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Determining the role of Tubby in feeding behavior

Obesity is a growing epidemic in the United States as over one-third of US adults are currently obese. [1] In 2008, the US spent $147 million on obesity related diseases including heart disease, stroke, type 2 diabetes, and certain types of cancer. [1] One gene that has been shown to function in obesity is *Tubby (Tub).* The *Tub* gene was first discovered in tubby mice that develop severe adult-onset obesity and insulin resistance. [2, 3] TUB belongs to a family of tubby proteins that include the four other members: TULP1, TULP2, TULP3, and TULP4. While the tubby family proteins all contain a highly conserved tubby domain in their C-terminus, TUB is the only family member that is associated with obesity. [4]

The *Tub* gene is highly expressed in the brain, particularly the hypothalamus, a region that is involved in energy regulation. This localization in the brain has suggested that obesity could be due to neuronal defects in the neuroendocrine control of feeding behavior. [5,6]. Tubby mice consume more food and human polymorphisms in the *Tub* gene also correlate with increased carbohydrate intake in women. [7, 8] **The mechanism by which TUB regulates this feeding behavior on the molecular level remains unknown.** Hunger neuropeptides (NPY, POMC, AGRP, and Orexin) that function to regulate feeding behavior are differentially expressed in tubby mice leading to a current **hypothesis** that TUB regulates the expression of hunger neuropeptides on a transcriptional level to control feeding behavior. [9, 10]

The **primary goal** of this study is to determine how TUB regulates feeding behavior on the molecular level. I will identify the protein motifs in Tubby that are important for this behavior as well as DNA motifs in the hunger neuropeptides that Tubby regulates. A better understanding of how Tubby regulates feeding behavior will lead to potential drug targets in the treatment of obesity.

SPECIFIC AIM 1: Determine why mutations in TUB, but not TULPs, result in increased food intake.
Approach: Alignments using Clustal Omega, T-Coffee, and Muscle of TUB and TULP1-4 in both humans and mice will be used to identify conserved and non-conserved regions between Tubby family members. MEME will then be used to identify protein motifs within regions unique to TUB. All homologues will then be searched for the unique protein motifs identified in mice and humans.
Hypothesis: The protein motifs that are unique to TUB will be involved in TUB's regulation of feeding behavior. This will be tested by mutating the identified motifs and observing feeding behavior.

SPECIFIC AIM 2: Identify DNA motifs in the differentially expressed hunger neuropeptides that are important for regulating food intake.
Approach: DREME will be used to identify DNA motifs that are common to all of the hunger neuropeptides that are differentially expressed in Tubby mice.
Hypothesis: TUB regulates the expression of hunger neuropeptides to control food intake by binding to the conserved DNA binding motifs. This will be tested using Restriction Endonuclease Protection Selection and Amplification (REPSA) with TUB and the identified hunger neuropeptide DNA binding motifs.

SPECIFIC AIM 3: Determine whether mutations in TUB affect food choice or food intake.
Approach: Tubby mice will be allowed to choose between high protein, carb, or fat diets and preference will be assessed by the amount of each food type consumed. Microarray will then be used to determine how food choice affects gene expression by assigning mice to a high protein, high carbohydrate, or high fat diet.
Hypothesis: Tubby mice will be more likely to choose high carbohydrate diets as compared to wild-type mice. Consumption of a high carbohydrate diet will affect the expression of TUB, its regulated hunger neuropeptides, and genes associated with metabolic processes and regulation according to Gene Ontology (GO) Biological Process classification.

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