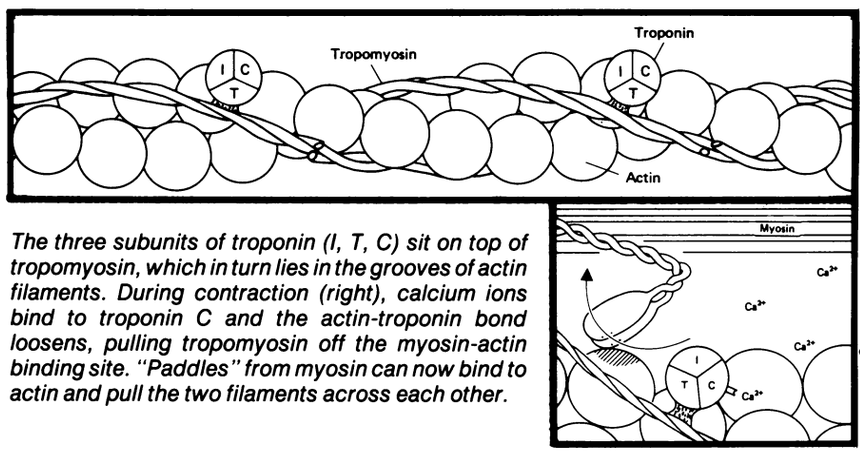
THE HEART AND HEREDITY

Unromantic as it may sound, genetics rules the normal, everyday function of the heart. And in that control lie clues to a basic mechanism of genetic expression.

By JOANNE SILBERNER



The three subunits of troponin (I, T, C) sit on top of tropomyosin, which in turn lies in the grooves of actin filaments. During contraction (right), calcium ions bind to troponin C and the actin-troponin bond loosens, pulling tropomyosin off the myosin-actin binding site. "Paddles" from myosin can now bind to actin and pull the two filaments across each other.

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Heart research spans everything from finding a replacement for the ailing organ to figuring out on a molecular level exactly what goes on in a normal heart. Working at the basic-research end of the scale, Bernardo Nadal-Ginard and co-workers at his Harvard University laboratory are delving into the way proteins in heart muscle cells respond to changes in the environment. While the work does not have any direct application to cures or treatments, it has enabled these researchers to delineate how a single gene could produce several dozen forms of a single type of molecule, and it is helping to clarify exactly how genes work.

Scientists once believed that an individual gene produced an individual protein. But with a finite gene bank, sometimes more diversity is needed, and researchers now know that certain cell types can generate more than one protein from a single gene. White blood cells called lymphocytes, for example, can produce billions of different immunoglobulins through rearrangement of their DNA.

There is a second way to generate diversity, called alternative RNA splicing. The Harvard group and others have found evidence of it in muscle proteins, and researchers at other laboratories have seen it in immune system proteins, some nervous system proteins and blood proteins.

Nadal-Ginard found the alternative RNA splicing in a muscle protein serendipitously, he says. He had originally set out to study how different proteins respond to calcium ions.

The scene for Nadal-Ginard's study is the contraction of muscle cells, a process that begins when an electrical change releases calcium stored within the cell. The contraction is effected by four key proteins in the cell — actin, myosin, tropomyosin and troponin.

The myosin molecules are aligned in thick parallel filaments; the other three molecules are intertwined in parallel thin filaments. Within each cell, rows of thin filaments jut out toward each other, like combs aligned teeth-to-teeth. The thick filaments lie between the teeth, bridging the gap between each set of "combs."

When the fiber receives a signal to contract, the thick filaments pull the combs together by "rowing" against the thin filaments. Globular ends of the myosin protein stick out toward the actin and act as little paddles. They grab hold of actin at an angle and pull the molecule slightly before the bond breaks. Then another bond is made a little farther down the actin filament and the process is repeated. Each cycle shortens the muscle by about 1 percent.

Troponin — which has subunits labeled I, T and C — and tropomyosin control binding between actin and myosin. When the fiber is at rest, the troponin sits on the tropomyosin, which in turn sits on the actin molecule in such a way that it blocks its binding site. But when the contraction-initiating calcium ions bind to troponin C, the system is displaced so that the tropomyosin is pulled off actin's binding site.

It's a complicated enough system. But as Nadal-Ginard, Roger E. Breitbart and their co-workers at Harvard have found, unraveling the genetics is even trickier. They are working specifically on the genetics of troponin T.

Troponin T comes in many different forms. The Harvard group has identified 10; in theory the gene has the capacity to encode an additional 54. "As far as we know, all can fulfill the same physical function," says Nadal-Ginard. But some may be "faster" than others — more responsive to the presence of calcium. In that case, a quicker move off the tropomyosin would result in a speedier contraction. However, quicker isn't always better. "There's a price to be paid for doing things faster," Nadal-Ginard explains. "The price for the heart in particular is that a faster contraction needs more oxygen." It's like a sports car: It may be faster, but it uses more gas.

Because they don't divide, heart muscle cells need to be plenipotentiary: The ones you're born with have to be able to grow and adapt to changing conditions both in the short term and over the course of a lifetime. A child's heart, for example, beats faster and works against a lower pressure than an adult heart. Even something as simple as going on vacation can significantly change the heart's work load.

The manufacture of troponin T begins normally — the DNA splits down the middle, and matching bits of RNA line up along it. The resulting RNA strand is essentially a mirror image of the DNA; the whole gene is transcribed, including pieces called introns that won't be a part of the final protein.

Sections that are represented in the final protein are called exons. Nadal-Ginard and his co-workers have found five exons at the beginning of the transcript and two at the end that may or may not wind up in the final protein. But the corresponding sections of protein al-

ways appear in the same order. If the segments are numbered 1 through 5, for example, there may be a 1,3,4, protein, or there may be a 2,3,4,5 protein, but there won't be a 3,1,4 protein.

The gene can be thought of as blocks connected by string. All of the blocks, they have found, have the same beginning and end, so that different combinations fit together. "You can remove blocks and still have a protein that makes sense," Nadal-Ginard says.

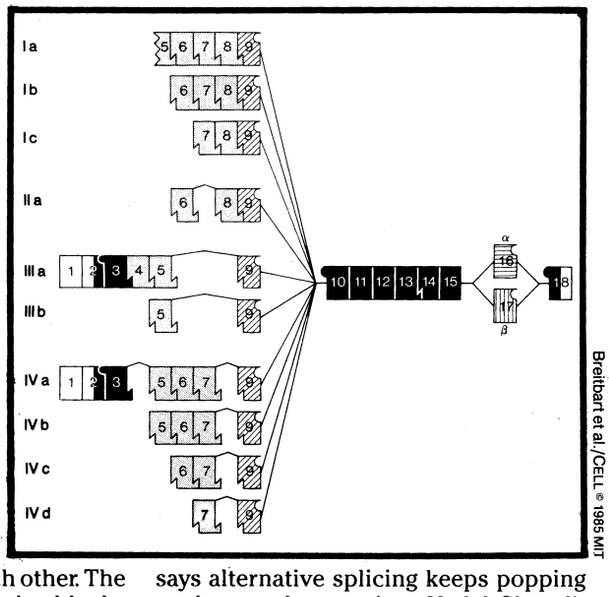
During the part of the process when the protein segments are lining up along the RNA, pieces of the string loop off and are left out of the protein-making. "We don't know for sure what controls this," says Nadal-Ginard.

Whether other proteins made by alternative splicing use the particular looping-off mechanism of the troponin system is possible but remains to be seen, says Nadal-Ginard. "In any system where generating diversity is important, this would be a good mechanism to have. You don't need a lot of DNA to do it – you've exploited the recombination power of DNA," he says.

Alternative RNA splicing also offers the advantage of speed. "It's difficult to turn on and shut off genes in a cell that doesn't divide," he says.

"To change from one gene to another in a cell that can't divide can take days to weeks," he says. But this method, he says, takes a matter of minutes.

Modular units of the troponin T gene fit together in various combinations to encode different proteins. The diagram represents the first 10 messenger RNAs found.



Breitbart et al./CELL © 1985 MIT

The process suggests one possible function of introns, the genetic sequences that do not code for proteins. Nadal-Ginard and his coworkers have found that introns between the "optional" blocks can match up with each other. The match-up pushes the intervening blocks out into the noncoding loops, suggesting that the pairing could be important in controlling gene expression. But they have also found similar matching sections in introns on the stable part of the gene, weakening the hypothesis.

Norman Davidson, a biologist at Caltech in Pasadena, who with Charles Rozek has found alternative splicing in myosin from fruit flies, says alternative splicing keeps popping up in muscle proteins. Nadal-Ginard's work, he says, "is one of the most fully documented and fully described."

When researchers first noticed alternative splicing, says Davidson, they thought it was exclusive to viruses. Then it was discovered in immunoglobulins and brain proteins. In muscle systems, he says, "Lo and behold, we keep running into alternative splicing.

"There's nothing heretical about the process," he says, "but it's still rather unusual." □