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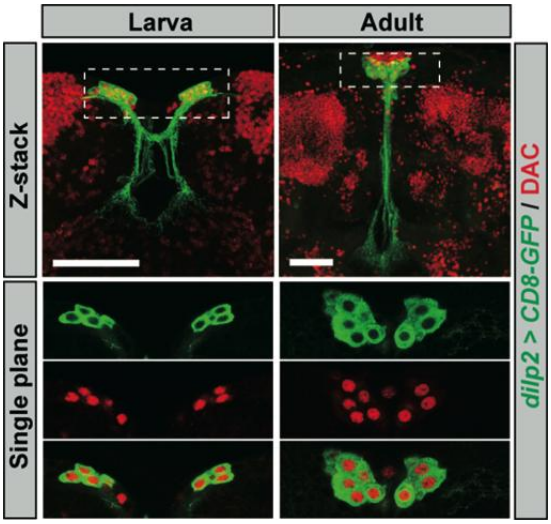
New conserved mechanism for insulin regulation

February 10, 2012 – Peptides of the insulin family are found across a broad range of taxa spanning both vertebrates and invertebrates, in which they play roles in the regulation of processes such as metabolism, growth, reproduction and longevity. The genome of the fruit fly *Drosophila melanogaster* includes seven genes encoding such peptides, known as *dilps* (*Drosophila* insulin-like peptides), which are primarily expressed in secretory insulin-producing cells (IPCs) within the brain. The expression of each *dilp* is independently regulated, but just how this level of coordination is achieved remains poorly understood.



Expression of Dac (red) in the IPCS (green) of larval (left) and adult (right) flies. Upper panels show IPCS and axons (green) with Dac labeled (red). Lower panels show the areas indicated by white dotted lines above at higher magnification.

Now, Naoki Okamoto and others in the Laboratory for Growth Control Signaling (Takashi Nishimura, Team Leader) have identified an important clue to this puzzle, showing that a highly conserved nuclear protein Dachshund (Dac) acts as a transcriptional regulator specific to *dilp5*. Published in the *Proceedings of the National Academy of Science*, these findings may open new avenues of understanding into the means by which diverse insulin-family peptides are kept working in harmony.

The IPCs of the fruit fly brain express several genes known to be involved in the development of the eye. The team knocked down the function of several of these genes in a tissue-specific manner to check for possible effects on *dilp* expression. They found that the knockdown of *dac* was accompanied by a dramatic down-regulation of *dilp5*, suggesting that Dac might function in Dilp5 regulation in IPCs.

An examination of Dac expression in IPCs showed the protein is expressed continuously throughout development. When Okamoto next analyzed homozygous mutants for *dac*, he found that while IPCs formed normally, the expression of *dilp5* in these cells was markedly lower in young larvae, but intriguingly its expression was normal in later larval stages, suggesting stage-dependent differential mechanisms for its regulation. A subsequent analysis using heterozygous *dac* mutants revealed that expression levels of that gene correlated closely with the expression of *dilp5*. A second protein called Eyeless (Ey) is known to control *dilp5* expression, and he found that *ey* mutants revealed similar phenotypes as found in the *dac* studies.

The team next focused on possible interactions between Dac and Ey, but found that the loss of function of one had no discernible effect on the other, suggesting their expressions are independently regulated. Their effects on *dilp5* were synergistic, however, as shown by tests in which the expression of both was perturbed. Looking next for an interaction between Dac, Ey and the *dilp5* promoter, they found that while Dac alone did not interact, the binding of Ey to the *dilp5* promoter was accelerated in the presence of Dac. Further experiments revealed Dac forms a physical complex with Ey and with itself through specific protein domains.

Knowing that Dac is evolutionarily conserved in mammals, Okamoto next turned to the insulin-

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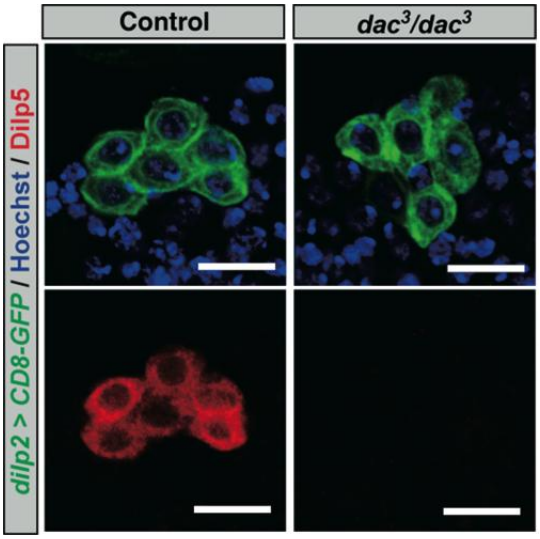
secreting β-cells in the islets of Langerhans of the pancreas, in which Dach and Pax6, homologs of the *Drosophila* Dac and Ey, play important developmental roles. Importantly, Pax6 is also involved in the transcription of genes encoding critical pancreatic hormones, such as insulin and glucagon. Using cultured rat β-cells-derived cell line, they examined the functions of these two regulatory proteins and found that, as in the fruit fly, Pax6 and Dach had similar combinatorial effects on the activation of insulin expression.



In wildtype young larvae (left) IPCs (green) express Dilp5 (red), while in *dac* mutants (right) Dilp5 expression is dramatically reduced.

"The expression of *dilp5* can change in response to nutritional status, and we still don't know how this gene is regulated in later stage larvae," says Nishimura, "suggesting that the regulatory situation is even more complex than we expected. We are looking forward to tackling the link between nutrition and *dilp5* expression in future work."

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