**All Eyes on RNA**

The list of RNA-binding proteins linked to amyotrophic lateral sclerosis is growing; RNA may also explain why a common mutation causes this fatal motor neuron disease—and a dementia

In the summer of 2011, a 3-year investigation into the genetics of amyotrophic lateral sclerosis (ALS) by research teams on two continents was coming to a head. This international consortium had narrowed the search for a mutation they knew caused ALS in families to just three genes on chromosome 9. Yet detailed and thorough gene analysis, using a variety of new sequencing technologies and massive computational power, failed to turn up any mutations that could be responsible.

Knowing that something unusual must lurk in this segment of DNA, Bryan Traynor, a geneticist at the U.S. National Institute on Aging in Bethesda, Maryland, took a closer look at one particularly suspicious stretch. When a computer algorithm clearly failed to correctly assemble the relevant DNA sequences of affected family members, Traynor arranged them manually. “I had to revert back to papyrus and pencil in order to actually work it out,” he jokes.

At that moment, Traynor became the first person to look upon the most common known cause of both ALS (sometimes called Lou Gehrig’s disease in the United States) and another, slightly rarer neurodegenerative disease called frontotemporal dementia (FTD). What he saw in the DNA of the ALS patients was the nucleotide sequence GGGGCC repeating itself over and over, far more than in unaffected family members. Because this type of mutation has been implicated in some other neurodegenerative diseases—repeated DNA sequences cause Huntington’s disease and fragile X syndrome, for example—Traynor was confident that the consortium’s long quest was over.

Indeed, a team led by Rosa Rademakers of the Mayo Clinic in Jacksonville, Florida, independently discovered the same repeat in the gene, provisionally dubbed C9ORF72, and the two groups published simultaneously in *Neuron* in September 2011. It quickly became apparent this was the most important ALS gene discovered to date, by far. The C9ORF72 mutation accounts for 40% of familial ALS and 21% of familial FTD. It has also been found in 7% of sporadic ALS, in which there’s no family history of the condition—the vast majority of cases—and in 5% of sporadic FTD. “For the first time, really, we are showing that genetics can underlie apparently sporadic disease,” Traynor says.

The whole field is waiting to find out how the mutation causes ALS—or how, in some people, indistinguishable mutations instead paradoxically trigger FTD. ALS robs a person of muscle control but spares the mind, whereas FTD does the opposite. (Many patients show symptoms of both diseases to varying degrees.)

The leading hypothesis is that the long DNA repeat spawns a bloated gob of RNA that creates a trap inside cells for one or more RNA-binding proteins necessary for a neuron’s function or survival. RNA-binding proteins have been under scrutiny in ALS since 2006, when one of them, TDP-43, was reported to make up the abnormal protein deposits, known as inclusions, found in motor neurons in almost all ALS cases (*Science*, 6 October 2006, p. 42). Mutations in the genes for TDP-43 and in FUS, a related RNA-binding protein, can cause ALS, and mutations in a third RNA-binding protein, ataxin-2, are a powerful risk factor for the disease.

Although ALS neurons suffer many types of dysfunction, the multiple recent discoveries have led to “a convergence of ideas” that errors in RNA processing are central to ALS and they could be linked to all the cellular problems, says biochemist Don Cleveland of the University of California (UC), San Diego. Others in the field caution that ALS might actually be several distinct diseases with different causes, RNA misprocessing being only one. Nevertheless, Cleveland says, “since last September, it’s been the most exciting time of discovery in ALS in the history of the planet.”

**Inward bound**

What RNA-binding proteins are doing—or not doing as the case may be—in ALS and FTD remains largely a mystery. That’s partly because each protein may bind so many RNAs, thousands in some cases, that it is hard to know which RNAs are important in disease. Among other duties, RNA-binding proteins trim, cap, escort, degrade, and otherwise process messenger RNA, which carries the transcribed DNA sequence from the cell nucleus to the cytoplasm for translation into protein. “There isn’t a step in the process [without] a halo of RNA-binding proteins surrounding the RNA,” says Robert Bowser, a neurobiologist at the Barrow Neurological Institute in Phoenix.

For ALS and FTD researchers, a key unknown is what triggers RNA-binding proteins to aggregate into the inclusions seen in the neurons and other nervous system cells of patients. One hypothesis is that the inclusions derive from stress granules, dense balls of RNA-binding proteins that arise normally in cells in response to cellular stress. Stress granules trap RNAs and prevent their translation into proteins, presumably to husband the cell’s resources until the stress is gone. “But as a consequence of having this great function, they’re more susceptible to aggregating...
in disease in an uncontrollable fashion,” says geneticist Aaron Gitler of Stanford University in Palo Alto, California. Normally, stress granules eventually dissolve and release the trapped RNAs, but under persistent stress—or owing to genetic risk factors—they may persist and transform into the massive cytoplasmic inclusions seen in ALS and FTD.

Genetic evidence supports this idea. In 2010, Gitler, then at the University of Pennsylvania (Penn), and colleague Nancy Bonini reported that about 5% of all ALS patients have a mutation in the gene encoding ataxin-2, an RNA-binding protein involved in stress granule assembly. More recent work suggests that mutated ataxin-2 indeed contributes to inclusion formation.

The six-nucleotide repeat sequence in the C9ORF72 gene may seed a different kind of aggregate—in the cell nucleus, not the cytoplasm. Researchers strongly suspect that this DNA sequence repeat, which is in a noncoding region of the gene, gives rise to an unnatural RNA structure that captures one or more RNA-binding proteins there. And the sheer number of repeat sequences—at least 30, and often hundreds or even thousands—could sequester enough protein to disrupt a neuron and provoke its demise. Indeed, Rademakers’s group has documented aggregates dubbed RNA foci in postmortem brain and spinal cord tissue from C9ORF72 mutation carriers.

“The race is really on to figure out what RNA-binding protein is being sequestered in there,” Gitler says.

There are precedents for such sequestration causing disease. The best-known case is myotonic dystrophy, an adult form of muscular dystrophy, in which an expanded three-nucleotide repeat sequesters an RNA-binding protein called muscleblind that’s involved in RNA splicing, forming RNA foci.

Whether or how RNA foci harm neurons is unknown. And researchers still can’t agree on whether the cytoplasmic inclusions in ALS and FTD neurons kill the cells because the captured RNA-binding proteins can’t carry out their normal function or because the aggregates gained a new, toxic function. The answer may be both. “I personally think that the best data out there indicate that it’s both loss and gain of function that’s important,” Gitler says.

**Unidentified captives**

Any definitive conclusion, says Zissimos Mourelatos, a pathologist at Penn, must wait until identification of the RNAs, if any, that are captured in the inclusions seen in people with ALS and FTD. Such work is now under way in several labs using a new technique called CLIP-seq. The technique uses ultraviolet radiation to create a chemical bond between RNA-binding proteins and their RNAs, enabling purification of the latter and then their sequencing and identification. In September, a group from UC San Diego reported in *Nature Neuroscience* that they had used CLIP-seq to reveal RNAs common to both TDP-43 and FUS. Several of these RNAs code for proteins important in synapse formation and function, suggesting that aggregation depleted these synaptic proteins, causing neurodegeneration.

TDP-43, FUS, and ataxin-2 won’t be the only RNA-binding proteins involved in ALS, Gitler and Mourelatos predict. “We think that this is going to be the tip of the iceberg,” Gitler says. Indeed, in September in *Acta Neuropathologica*, Bowser’s group reported finding inclusions containing the RNA-binding protein RBM45 in the cytoplasm of spinal cord cells from 21 of 23 ALS patients, versus none in seven control cases. (RBM45-containing inclusions were also present in all six FTD patients tested.) No mutations in the gene for RBM45 have yet been found in ALS, but that was also true of TDP-43 when first found in ALS and FTD inclusions back in 2006.

At Penn, Gitler’s group cloned the genes for almost 200 RNA-binding proteins and introduced them individually into yeast cells, checking for aggregation and toxicity. Gitler so far has found mutations in two of them, TAF15 and EWSR1, in several people with ALS or FTD, suggesting possible roles in the disease. “I’m pretty confident that there will be additional RNA-binding proteins that you’ll be seeing in the literature soon,” he says.

**What’s special about neurons?**

All this begs the question of why neurons are so sensitive to changes in RNA-binding proteins, while most other cell types appear unaffected. “It’s a big unknown,” says Mourelatos, who suggests that the large amount of gene splicing required for the generation of specialized protein isoforms at synapses, the connection points between neurons, could be a factor. “There’s a lot of RNA processing going on in neurons,” he says. Gitler speculates that the sheer length of motor neurons—in humans, a single motor neuron axon can extend several feet—may make them more dependent on RNA trafficking and processing.

Other researchers stress that, for ALS at least, defects in RNA processing may not be the whole story. “I think it’s a much more complicated disease than that,” cautions Lucie Bruijn, chief scientist at the ALS Association, which is headquartered in Washington, D.C. Bruijn cites mitochondrial dysfunction and defects in transport along motor neuron axons as two important features of ALS disease pathology. For example, a recent paper in *Nature* described ALS-causing mutations in the profilin-1 gene. Profilin-1 affects axon growth and regulates actin, an abundant and critical protein not involved in RNA processing. “It’s not clear to me how we would fit that discovery into an RNA-binding protein world,” Cleveland admits. “So it’s just a little perplexing.”

And one camp of ALS researchers maintains that it’s not RNA misprocessing but a failure of the cell’s protein-disposal system that causes the disease; mutations in several genes involved in protein clearance cause a small proportion of familial ALS. Most researchers believe that a combination of RNA misprocessing and failed protein clearance is responsible for most cases. “How the two meet up in the middle, we do not know,” Traynor says.

The current intense focus on disease mechanism should be accompanied by efforts to develop therapies now, even before these controversies are resolved, Bruijn says. The ALS Association, besides funding mouse models of the C9ORF72 expansion, is also collaborating with Isis Pharmaceuticals on developing “antisense” DNA drugs to bind and neutralize the repeat sequences. Although uncovering the mutation’s function is important, Bruijn says, “we might never find out exactly, or all agree. And maybe if we get rid of that large expansion there will be therapeutic benefit.”

—KEN GARBER

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