

Research Roundup

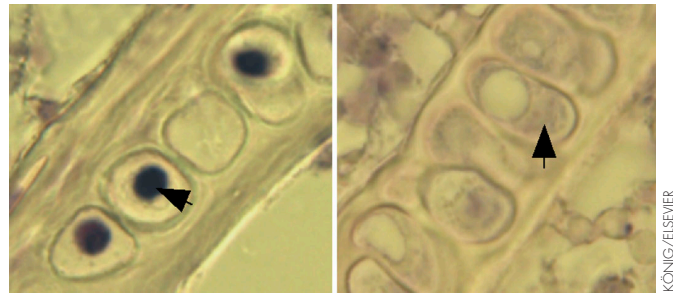
Minor spliceosome, major surprise: it's cytoplasmic

One of the great surprises of modern biology was the discovery of introns and the consequent understanding that gene transcripts are spliced to form mature messenger RNA (mRNA). A further surprise was the recent discovery that there are two kinds of splicing systems, the major and minor, which act on different types of introns. Now, Harald König, Ferenc Müller (Institute for Toxicology and Genetics, Karlsruhe, Germany), and colleagues uncover yet one more surprise: the minor system acts not in the nucleus, but in the cytoplasm.

The minor spliceosome, found primarily in plants and animals, edits less than 1% of all introns, which are characterized by unique sequences at the splice sites. All genes containing minor introns also contain major introns, which are processed by the major spliceosome in the nucleus. Despite their rarity, minor introns are evolutionarily conserved, suggesting they have some important properties. Genes with minor introns include the E2F transcription factors and genes of the MAP kinase pathway.

The authors used *in situ* hybridization in zebrafish and mammalian cells to show that snRNAs of the minor spliceosome are primarily cytoplasmic. mRNAs that had their major introns removed but still contained minor introns were transported to the cytoplasm. The minor spliceosome can be inhibited by an antisense morpholino that obstructs access to the minor introns. Attaching a nuclear export signal to the morpholino, so that it primarily localized to the cytoplasm, further inhibited the spliceosome.

The authors reason that its cytoplasmic location might allow the minor spliceosome to continue to function during mitosis, when the nucleus is in disarray. They found that transcripts containing major introns accumulated during mitosis



In zebrafish, snRNAs (black) of the major spliceosome accumulate in the nucleus (left), while those of the minor spliceosome are cytoplasmic (right).

as expected, but transcripts containing only minor introns did not, suggesting that minor splicing continues even while the nucleus is being reorganized.

Suppression of minor spliceosome activity suggested that the minor spliceosome might regulate cell proliferation. In zebrafish, its suppression halted development during the formation of muscle and vertebra precursors, apparently due to increased apoptosis and a block in cell cycle progression. In human cells, the suppression prevented cells from progressing beyond G1.

So why is the minor splicing system segregated to the cytoplasm? “The minor system is much slower,” says Koenig. “It could be that its cytoplasmic localization evolved to cope with that slower processing, by following partially spliced transcripts into the cytoplasm.” Its segregation and specialization may explain the evolutionary conservation of the minor spliceosome in the face of a far more efficient nuclear system. **JCB**

Reference: König, H., et al. 2007. *Cell*. 131:718–729.

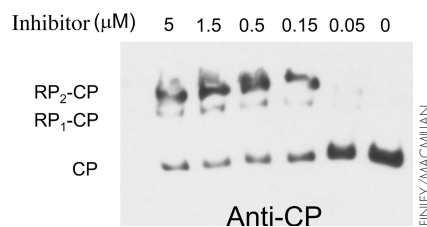
A chewing proteasome is stabilized

To do its job of degrading misfolded proteins, the proteasome's core particle (CP) and regulatory particle (RP) must link up. But what keeps them together while they work? According to new research by Maurits Kleijnen, Jeroen Roelofs, Daniel Finley (Harvard Medical School, Boston, MA), and colleagues, having something to chew on might keep the proteasome intact until the job is finished.

The proteasome's active sites sit deep within its core, far removed from its regulatory particles, which cap the ends of the proteolytic tunnel. Nonetheless, proteasome inhibitors that bind to the core's active site, such as epoxomicin, make it more likely that the core and regulatory units coprecipitate, suggesting that inhibitors may stabilize the interface between the two despite their distance.

To test this theory, the authors treated

purified proteasome constituents with apyrase, which destabilizes and inactivates the proteasome by hydrolyzing its bound ATP and ADP cofactors to AMP. With apyrase alone, the two complexes separated readily. But if the authors also added increasing concentrations of epoxomicin, the proportion of linked and active units increased. “No one had noticed this before,”

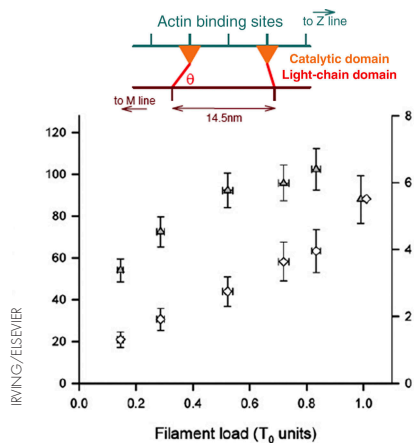


The proteasome remains assembled (RP₂-CP and RP₁-CP) in the presence of its inhibitor. But as inhibitor concentration falls, the core particle (CP) separates.

Finley says. “Only by following both the assembly state and the activity state at the same time can you see this effect.”

It is not yet clear whether the protein substrates normally degraded by the proteasome exert the same linking-activating effect, although Finley expects they will. The group is also not sure how a conformational change in the buried active site alters the binding of core and regulatory particles. Experiments to answer both questions are in progress. But it would make sense that protein substrates prevent subunit dissociation, Finley says, since once degradation of a protein begins, its stabilizing effect on the proteasome will both ensure the job gets finished and prevent harmful intermediaries from lingering in the cell. **JCB**

Reference: Kleijnen, M.F., et al. 2007. *Nat. Struct. Mol. Biol.* doi:10.1038/nsmb1335.



In a muscle fiber, myosin heads (orange triangles) attach to the actin filament. When loads are low, the motors pull with the same force (gray triangles), but fewer motors are attached (diamonds).

Myosin remains strong as muscle contracts

A muscle held at fixed length under a heavy load will contract rapidly if that load is suddenly decreased, reducing the force the muscle exerts as its velocity increases. During this contraction, what happens within the muscle fiber? According to the prevailing model, all the myosin motors remain attached to actin filaments, and the elasticity of individual myosins accounts for this force reduction—like a rubber band, they pull less as they contract further. New work by Malcolm Irving (King's College, London, UK), Vincenzo Lombardi (University of Florence, Italy), and colleagues now shows that, on the contrary, myosins maintain a constant force during shortening. Fewer, not weaker, myosins reduce the overall muscle force during shortening.

The authors combined precise mechanical measurements of individual muscle fibers with real-time x-ray diffraction, allowing them to measure myosin's force and velocity during contraction while imaging changes in the highly regular myosin array. They showed that only a proportion of myosins remained attached, reducing the total force generated by the fiber. Those myosins remaining attached to actin continued to exert a steady force even as they changed shape. This previously described shape change is an active process that continuously maintains the motor force.

These results are counter to a model proposed 50 years ago by Andrew Huxley, who suggested that the force reduction of a contracting muscle fiber was due to reduced force from individual myosins. But later in his career, Huxley also suggested that conformational changes in myosin would allow it to generate active force. Irving notes that the new model supports that concept.

Reducing the number of active myosins during low-load contraction makes sense, Irving says, since it matches ATP expenditure to muscle output. Since less force is needed, the cell can save on ATP by reducing the number of active myosins. **JCB**

Reference: Piazzesi, G., et al. 2007. *Cell*. 131:784–795.

Misfolding in muscle when neuron misfires

An overactive neuron can cause protein aggregation in its target cell, according to new work by Susana Garcia, Richard Morimoto (Northwestern University, Evanston, IL), and colleagues, indicating that actions of one cell may disrupt protein homeostasis in another.

The authors discovered that mutations in a transcription factor found only in neurons increased aggregation of polyglutamine-containing proteins in muscle cells in *C. elegans*. This factor, UNC-30, boosts synthesis of GABA, which inhibits neuronal firing. Increased protein aggregation also resulted from other GABA-reducing (and thus neuronal stimulating) mutations, including one in the muscle cell's GABA receptor.

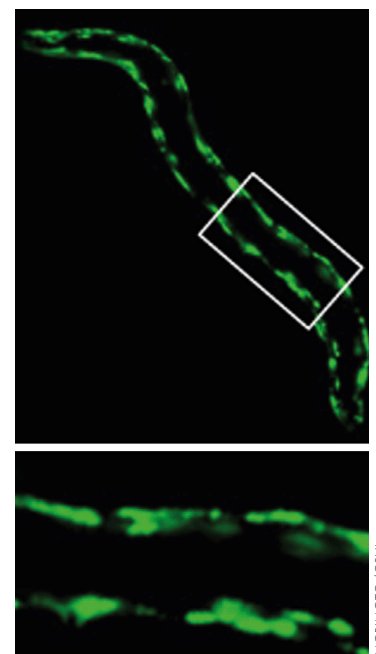
GABA's normal actions are counteracted by the stimulatory neurotransmitter acetylcholine. Mutation-induced overactivity of the acetylcholine system had the same effect on polyglutamine aggregation as too little GABA activity. Small molecules

also had similar effects: nicotine, which stimulates neurons, promoted aggregation, as did an insecticide called lindane, which inhibits GABA.

"It is a stunning surprise that a transcription factor expressed only in the presynaptic cell can have such a profound effect on aggregation in the postsynaptic cell," Morimoto says. The researchers are planning to seek evidence for similar effects in other communicating cells.

The polyglutamine protein used in these experiments contains just enough glutamines to be at the threshold of aggregation and is therefore exquisitely sensitive to outside influences. "Environment can play a big role in tipping the balance in such systems," says Morimoto. The effects may help explain why polyglutamine diseases such as Huntington's arise at different times in siblings with identical repeat lengths. **JCB**

Reference: Garcia, S.M., et al. 2007. *Genes Dev.* 21:3006–3016.



Mutation of a neuronal transcription factor that activates neurons causes protein misfolding and aggregation (green) in worm muscle cells.