Biology

Julie Ann Miller reports from San Francisco at the meeting of the American Society of Biological Chemists

A novel enzyme in basic metabolism

After almost a century of detailed study, biochemists have just discovered a new twist to an important and nearly universal biological pathway. A sugar called fructose-2, 6-diphosphate (fru-2, 6-P_2) plays a significant role in regulating the generation of metabolic energy from glucose — the process called glycolysis — and in the formation of glucose from compounds other then sugars — gluconeogenesis. And the enzyme that synthesizes fructose-2, 6-P_2 has unique and interesting properties, reports Simon J. Pilkis of Vanderbilt University in New Orleans.

The sugar fru-2, 6-P_2 was discovered in 1980. It has been found in all mammalian tissue examined, as well as in plants and yeast. This sugar activates the enzyme most important for regulating glycolysis. Pilkis now reports that both synthesis and degradation of fru-2, 6-P_2 in liver is catalyzed by "a unique bifunctional enzyme" that can both add a phosphate group from adenosine triphosphate (ATP) to fructose-6-phosphate and remove a phosphate group from the diphosphate sugar. He and colleagues have purified this enzyme and find that under some conditions one activity can be enhanced and the other depressed.

"The finding of two opposing activities in a single enzyme presents interesting possibilities," Pilkis says. For example, the product of the synthesis reaction can be immediately broken down by the degradation reaction, resulting in wasteful consumption of ATP.

Further research indicates another enzymatic reaction controls which activity of the bifunctional enzyme prevails. This control also is the basis for hormonal effects on the reaction. The enzyme, called cyclic AMP-dependent protein kinase, adds a phosphate group to the bifunctional enzyme. The phosphate group makes the bifunctional enzyme more likely to break down fru-2, 6-P_2 than to synthesize it. Another enzyme removes the phosphate from the bifunctional enzyme, making it more likely to synthesize fru-2, 6-P_2. "This is the first bifunctional enzyme shown to be modified by phosphorylation," Pilkis says.

Molecular details of cell division

Recombinant DNA techniques have now reached the most basic levels of biology—the classical description of cell division. Scientists are identifying sequences of the nucleotide subunits of DNA required for proper inheritance. John A. Carbon of the University of California at Santa Barbara and Molly Fitzgerald-Hayes of the University of Massachusetts in Amherst have analyzed the part of certain chromosomes that during cell division attaches to a fiber and thus is drawn into the appropriate daughter cell. The investigators have isolated and reproduced in bacteria these centromeric regions of three of yeast's 17 chromosomes. When copies of a centromeric region, in rings of bacterial DNA (plasmids), are returned to yeast, they still participate in accurate segregation; in cell division one copy goes to each cell. "The small segment of DNA allows the plasmid to segregate like a mini-chromosome," Fitzgerald-Hayes says.

The nucleotide sequence of the centromeric regions have some striking similarities. One area of 11 nucleotides is homologous in all three centromeres. This sequence also has been observed, repeated many times, near centromeres of human, baboon, cow, rat and fruit fly chromosomes. Another stretch of 14 nucleotides is homologous in two of the yeast centromeres and is present in an altered form in the third. Finally each of the three centromeres has a stretch of DNA about 87 nucleotides long made up almost entirely of adenosine (A) and thymidine (T), two of the four DNA nucleotides. The scientists have removed various parts of the centromeric regions in the plasmids and find that the homologous and the AT-rich stretches are essential for proper segregation during cell division. These regions seem necessary for the binding of special protective proteins to the DNA.

Finding factors for fast fetal growth

From whence comes the signal for rapid growth of fetal tissue? The period of fastest growth, the first 14 weeks, requires insulin or a similar hormone to promote the use of glucose in tissue-building metabolic processes. But this growth occurs well before the fetus is mature enough to make growth-promoting factors of its own, and the placental barrier keeps insulin made by the mother's pancreas from traveling to the fetus.

Kwang-San Liu, Judith Ilan and colleagues at Case Western Reserve School of Medicine in Cleveland now suggest that the placenta produces insulin-like substances and supplies them, along with steroid and peptide hormones, to the fetus. Specimens of placenta taken from the first trimester of pregnancy and incubated in the laboratory were found to produce both messenger RNA and protein related to insulin. "We don't know yet if this material is efficacious or if it is different from insulin in adults," says Ilan. She plans to determine the sequence of nucleotide subunits for the placental messenger RNA related to insulin and to translate it into protein.

Insufficient supplies of such an insulin-like factor might be responsible for many first-trimester miscarriages by preventing growth of crucial tissue, Ilan speculates. "The fetal pancreas is fairly late to develop and even later to produce insulin and become vascularized. But brain development is an early event that obviously requires glucose as the source of energy," she says. "We have now identified an insulin-like protein synthesized and secreted by the human placenta."

Mix and match antibodies

The business of making specific antibodies is a gamble, and success requires some luck. Scientists expose antibody-making cells to the desired target molecule and then hope to harvest at least one line of cells specific to that target. But antibodies vary not only in the variable region, the portion that confers specificity, but also in the constant region, the area responsible for the characteristics that determine an antibody's class. For some uses of antibodies, scientists need to obtain material of a certain class.

Leonard A. Herzenberg of Stanford University in California gave a talk on the topic "How to modify monoclonal antibodies after you have them to get what you really want." For example, a routine assay for tissue typing requires antibodies of groups designated IgG_2 or IgM. The blood factor used in the assay will kill cells only when they bind antibodies of these types.

Herzenberg has devised a procedure for changing the class of an antibody with a desirable specificity. In normal immunity, the gene for the variable region of an antibody moves within the chromosome to a location adjacent to the gene for one of the constant regions. Herzenberg and others have observed that in hybridomas, the cells used to make monoclonal antibodies, the genetic material will somehow rearrange at a low frequency (about once per 100,000 cells), putting the variable region gene adjacent to a different constant region and thus changing the class of the antibody product.

To select such altered cells, Herzenberg exposes the cells to a fluorescently tagged antibody that binds to the constant region desired. Then a device called a fluorescent cell sorter selects individual cells producing the desired antibody and they are grown into a cell line. Using this method Herzenberg reports he and colleagues have changed an IgG_1 antibody to each of two IgG_2 subtypes. This switch gives an antibody suitable for clinical assays. Herzenberg also has used genetic engineering techniques to introduce new genes for the constant and variable regions into antibody-producing cells. "You can now mix and match chains [subunits] and you can mix and match domains [within the subunits] to get new types of hybridoma cells," Herzenberg concludes.

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