SCIENCE NEWS of the week

Muscular Dystrophy Protein Identified

Researchers last week reported the discovery of a protein whose absence triggers the onset of Duchenne muscular dystrophy (DMD), the most common and devastating of the muscular dystrophies. The discovery comes only five months after the muscular dystrophy gene was first cloned, and provides new and intriguing clues about the biochemical mechanisms behind the incurable disease. Discovery of the missing protein, which the researchers have named dystrophin, represents a major step toward finding a treatment for the disease, which is characterized by progressive muscle weakness in young boys. Affected individuals rarely live beyond their early 20s.

"People have been working on this disease for 130 years, and very intensively, and have never been able to find anything consistently wrong," says Eric Hoffman, a researcher at Children's Hospital in Boston and part of the team reporting the discovery. Part of the difficulty in identifying the critical protein, the researchers found, was that even in normal individuals it accounts for only 0.002 percent of the total amount of protein in skeletal muscle. The protein's absence in muscular dystrophy is the result of a defective DMD gene.

Despite its low concentration in normal muscle, dystrophin is clearly an important ingredient. Earlier work had hinted at the essential role the missing protein might play in normal muscle structure – a role many researchers believed was related either to calcium regulation in muscle tissue or to inhibition of certain protein-dissolving enzymes (SN: 1/17/87, p.41). The latest research, led by Louis Kunkel of Harvard Medical School, provides strong evidence that dystrophin depletion is but the first step in a cascade of events that ultimately leads to muscle wasting. The research is described in the Dec. 24 issues of CELL and NATURE.

To identify the protein, the researchers took a normal DMD gene and inserted it into a bacterial cell. The bacteria created a fusion protein – a mixture of bacterial proteins and the protein normally programmed by the DMD gene. Using custom-made antibodies, the researchers identified the dystrophin portion, which they then studied in an effort to determine its function in normal muscle.

They found that dystrophin is normally a part of microscopic structures called triads, deep within the contractile fibers in muscle tissue. These triad structures are critical to muscle function; they sense electrical signals from incoming nerves and respond by triggering the release of calcium ions from storage areas, thus initiating muscle contraction. A biological "pump" constantly returns the reusable calcium ions to storage areas. The researchers hypothesize that a lack of dystrophin leads to defects in triad membrane structure and a constant leaking of calcium ions. However, says Hoffman, the membrane defects themselves are probably not the cause of muscle wasting in individuals with DMD.

"We know that whenever you upset the calcium balance you can activate phospholipase A," an enzyme that dissolves muscle fibers, he says. And in the body's attempt to repair this damage, human skeletal muscle is subject to fibrosis – a "hardening" process that impairs muscle function.

"We think that despite a lack of dystrophin, the muscle cells regenerate themselves," Hoffman says. "But then there is this secondary pathological reaction [fibrosis]. This ends up restricting the vascular supply, so the cells can't get nutrients. You end up with more and more cells starving to death, which is actually causing the weakness and killing the patient."

In support of this hypothesis, the researchers provide new evidence that certain healthy mice have the same dystrophin-lacking genetic defect as do humans with DMD. Although these mice lack dystrophin, they survive – probably because mice typically do not develop fibrosis.

"Fibrosis is a very common reaction throughout the [human] body, but we know very little about it," Hoffman says, adding that physicians may someday be able to treat DMD by replacing dystrophin or by learning how to control fibrosis. In either case, he says, the mouse model may prove to be an invaluable research tool. *– R. Weiss*

New gene may solve the Y (and X) of sex

Whether a person is male or female apparently depends upon a very small portion of the Y chromosome, scientists reported last week. By using abnormal human sex chromosomes and a "Noah's ark" of Y chromosomes from other species, the international research team has cloned an area containing what they say is the gene responsible for at least the first step in sex differentiation.

Although further studies are needed to confirm the gene's exact function, its discovery – coupled with the concurrent identification of a similar segment on the X chromosome – should answer some fundamental questions about what determines sex in humans and other species.

Scientists from the University of Helsinki in Finland, the University of British Columbia in Vancouver and the Massachusetts Institute of Technology and the Whitehead Institute for Biomedical Research in Cambridge, Mass., report in the Dec. 24 CELL that the newly cloned segment contains the testis-determining factor gene (TDF). Scientists have said for years that there must be such a factor, a "master" protein coded by the Y chromosome that can determine sex by its presence or absence. The newly identified gene, which the authors conclude "is probably TDF," apparently resides in a DNA segment only 1.3 kilobases long. In comparison, the length of the entire human Y chromosome is about 70,000 kilobases (a standard unit of measure in genetics).

With TDF's hypothesized existence as a starting point, the researchers collected data from a wide range of chromosome studies, searching for the genetic segment most likely responsible for determining sex in developing embryos. Included in the study was material from individuals with abnormal configurations of sex chromosomes.

If developed from an egg fertilized by Y-chromosome-bearing sperm, a human embryo usually becomes obviously male. In other words, the presence of both an X and a Y chromosome normally mandates male sexual development, while two X chromosomes mean an individual will be female. But in a relatively small proportion of births, the normal course of events goes awry. For example, there are XX individuals who are essentially male, as there are XY females.

Using dozens of DNA probes that attach to specific genetic sequences, the scientists compared the structures of Y chromosomes from an XX male and an XY female. They found a small portion that "is necessary and sufficient" to induce testis formation: If present, the individual is male; if absent, female. They also found what appears to be a related gene on the X chromosome. To further pinpoint the TDF gene, the researchers looked for the same DNA sequences in other mammals, and found a "striking" degree of similarity among the species, further evidence of the newly described structure's importance. *– D.D. Edwards*