Cell, Vol. S116, S121–S122, January 23, 2004

A Thin Commentary on a Fat Receptor

Commentary

Louis A. Tartaglia* and Robert I. Tepper
Millenium Pharmaceuticals
38 Sidney St.
Cambridge, Massachusetts 02139

Looking back on an exciting scientific enterprise can be a very nostalgic experience for any scientist, and this is particularly true for us in the case of cloning the leptin receptor. We had been very interested in tapping into the molecular mechanisms that regulate body weight. The molecular biology of body weight regulation was still largely a black box in the early 1990s when compared to other fields in which we had worked, such as immunology. This was largely due to the fact that body weight regulation is a "whole organism" phenomenon that is difficult to break down into convenient in vitro systems. Therefore, the identification of "factors" and their corresponding genes was quite challenging. Fortunately, revolutions in genomics technologies were looming and, therefore, we were optimistic about gaining a molecular foothold in this area. It was clear to many of us at the time that cloning the genes that corresponded to the mouse *ob* and *db* loci would provide such a key entry point. Mice mutant for the *ob* and *db* loci (which have a dramatic obesity syndrome) had been studied for decades. Obtaining a molecular understanding of the severe consequences on whole body weight regulation seen in these mutant mice would be a pivotal advance in the field. The first breakthrough came from the work of Jeff Friedman and colleagues when they used a positional cloning approach to clone the *ob* locus and define its gene product, leptin. This made it very likely that the receptor for leptin would correspond to the genetically defined *db* locus.

There was immediate and very justified excitement about the identification of the leptin protein. However, it soon became clear from ongoing work in a number of laboratories (including our work at Millennium) that most obese humans are not deficient in leptin and actually produce it at higher levels. This implied that a better understanding of mammalian body weight regulation and, hence, human obesity, would come from the cloning of the leptin receptor and an understanding of signaling events downstream of this receptor. Needless to say, we were not the only ones to recognize the implications of this. In fact, nearly every pharmaceutical company, large biotechnology company, and many academic labs began a pursuit of the leptin receptor.

Given the intense interest and clarity of the goal, we then found ourselves in a race. It may not be politically correct to refer to an exciting scientific endeavor as a race, but in this case we think it is justifiable. However, this in no way should detract from the sensibility of such an intense scientific effort, because it is the entire group of scientists participating in this endeavor that directly or indirectly moves the field forward by assuring eventual success.

*Correspondence: lou.tartaglia@mpi.com

Immediately after the cloning of leptin was published, we began mapping out an initial strategy to clone the leptin receptor. It was clear we were not in a position to further refine the position of the *db* locus on the mouse genome in time, and, therefore, we could not use a positional cloning strategy with the tools in hand. Using a biochemical approach, with leptin binding as a tool, seemed the appropriate strategy. We had experience with several expression cloning techniques from our days at either Genentech in David Goeddel's lab (Lou) or Harvard in Philip Leder's lab (Bob). We tinkered with several specific approaches with which we were familiar, including a relatively novel approach (at the time) successfully used by John Flanagan. This approach involved tagging the leptin protein with alkaline phosphatase (AP), which could then be easily tracked in binding studies. John and his post-doc (Hwai-Jong Cheng) talked us through this approach in more detail, and it became clear that this approach had potential advantages in the case of the leptin receptor (which would later become one of the earliest examples of successful expression cloning of a receptor from an actual tissue source, as opposed to a cell line). We therefore took the gamble of putting the majority of our efforts and resources into this approach.

The longest period of time was spent identifying, via binding studies, an appropriate tissue source from which to make the expression cloning libraries. This key data came from both an extensive search of tissues and cell lines using our AP-tagged leptin proteins and also 125I-leptin binding data from our collaborators at Roche. The AP-leptin tissue binding data from our team came through the efforts of Suzy Dembski and Jim Deeds, and the 125I-leptin binding data came through the efforts organized by Rene Devos. Through these studies we identified the choroid plexus (a thin layer of tissue within the ventricles of the brain involved in transport across the blood brain barrier) as a tissue that had significant leptin binding. The presence of this binding activity in *db/db* mice caused us considerable concern. The reason for this became apparent later when we found that the mutation in this mouse strain only affects signaling of the receptor and not leptin binding. Given the small tissue mass of the choroid plexus, it was necessary to sacrifice 600 rodents, which required 6 people from our team to dissect these cells from within the rodent brain ventricles over the course of a week (our binding studies in larger animals, which involved several trips to the slaughter house, were unconvincing).

The library itself was made from the isolated choroid plexus RNA by Janice Culpepper and Frederick Clark. Craig Muir and Sean Sanker supplied the automation technology to create large numbers of pooled cDNA. With the team having prepared both the expression library and also large stocks of AP-tagged leptin, we then began the expression cloning. After about six weeks we began to get enticing positives from one of the library fractions. Further pool subdivision and careful binding studies with specific individual clones made it clear that we had cloned the leptin receptor. Sequence analysis

showed it to be a class I cytokine receptor. The first receptor we cloned had a very short intracellular domain. This was confusing at first, since it was unlikely to be capable of signal transduction. However, over the next week or so, we identified otherwise identical forms of the receptor except with a large intracellular domain. We surmised from this that some forms of the receptor are used for signaling in the hypothalamus, while others are used for transport across the blood brain barrier. Genome mapping studies using this cloned gene as a probe (done by Karen Moore) also indicated that this clone was likely the *db* locus.

We wrote the manuscript in one weekend and it appeared in print three weeks after we submitted it to *Cell*, in recognition of the well-known intensity of this race. Interestingly, the only referee comments of significant concern stemmed from rumors the referees had heard about competing groups also cloning the receptor; specifically, that these "other" receptors looked nothing like ours. Fortunately, a quick conversation with Ben Lewin made it clear that *Cell* did not expect us to address such rumors. This was fortunate, because these rumors later turned out to be incorrect. We then established definitively the relationship between our receptor and the *db* locus and described the nature of the mutation in the original *db/db* mouse. This work was published in a follow-up paper in *Cell* six weeks after the publication of the cloning and genomic mapping of the receptor. During the period between the construction of the expression library and sending off the completed manuscript to *Cell* (about two months), Lou worked approximately 18 hr a day and learned a lot about the biology of sleep deprivation as well. Nevertheless, it was one of the most exciting periods of our scientific careers, and a memory that our entire team holds very dear.

It has also been very exciting to see the explosion of obesity research and the progress that has been made since the initial leptin/leptin receptor papers. The fuse for this explosion has not only been these papers, but also a number of others, including the characterization of other mouse monogenic obesity syndromes and the identification of human counterparts. The new genomics technologies that are now available, such as whole genome transcriptional profiling of pathways and mutation detection in humans, have also accelerated work dramatically compared to what would have been possible a decade ago. As a result, not only has our scientific understanding of the molecular mechanisms regulating body weight improved, but even in the lay population there is a greater acceptance of obesity as a chronic disease in which genetics plays an important role, as opposed to simply a weakness of will. Also, the impact of these many discoveries on anti-obesity efforts within the pharmaceutical industry cannot be overstated. Most pharmaceutical companies have increased their efforts in metabolic disease, since it now appears possible that manipulation of specific protein molecules may reduce body weight. We are confident that in the science and treatment of obesity, the best is yet to come.

Cell, Vol. 83, 1263–1271, December 29, 1995, Copyright ©1995 by Cell Press

Identification and Expression Cloning of a Leptin Receptor, OB-R

Louis A. Tartaglia,* Marlene Dembski,* Xun Weng,* Nanhua Deng,* Janice Culpepper,* Rene Devos,† Grayson J. Richards,‡ L. Arthur Campfield,§ Frederick T. Clark,* Jim Deeds,* Craig Muir,* Sean Sanker,* Ann Moriarty,* Karen J. Moore,* John S. Smutko,* Gail G. Mays,* Elizabeth A. Woolf,* Cheryl A. Monroe,* and Robert I. Tepper*

*Millennium Pharmaceuticals, Incorporated
640 Memorial Drive
Cambridge, Massachusetts 02139
†Roche Research Gent
Jozef Plateaustraat 22
B-9000 Gent
Belgium
‡Hoffmann–La Roche Limited
CH-4002 Basel
Switzerland
§Hoffmann–La Roche Incorporated
Department of Metabolic Diseases
Nutley, New Jersey 07110

Summary

The *ob* gene product, leptin, is an important circulating signal for the regulation of body weight. To identify high affinity leptin-binding sites, we generated a series of leptin–alkaline phosphatase (AP) fusion proteins as well as [^{125}I]leptin. After a binding survey of cell lines and tissues, we identified leptin-binding sites in the mouse choroid plexus. A cDNA expression library was prepared from mouse choroid plexus and screened with a leptin–AP fusion protein to identify a leptin receptor (OB-R). OB-R is a single membrane-spanning receptor most related to the gp 130 signal-transducing component of the IL-6 receptor, the G-CSF receptor, and the LIF receptor. OB-R mRNA is expressed not only in choroid plexus, but also in several other tissues, including hypothalamus. Genetic mapping of the gene encoding OB-R shows that it is within the 5.1 cM interval of mouse chromosome 4 that contains the *db* locus.

Introduction

The recently cloned mouse obesity (*ob*) gene appears to encode an adipose tissue–derived signaling factor for body weight homeostasis (Zhang et al., 1994). Mice that are homozygous for mutations in this gene exhibit a profound obesity resulting from defects in energy expenditure, food intake, and nutrient partitioning (reviewed by Bray and York, 1979). Several recent studies have shown that recombinant OB protein (leptin) purified from Escherichia coli can correct the obesity related phenotypes in *ob/ob* mice when exogenously administered (Campfield et al., 1995; Pelleymounter et al., 1995; Halaas et al., 1995; Stephens et al., 1995). Weight-reducing effects of recombinant leptin were also observed in normal mice and mice with diet-induced obesity. Although the target tissues that mediate the effects of leptin have not yet been defined, the work of Campfield et al. (1995) and Stephens et al. (1995) demonstrate that leptin introduced into the lateral or third brain ventricle is effective at low doses, arguing for a direct central affect of the leptin molecule.

Another well-characterized recessive obesity mutation, diabetes (*db*), also results in profound and early onset obesity (reviewed by Bray and York, 1979). Mice homozygous for the *db* mutation exhibit an obesity phenotype nearly identical to the phenotype of *ob/ob* mice (Coleman, 1978). Parabiosis studies with *ob/ob* and *db/db* mice indicate that *db/db* mice may be defective in reception of the *ob* gene product signal (Coleman, 1973). These data have led to speculation that the *db* gene may encode the receptor for leptin, although the available data would also be consistent with *db* encoding a component of the leptin signal transduction pathway.

Recent studies have suggested that obese humans and rodents (other than *ob/ob* mice) are not defective in their ability to produce leptin mRNA or protein and generally produce higher levels than lean individuals (Maffei et al., 1995; Considine et al., 1995; Lönnqvist et al., 1995; Hamilton et al., 1995). These data suggest that resistance to normal or elevated levels of leptin may be more important than inadequate leptin production in human obesity. We have therefore begun a characterization of the mechanism of leptin signal reception. As a first step toward this goal, we have identified and cloned a high affinity receptor for the leptin molecule.

Results

Localization of Leptin-Binding Sites

To search for the leptin receptor, we generated both purified [^{125}I]leptin and a series of leptin–alkaline phosphatase (AP) fusion proteins. To generate the leptin–AP fusion proteins, cDNAs encoding the mouse and human leptin proteins were inserted into the expression vectors APtag-2 and APtag-3 (see Experimental Procedures). Insertion into the expression vector APtag-2 resulted in fusion proteins with leptin at the N-terminus and placental AP at the C-terminus (OB–AP). Insertion into the vector APtag-3 resulted in fusion proteins with AP at the N-terminus fused to the predicted mature form of the leptin protein at the C-terminus (AP–OB) (Figure 1A). Figure 1B shows that both murine AP–OB and OB–AP are secreted proteins and that each is produced at the predicted molecular mass of approximately 81 kDa.

After a search of mammalian cell lines and tissues, reproducible binding of the AP–OB fusion proteins and [^{125}I]leptin was observed in the mouse choroid plexus. For the [^{125}I]leptin studies, coronal brain sections were taken and tissue slices incubated with 0.5 nM [^{125}I]leptin. Tissues were then washed and exposed to film. Figure 2 shows strong [^{125}I]leptin binding to the choroid plexus in both the