CALCIUM's PROTEIN PARTNER

From protozoa to people, the same protein teams up with calcium to control an amazing range of biological processes

BY JULIE ANN MILLER

There's much more to calcium's role in the body than building bones and teeth. Only 1 percent of the body's calcium is not bound into those hard tissues, but that small fraction is crucial in controlling nerve and muscle action, cell motility, blood clotting and cell membrane activities.

How a simple ion could have so many different actions baffled scientists until it was suggested that calcium operates not as a free ion, but teamed with a variety of special proteins. Research teams soon identified proteins that combine with calcium to activate enzymes in the brain, red blood cells and skeletal muscle. Further investigation revealed that the same calcium-binding protein acts in each of these and many other systems—including secretion, cell division and cyclic nucleotide and glycogen metabolism. In fact, that protein plays its role in cells throughout the plant and animal kingdoms. Because the protein acts to modulate enzyme activities in a calcium-dependent style, it is now called "calmodulin."

"Calmodulin is in every cell from the most primitive plant or animal to man," says Anthony R. Means of Baylor College of Medicine in Houston. "It is not secreted from cells, so it is not found in biological fluids, such as blood plasma and urine, but we find it in all cells."

Sources of calmodulin so far identified include, in addition to vertebrates, sea anemones, clams, snails, earthworms, starfish, slime molds, protozoa, a fungus, pea plants, mung beans and wild carrots.

The similarity of calmodulin throughout the animal kingdom is clear in its amino acid sequence. The protein is a string of 148 amino acids—142 are exactly the same in rats, cows and the sea pansy, a marine coelenterate. In each of the six other positions, the amino acids observed are similar in their properties. Means says, "Calmodulin is as extremely highly conserved as any protein in evolution." So far no related calcium-binding protein has been found in bacteria, but Means expects a homologue to turn up.

Calmodulin itself is evolutionarily a very old protein and vertebrates contain, in addition to calmodulin, a skeletal muscle protein called troponin C that is derived from it. In the interior of the troponin C and calmodulin molecules, more than 70 percent of the positions are filled by exactly the same amino acids.

How can a single protein guide calcium in its diverse interactions? One possibility is that the number of calcium ions bound to a calmodulin molecule dictates the activity. Calmodulin has four sites that bind calcium. The similarity in amino acid sequence at the sites suggests that the protein evolved by gene duplication from a smaller ancestral product that bound a single calcium ion.

In the modern calmodulin, when one calcium ion binds it changes the conformation of the molecule. Some of calmodulin's effects require that only one calcium site be filled, others require a full house of four calciums. In theory, there are 16 possible conformations of calmodulin bound to between zero and four calcium ions. Each of the conformations may be recognized by a different protein or set of proteins in some cell.

To locate calmodulin within cells,

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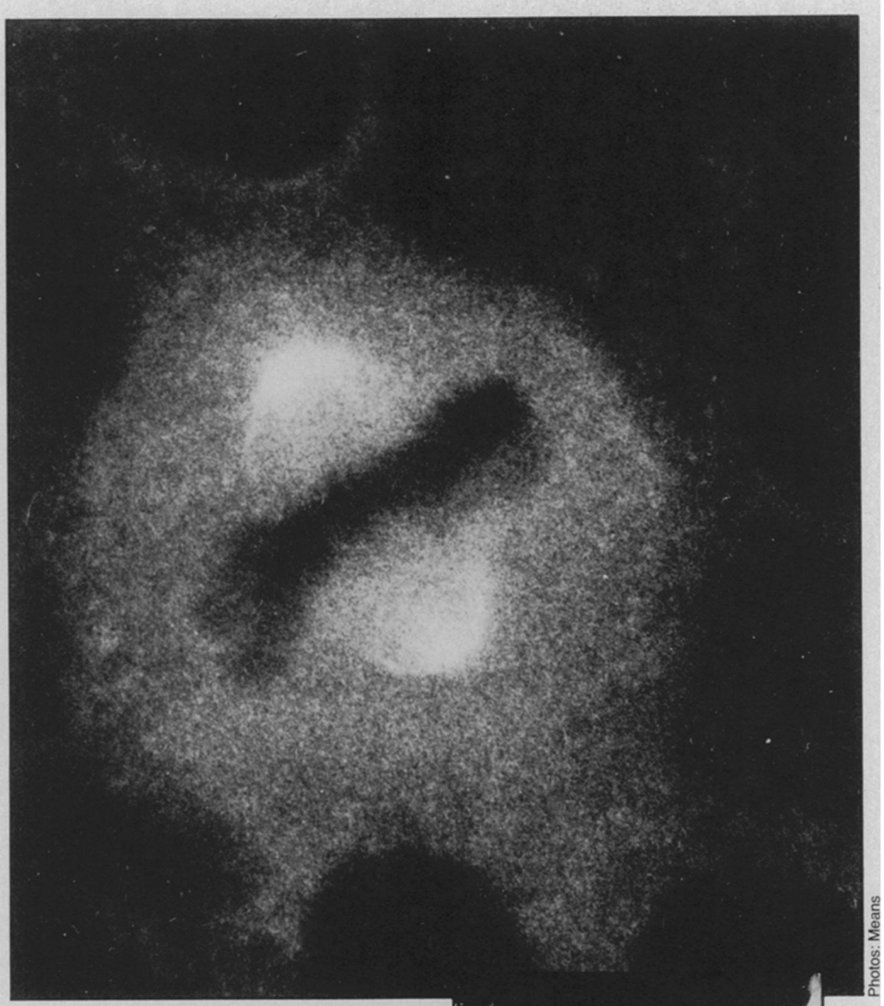

Photos: Means

Distribution of calmodulin changes during cell division. Fluorescence labels calmodulin during cell division when the chromosomes line up across the equator of a rat kangaroo cell (top). Between cell divisions calmodulin is located throughout the cytoplasm, as in this cell from an African clawed toad (bottom). In these experiments, antibodies link the fluorescent dye to calmodulin in the cells.

. . . Mistakes

In addition to having hospital personnel pay closer attention to the medications they choose, Wang also proposes innovations in bottling, including distinct sizes, shapes and colors for specific drugs. But perhaps his most intriguing idea is to incorporate a sort of organic relief map on the ampule itself. Each such bottle would be embossed, Wang says, with "the principal site of action of the drug" — i.e., the shape of a kidney, heart, brain, etc. — primarily to aid persons who might have trouble reading the print on the label. "God has given us five senses — we can utilize touch on the outside of containers," says Wang, who also advocates using different colored intravenous lines for different substances.

Confusing medication labeling, however, constitutes just one aspect of medical errors. A Johns Hopkins Hospital survey of 178 medication errors divided the incidents into seven categories, in order of frequency (a, b and c occurred with equal frequency):

a. The wrong patient received or almost received a medicine.

b. A patient received or almost received the wrong dose.

c. A patient received or almost received an extra, unordered, dose of medicine.

d. A patient's medicine was omitted or almost omitted.

e. A patient received or almost received the wrong drug.

f. A patient received or almost received a medication at the wrong time.

g. A patient received or almost received a medicine through an improper route.

Researchers Alphonse Chapanis and Miriam Aronstein Safren found that such incidents often happened not only during "stress periods" such as shift-changes and meal times but, somewhat surprisingly, when a nurse had to administer medication to just a single patient, rather than to several. Single dose administrations, they note, are almost always unscheduled and tend to break up the nurse's routine — apparently contributing to the error.

The researchers recommend that:

- The pharmaceutical industry should set up clear standards for drug nomenclature. Errors often occurred because certain drug names — generic, brand or both — resemble others of different function.
- The decimal point labeling of dosages — where .1 cc can be misread as 1 cc or 2.5 mg as 25, etc. — should be improved, or changed, possibly by writing the dose as a fraction to avoid perceptual errors.
- Confusing medical abbreviations pertaining to dosages should be changed. Terms such as q.n. (every night) were confused with q.h. (every hour), and q.o.d. (every other day) with q.d. (every day).
- Drug labels should be improved. Small print, poor area lighting and ambiguous wording all contribute to label problems.
- Arrangement of drugs in ward medication cabinets should be standardized. Such arrangements can vary greatly within a hospital as well as among hospitals. It is often difficult to see or reach a drug; drugs with similar names are frequently placed next to each other or in the same section of the cabinet.

Finally, the researchers suggest a more efficient cross-checking system regulating the flow of medication orders between doctors and other hospital personnel.

The fact that the Hopkins study was performed in the 1960s and still remains among the most "recent" formal studies cited by current human error investigators testifies to what Wang and others say is a neglect of the medical errors problem. And though Chapanis says the situation has "improved at this hospital [Johns Hopkins]," he adds, "I don't know about other places."

Wang suggests that in many hospitals, most of the medication problems identified some two decades ago still exist. "This is why I go around to these meetings, exhibiting," he says. Although it may be impossible to determine exactly how often mistakes are made in hospitals, one study in Little Rock, Ark., estimated the error-incidence rate at 15 percent. Says Wang: "I would say it's maybe even more." □

. . . Calmodulin

Means and colleagues Bill Brinkley and John Dedman have used antibodies that bind to it. Antibodies to those antibodies are labeled with a fluorescent dye, and striking patterns of calmodulin distribution can been seen. For instance, during cell division the fluorescence is associated with the mitotic spindle, the cell's apparatus for organizing and distributing chromosomes. The fluorescence is most intense at the poles of the spindle and projects toward the chromosomes lined up across the center of the cell. A variety of experiments indicate that calmodulin bound to calcium disassembles, and thus shortens, microtubules of the mitotic apparatus. The chromosomes therefore are drawn toward opposite sides of the dividing cell under calcium-calmodulin direction.

Calmodulin's many roles make it, and related compounds, key candidates for diagnostic and therapeutic use. For example, calmodulin, which has been found in both the head and the tail of sperm, may provide a new approach to contraception. Proportionally more calmodulin is measured in sperm (and curiously in the electric organ of eels) than in any other cells. In sperm 7 percent of the total cellular protein is calmodulin.

Several important reactions in sperm, including motility, are inhibited by calmodulin-binding drugs, such as phenothiazines. Milton Cormier and co-workers at the University of Georgia have made progress toward a calmodulin-directed contraceptive.

Calmodulin may also play a role in development of new antipsychotic tranquilizers. The ability of individual phenothiazines to bind calmodulin, in a calcium-dependent fashion, correlates to the drug's activity as an antipsychotic agent.

Calmodulin also may provide a tool for diagnosing cancer, Means says. It is present in elevated levels in a wide variety of tumor cells, including those made cancerous by virus, hormone and chemical carcinogens. The boost in calmodulin in tumor cells seems to be due to a general acceleration in protein synthesis. The rate of degradation of most proteins in the tumor cells increases to compensate, but the rate of calmodulin degradation remains fixed.

The role of hormones in calmodulin's actions is not yet clear. Means says it has long been known that hormones influence calcium ion's distribution. And now it looks as if hormones also affect the distribution of calmodulin within each cell. Speaking at the recent Endocrine Society meeting in Washington, Means emphasized the potential importance of calmodulin's role in the endocrine system. "It opens an entirely new field of endocrinology and of cell biology," he says.

One speculation about how calcium carries out its many roles is that it acts as a messenger inside a cell. When specific events raise the level of free calcium in a cell, those additional ions bind to calmodulin, which acts as an intracellular calcium receptor. That protein then interacts with a distinct set of proteins in each tissue, amplifying the calcium-calmodulin impact.

The more traditional example of a "second messenger" is the cyclic nucleotide cAMP, which stimulates enzymes that add phosphate groups to proteins. Because the calmodulin-calcium complex regulates cyclic nucleotide metabolism, the two messenger systems in cells seem to be coupled. Moreover, many of the enzymes regulated by the calmodulin-calcium complex are also the substrates for enzymes activated by cAMP.

"At the moment most of the data concerning calmodulin regulation must be considered as phenomenological," Means and Dedman say in a review article in the May 8 NATURE. "What is now required is a concerted effort to understand the chemical basis for each of calmodulin's actions."

Claude B. Klee, Tom H. Crouch and Paul G. Richman of the National Cancer Institute conclude in the 1980 ANNUAL REVIEW OF BIOCHEMISTRY, "It may soon become more interesting to ask which cellular processes are not under calmodulin control than which are, since it is already clear that a large part of cellular metabolism and function is under the direct or indirect control of this small but precisely designed protein." □