Muscular Dystrophies: Diseases of the Dystrophin-Glycoprotein Complex

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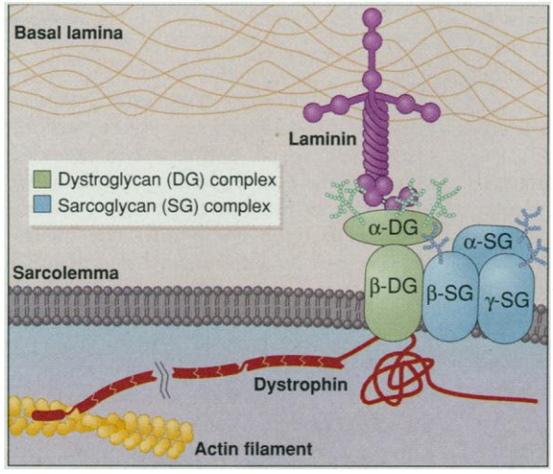
Muscular dystrophy. These words are devastating to parents hearing that their child has a genetic defect that dooms him or her to progressive muscle degeneration, loss of the ability to walk, often the loss of lung or cardiac function, and, frequently, early death. The muscular dystrophies, long known to be genetic in origin because of the transmission of the disease in families, can be X-linked, autosomal recessive, or dominant, but the precise genetic defect remained a mystery until 1986, when the gene that is defective in severe X-linked Duchenne muscular dystrophy (DMD) was identified. Its protein product, dystrophin, is a large rodlike cytoskeletal protein found at the inner surface of muscle fibers and is missing in DMD patients and altered or reduced in amount in the milder Becker muscular dystrophy (BMD). A simplistic model emerged in which dystrophin provided structural support to the muscle membrane; contraction-induced tearing of the membrane was the consequence of no dystrophin.

However, this only explained one form of the disease. What about the other muscular dystrophies? Enter the dystrophin-glycoprotein complex. Dystrophin is actually part of an elaborate protein complex that bridges the inner cytoskeleton (F-actin) and the extracellular matrix (laminin). It is now clear that defects in other proteins of this complex are responsible for the other forms of muscular dystrophy, as reported by Noguchi *et al.* (*1*) in this issue of *Science* and by Lim *et al.* (*2*) and Bonnemann *et al.* (*3*) in this month's *Nature Genetics*.

Campbell's laboratory initially described components of the dystrophin-glycoprotein complex as dystrophin-associated proteins (DAPs) and glycoproteins (DAGs) (*4*) and gave them names reflecting their approximate molecular weight: 156DAG, 59DAP, 50DAG, 43DAG, 35DAG, and 25DAP. Ozawa and his colleagues identified the same complex (*5*), referring to the proteins as A0, A1 (59DAP), A2 (50DAG), A3 (43DAG), and A4 (35DAG), separating A3 into two distinct proteins, A3a and A3b.

They further described two subcomplexes: the dystroglycan and sarcoglycan complexes (*6*).

The dystroglycan complex is composed of α- and β-dystroglycan (previously 156DAG and 43DAG/A3a) derived by proteolytic processing of a single precursor protein (*6*). α-Dystroglycan is extracellular and binds to merosin, a laminin subunit, in the basement membrane (*7*), and β-dystroglycan, a transmembrane protein, binds to the cysteine-rich and carboxyl-terminal domains of dystrophin inside the cell (*8*) (see figure).



The dystrophin-glycoprotein complex. Dystrophin contacts F-actin in the cytoplasm of the cell, and the dystrophin-glycoprotein complex forms a bridge across the membrane to the merosin subunit of laminin in the extracellular matrix.

The associated sarcoglycan complex is composed of α-, β-, and γ-sarcoglycan, a new nomenclature agreed upon by the field (*1–3*). In the old nomenclature these proteins were A2/50DAG (α), A3b (β), and A4/35DAG (γ). (α-Sarcoglycan has also been called adhalin.) All are transmembrane glycoproteins, but the nature of their relation to the dystroglycan complex is unclear. Proteins of both the dystroglycan and the sarcoglycan complex are deficient in muscle membranes from DMD patients. These patients are genetically deficient only for dystrophin, implying that dystrophin is necessary for the correct formation of the complex.

Alterations in the sarcoglycan complex now appear to be a significant cause of autosomally inherited muscular dystrophy, with phenotypes ranging from Duchenne-like muscular dystrophy to later onset limb-girdle muscular dystrophy (LGMD) (see table). The gene for α-sarcoglycan maps to chromosome 17, and four affected members of a French family with late-onset autosomal recessive muscular dystrophy (ARMD) have missense mutations in both alleles of this gene (*9*). Other mutations have been subsequently described in families with ARMD or LGMD (*10*), and in a survey of 30 patients with muscular dystrophy and normal dystrophin, only one showed mutation in the α-sarcoglycan gene, suggesting that less than 5% of non-Duchenne patients have defects at this locus (*11*).

In contrast, a large group of Tunisian patients with severe childhood autosomal recessive muscular dystrophy (SCARMD) show reduced immunostaining for α-sarcoglycan, but mapping of the Tunisian gene to chromosome 13 ruled out a genetic defect in the α-sarcoglycan gene. As now reported by Noguchi *et al.*, the γ-sarcoglycan gene maps to 13q12, and a deletion of a single base pair is found in both alleles of this gene in Tunisian SCARMD patients (*1*). This mutation results in a stop codon, eliminating the carboxyl-terminal end of the extracellular domain. In the muscle membrane of SCARMD patients, γ-sarcoglycan is absent and α- and β-sarcoglycan are deficient, indicating destabilization of the complex. Another mutation in a Japanese patient with sporadic muscular dystrophy suggested that alterations in the γ-sarcoglycan gene are not confined to the Tunisian patients.

The remaining member of the complex, β-sarcoglycan, has now also been cloned (*2*, *3*). The gene maps to 4q12 and has been found to be defective in a subset of patients with LGMD, a heterogeneous group of diseases with progressive weakness of pelvic

Protein	Location	Disease
Dystrophin	Xp21	DMD, BMD, mdx mouse
α-Sarcoglycan (50DAG; A2; Adhalin)	17q12-q21	ARMD, LGMD-2D
β-Sarcoglycan (43DAG; A3b)	4q12	ARMD, LGMD-2E
λ-Sarcoglycan (35DAG; A4)	13q12	SCARMD, LGMD-2C
Merosin (α-2 laminin)	6q22-q23	CMD, dy/dy mouse
Calpain-3	15q15	LGMD-2A

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and shoulder girdle muscles.

LGMD is genetically heterogeneous with a dominant form (LGMD-1A) mapping to 5q and four recessive forms (LGMD-2A, 2B, 2C, 2D) mapping to 15q, 2p, 13q, and 17q, respectively; the latter two co-map with the γ- and α-sarcoglycan genes. Lim *et al.* (*2*) have now demonstrated that LGMD in the old order Amish of southern Indiana is linked to markers on 4q, indicating the existence of another locus (LGMD-2E), and have shown that this locus is the β-sarcoglycan gene at 4q12. The homozygous missense mutation in the affected members results in loss of all three sarcoglycans from the muscle membrane. This result was surprising because the Amish of northern Indiana, despite a common European origin (the Canton of Bern, Switzerland), exhibit LGMD-2A caused by mutations in the gene at 15q15 that encodes the muscle-specific proteolytic enzyme calpain-3 (*12*).

Further evidence for β-sarcoglycan involvement comes from Bönnemann *et al.* (3), who analyzed cDNA from 62 unrelated dystrophin-positive patients with muscular dystrophy and found one individual with mutations in the β-sarcoglycan gene. This was a 3-year-old female with moderate muscle weakness and dystrophic changes similar to a DMD or SCARMD phenotype. One allele carried a stop codon mutation and the other an 8-bp duplication that resulted in a stop codon. Her muscle biopsy was positive for dystrophin immunostaining but negative for the three sarcoglycans, similar to the biopsies from the Amish, indicating that the sarcoglycan complex may act as a functional unit distinct from the dystroglycan complex. This complex is also deficient in the cardiomyopathic hamster, although a specific mutation has not yet been reported.

The dystrophin complex is clearly required for the maintenance of normal muscle. The sarcoglycan complex is certainly as important as dystrophin, and loss-of-function mutations in the dystrophin gene or any of the three sarcoglycan genes results in a severe phenotype. Mutations that cause partial loss of function (missense or non–frame-shifting deletions) in dystrophin produce a milder BMD phenotype and in the sarcoglycans appear to cause a mild LGMD. To complete the picture, loss-of-function mutations in the merosin gene have recently been described in severe congenital muscular dystrophy (CMD) (*13*), and merosin is deficient in the dystrophic dy/dy mouse (*14*, *15*). To date, no genetic lesions have been described for α- or β-dystroglycan that bridge between dystrophin and merosin.

The function of the complex remains a mystery. Is it merely structural, protecting the integrity of the membrane? Or do the proteins of the complex have other, nonstructural roles? Could they form the stretch-activated calcium channel that is defective in DMD muscle? Many questions remain without answers, but at least we now know the questions.

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Cracking the Neuronal Code

David Ferster and Nelson Spruston

To control behavior, the central nervous system employs approximately one trillion (10^{12}) neurons, all connected in networks of unfathomable complexity. The challenge for neuroscientists is to learn how these networks do their job. For decades, most neurophysiologists have assumed that a neuron's information content is contained solely in its firing rate, the number of action potentials it sends down its axon in any given period. An alternative view—that temporal firing patterns contain information—although considered somewhat heretical, is gaining attention as a result of new theoretical and experimental approaches.

Consider the firing pattern of the neuron in the figure. Three groups of 10 action potentials occurring in a 100-ms period travel down the axon, each group occurring in a different temporal pattern (three insets). According to the rate code hypothesis, the timing of each change in firing rate would indicate when an event occurred, and the strength of the increase might report how strong the stimulus was (*1*). In each case, however, the "what" of the stimulus would be the same; any single neuron could code for the presence of only a single stimulus property. By averaging the firing rates of a number of neurons responding to the same stimulus property, the nervous system could determine the strength of that stimulus at any point in time. By considering the activity of many such populations responding to different stimulus properties, the exact nature of a complex stimulus could be deciphered, as originally postulated in the line-labeling models of neuronal coding in peripheral nerves (*2*, *3*).

In contrast to the rate code model, the temporal code hypothesis holds that the firing pattern of an individual neuron could report different "whats," even while the average firing rate remained unchanged. A single neuron like the one in the figure could report the presence of three different stimuli with the three different temporal firing patterns shown in the insets. Such a temporal code could, in principle, resolve some of the apparent ambiguity of the information provided by neurons of the visual cortex. The response of each cortical neuron is dependent on many different features of a stimulus—for example, its orientation, its length, or its contrast. A rate code requires that the brain determine the exact nature of the stimulus by comparing the output of many different neurons; in a temporal code, one neuron could unambiguously code changes in a single feature of the stimulus by emitting one of a large repertoire of temporal output patterns (*4*).

Despite the appeal of packing a large amount of complex information into the spike train of a single neuron, the temporal code hypothesis has yet to be universally accepted. In many parts of the brain, neurons fire in highly irregular temporal patterns. But do such patterns encode different events, or are they merely random noise superimposed on a basic firing rate?

One approach to this question is to consider the mechanisms whereby irregular firing might arise. The long-standing "integrate and fire" models of neuronal function produce highly regular firing patterns (*5*). Two alternative models (*6*–*8*) have suggested different mechanisms for generating the highly irregular spike intervals observed in the visual cortex. One model is well suited to precise temporal coding; the other is a random process that precludes such coding.

The first model is based on the concept that rapidly rising depolarizations are required to trigger action potentials that accurately reflect the timing of synaptic in-

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