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NEWS AND VIEWS

RNAi screen in Drosophila yields a fat catch of Hedgehog

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Obesity, the scrooge of technological advances of the human society, just had its genetic makings revealed once again. A large international team led by Josef M. Penninger and Harald Esterbauer from the Austrian Academy of Sciences and Medical University of Vienna have conducted a systemic hunt for obesity genes in adult Drosophila using an ingenious design of RNAi-based screening strategy (Pospisilik et al., 2010). This study covered 78% of Drosophila genome, including 30% of genes that would otherwise cause embryonic lethality if inactivated by conventional mutations. The findings included ~500 obesity genes that have their functions linked to control of differentiation along neuronal, muscle, and oenocyte lineages, but components of the Hedgehog (Hh) signaling distinguished themselves as the top-scoring hits, underscoring the significant involvement of this pathway in generating the fat tissue.

For the most part of evolutionary history, animal species, humans included, have lived on what little the nature has to offer—it is a species survival necessity to evolve ways to store excess energy in the form of fat for future use when the going becomes tough (Gesta et al., 2007). As humans developed agriculture and began using tools, food supplies became stable, but the abundance in food sometimes rendered the energy intake exceeding the expenditure, and as the result of this energy imbalance, the bodies gained excess weight. This problem is now exacerbated by the modern way of life to the point that it has becomes a worldwide pandemic. Human bodies contain two types of fat, the white adipose tissue (WAT) primarily used for the storage of triglyceride/energy and the brown adipose tissue (BAT), which is important in both basal and inducible energy expenditure. BAT can be mobilized rapidly to generate heat, and is thus the so-called “good fat”, but once acquired, the WAT is difficult to be rid of. Since the epic discovery of leptin by Jeffery M. Friedman and colleagues, much has been learned about the physiology and metabolic mechanism leading to weight gain, which is achieved primarily through size increase in adipocytes. However, the developmental origin and the precise cell lineages of the fat tissues in the postnatal human body are largely unknown. Neither is it clear about the genetic control of the amount of white fat relative to brown fat. A comprehensive understanding in these areas will enable us to track the obesity to its cellular source, and may offer new treatments that are gentler and more civilized than some of the today’s horrific interventions exemplified by liposuction!

The study by the Esterbauer and Penninger team is highly significant precisely because it made a far-fetching inroad into the genetic basis of fat tissue generation. It is also a success story of the *in vivo* RNAi screening technique, a new addition to the awesome power of the Drosophila genetics. In the brief history since its discovery some twelve years ago, RNA interference or RNAi has already leap-frogged into the method of choice when comes to hunting for gene functions. A plethora of RNAi tools ranging from synthetic double stranded siRNA to plasmid-based small hairpin RNA (shRNA) expression vectors have been developed for investigating individual gene functions as well as for systemic annotation of gene functions in genome-wide screens. Coupled with high-throughput techniques and instrumentation, RNAi screening has proven its usefulness and potential directly in mammalian cell culture systems. However, the application of RNAi in mammalian cell culture systems is not without its limitation, chief among which are the type of biological processes that can be studied and the need to select and validate a particular small interfering RNA (siRNA) sequence that can mediate efficient silencing of the targeted gene. These limitations are complimented by *in vivo* RNAi screening in model animals. In *Caenorhabditis elegans* in which the RNAi phenomenon was originally discovered, RNAi can be initiated by feeding the worm with genetically engineered bacteria that express double stranded RNAs (dsRNA) containing complimentary sequences to targeted genes. In *Drosophila melanogaster*, RNAi is also initiated with long dsRNAs derived from the

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target gene sequences. Because the dsRNAs are usually made against the full length of a target gene, they alleviate the need to identify an effective siRNA sequence, which often is the most burdensome step in the application of RNAi approach in the mammalian systems. In 2007, a research group led by Barry J. Dickson at the Institute of Molecular Biotechnology of the Austria Academy of Sciences constructed a library of transgenic fruitflies expressing dsRNAs under the control of the two-factor Gal4/UAS system for each of the 12,251 Drosophila genes covering about 88.2% of the genome (Dietzl et al., 2007). This valuable resource, which is freely available to researchers in academic institutions throughout the world, has combined the power of precision in the temporal and spatial control of gene expression offered by the traditional Drosophila genetics and the specificity and the ease of RNAi to inactivate the expression of any gene at will. Now the time to systemically cataloging gene functions in complex biological processes on a genomic scale has finally arrived.

Armed with the UAS-RNAi transgenic library that the Dickson's group has developed, the team led by Harald Esterbauer and Josef M. Penninger set out to identify candidate obesity genes defined as those that alter whole body Triglycerides levels when inactivated. They used the Hsp70-Gal4 line to drive the expression of UAS-RNAi ubiquitously in adult fruitflies, bypassing the developmental stages. Out of the 11,594 different UAS-RNAi lines they tested, 516 passed three rounds of screening tests, and 319 of which have matching human homologous genes. The functions of these candidate obesity genes fall into cell fate determination, cellular protein metabolic processes, signal transduction, intracellular transport, and regulation of Smoothened (Smo), which is a membrane receptor in the Hh signaling pathway. A similar RNAi screen conducted on *C. elegans* in Gary Ruvkun's laboratory of the Massachusetts General Hospital in Boston also revealed 417 worm obesity genes, and the functions of which include metabolic enzymes, fat/lipid interacting proteins, transcription factors, signal transduction, receptors, and neuronal specific proteins (Ashrafi et al., 2003). It may be an uncanny coincident, however, that both screens identified components in the Hh signaling pathway, an area which has so far largely eluded the scrutiny of the "fat" researchers.

The Hh pathway is a major driving force behind the tissue patterning during embryonic development. It is frequently associated with how body shapes are formed and how cells are determined to take on a fate of differentiation. The finding of Hh as one of the cell signaling systems that regulate adult body fat content was both surprising and insightful, but given the role of Hh in cell fate determination, makes perfect sense. Although the body weight gain is primarily accomplished by adipocyte size increase, frequently new adipocytes are needed for replenishing the adipose tissue. Shepherding cells down a particular lineage is in the realm of developmental morphogens, such as Hh. It is entirely possible that Hh may play a specific role in directing the differentiation of preadipocytes, or multi-potential mesenchymal stem cells. In fact, this has been proven to be the case. A study appeared in Cell Metabolism in 2006 from Jonathan M. Graff's laboratory of UT-Southwestern Medical Center reported that an enhancer trap line with a P-element carrying GFP decorated the Drosophila fat body (Suh et al., 2006); because this P-element landed in the 5'UTR of the Smo gene, this line revealed a specific expression of Smo in the fat tissue, implicating a role of Hh signaling in adipogenesis. This group went on to show in the mouse pre-adipocyte cell line, 3T3-L1, that Hh signaling inhibits adipogenesis, possibly by inducing the expression of anti-adipogenic transcription factors such as Gata2. The Esterbauer and Penninger team also moved onto the mammalian systems with their Drosophila study and constructed a mutant mouse line, in which they activated Hh signaling in both the white and the brown adipose tissues by specifically deleting Sufu, a potent negative regulator of Hh signaling. Surprisingly, the mouse progenies showed a complete absence of the WAT fat while the BAT fat remained intact. Moreover, the mice with dysregulation of Hh signaling in adipose tissues nevertheless exhibited a normal glucose tolerance and energy metabolism. This finding validated the notion that Hh signaling is a strong inhibitor of adipogenesis and suggests that the white and the brown adipose are likely derived from distinctive precursor cells.

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