ± SEM; $*P < 0.05$, $**P < 0.01$ for differences between treated and control groups. (**A**) L-Threoninol decreased threonine BAS intake starting at 20 min (left), reaching significance at 40 min (right; $P < 0.01$) ($n = 11$/group). (**B**) Effects of L-leucinol injections and leucine BAS diet. L-Leucinol ($n = 11$) decreased food intake at 20 min ($P = 0.05$) and 40 min ($P < 0.05$) (controls, $n = 13$). (**C**) Injection of D-threoninol had no effect on threonine BAS intake at any time ($n = 7$/ group). This indicates a stereospecific effect of L-threoninol. (**D**) Rats ($n = 7$/group) that were preloaded with threonine via the corrected diet for 3 days did not respond to L-threoninol, when eating BAS, as did those prefed threonine BAS (Fig. 1A). Reciprocally, those prefed threonine BAS but offered corrected after injection also did not respond (data similar to Fig. 1C); this shows competitive inhibition of L-threoninol by dietary threonine.

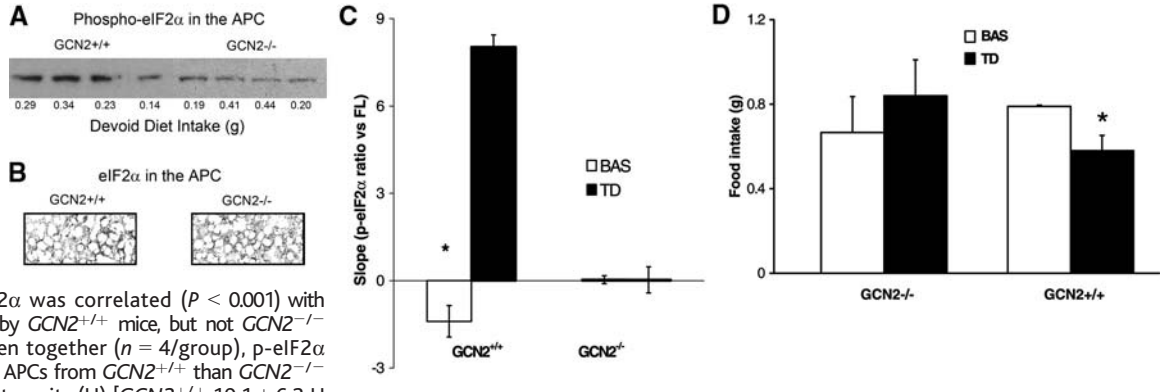


**Fig. 2.** Phospho- and total eIF2α in mouse APC; feeding responses of wild-type and GCN2 null mice. (**A**) Phospho-eIF2α in tissue from individual 42-day-old mice after 40-min access to TD. Grams of TD eaten by each mouse are given below the protein band for that mouse. Phosphorylation of eIF2α was correlated ($P < 0.001$) with the amount of TD eaten by $GCN2^{+/+}$ mice, but not $GCN2^{-/-}$ mice [see also (C)]. Taken together ($n = 4$/group), p-eIF2α was greater ($P < 0.04$) in APCs from $GCN2^{+/+}$ than $GCN2^{-/-}$ mice, calculated as density units (U) [$GCN2^{+/+}$ 19.1 ± 6.2 U (mean ± SD) versus $GCN2^{-/-}$ 9.8 ± 2.0 U]. (**B**) Cell body layers in APC of both genotypes contained ample and equal amounts of total eIF2α protein (black punctate areas surrounding white nuclei.) Immunoblot values for total eIF2α protein, as density units (U), also did not differ (means ± SEM: $GCN2^{+/+}$ 66.2 ± 6.6 U versus $GCN2^{-/-}$ 71.0 ± 4.8 U, $P$ = NS). (**C**) Slopes of the phospho/total eIF2α ratio in APC are correlated with 40-min intake of TD in $GCN2^{+/+}$ (left), but not $GCN2^{-/-}$ mice (right), and not in either genotype fed threonine BAS. With increased ingestion of TD in $GCN2^{+/+}$ mice, the phosphorylation ratio of eIF2α (*19*) was increased (slope 8.03, $R^2 = 0.99$, $P = 0.001$). With ingestion of BAS by these mice, p-eIF2α decreased slightly (slope –0.25, $R^2 = 0.02$, $P$ = NS). (**D**) Food intake of $GCN2^{+/+}$ and $GCN2^{-/-}$ mice (42 to 78 days of age, $n = 7$ or 8 per group) eating threonine BAS or TD for 40 min. The control $GCN2^{+/+}$ mice decreased their intake of the TD ($P = 0.015$) whereas the $GCN2^{-/-}$did not. Values are means ± SEM for grams eaten.

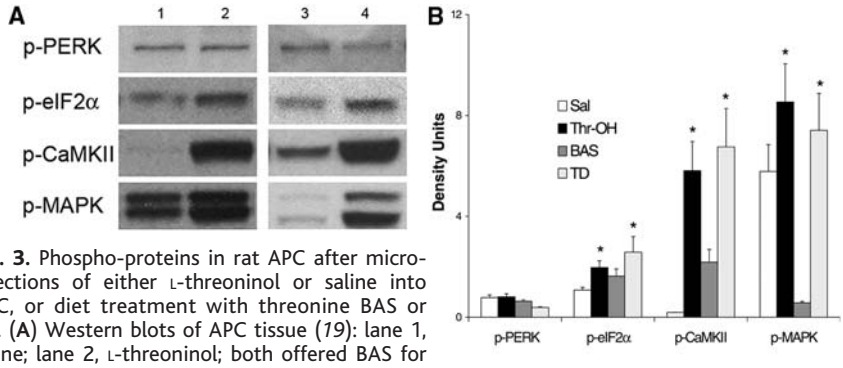


**Fig. 3.** Phospho-proteins in rat APC after microinjections of either L-threoninol or saline into APC, or diet treatment with threonine BAS or TD. (**A**) Western blots of APC tissue (*19*): lane 1, saline; lane 2, L-threoninol; both offered BAS for 20 min; lane 3, uninjected rats that ate threonine BAS; lane 4, uninjected rats offered TD, both for 20 min. Animals that failed to eat > 0.8 g of diet were excluded. Phospho- (p-) proteins (defined in the text) are indicated at the left of each band. (**B**) Western blot data from (A): Values are U (means ± SEM) for each protein. Stars indicate significant differences for the experimental groups compared with their respective controls ($P < 0.05$).

stressors. Double-stranded RNA-activated protein kinase (PKR) is not involved in IAA starvation (*28*), and heme-related inhibitor (HRI) is not found in brain (*18*). Pancreatic endoplasmic reticulum–resident kinase (PERK), is associated with malfolded proteins in the ER, and it is found in brain. We found no increase in phospho-PERK (p-PERK) in Western blots of APC taken 20 min after either L-threoninol injection or eating TD (Fig. 3). This supports GCN2 as the eIF2α kinase in this system. Downstream signaling (*29*, *30*) was also seen similarly in animals fed TD or injected with L-threoninol, including increased phosphorylation of calcium calmodulin kinase II (p-CAMKII) (*29*) and mitogen-activated protein kinase (p-MAPK) (*30*) (Fig. 3).

We conclude that the tRNA/GCN2/p-eIF2α system signals IAA deficiency in the output neurons of the brain area essential for the adaptive rejection of an IAA-deficient diet within the 20-min time frame of the behavioral response (*6*). These unique neurons of the APC, the vertebrate IAA chemosensors, respond to IAA starvation as do yeast: one by feeding behavior, the other by biosynthesis. The responses in both systems lead to restoration of IAA homeostasis and show conservation of this crucial nutrient sensor across evolution, from yeast to mammals.

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# Human Symbionts Use a Host-Like Pathway for Surface Fucosylation

**Michael J. Coyne, Barbara Reinap, Martin M. Lee, Laurie E. Comstock***

**The mammalian intestine harbors a beneficial microbiota numbering approximately $10^{12}$ organisms per gram of colonic content. The host tolerates this tremendous bacterial load while maintaining the ability to efficiently respond to pathogenic organisms. In this study, we show that the *Bacteroides* use a mammalian-like pathway to decorate numerous surface capsular polysaccharides and glycoproteins with L-fucose, an abundant surface molecule of intestinal epithelial cells, resulting in the coordinated expression of this surface molecule by host and symbiont. A *Bacteroides* mutant deficient in the ability to cover its surface with L-fucose is defective in colonizing the mammalian intestine under competitive conditions.**

The ability of humans to tolerate a complex gut microbiota despite their exquisite ability to distinguish self from nonself has been called an "immunological paradox" (*1*). One mechanism that may contribute to the tolerance of these resident microorganisms is molecular mimicry, whereby the bacteria display surface molecules resembling those of the host's surface to render them immunologically inert. Immunologic similarities between the abundant colonic microorganisms *Bacteroides* and tissues of the host are known (*2*, *3*).

The surfaces of intestinal epithelial cells are covered with an abundance of terminally fucosylated glycoproteins and glycolipids (*4*, *5*), which are induced by the intestinal microbiota and specifically by *Bacteroides* (*6*), which in turn cleave L-fucose moieties from the host's surface and internalize them for use as an energy source (*7*). Here we show that *Bacteroides* convert exogenously acquired L-fucose to guanosine diphosphate (GDP)–L-fucose to incorporate it into multiple

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# Uncharged tRNA and Sensing of Amino Acid Deficiency in Mammalian Piriform Cortex

Shuzhen Hao *et al.*

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