NEWS.

spying on a cellular director in the cutting room

ANN ARBOR, Mich.—Like a film director cutting out extraneous footage to create a blockbuster, the cellular machine called the spliceosome snips out unwanted stretches of genetic material and joins the remaining pieces to fashion a template for protein production.

But more than box office revenues are at stake: if the spliceosome makes a careless cut, disease likely results.

Using a new approach to studying the spliceosome, a team led by University of Michigan chemistry and biophysics professor Nils Walter, collaborating closely with a team led by internationally recognized splicing experts John Abelson and Christine Guthrie of the University of California, San Francisco, spied on the splicing process in single molecules.

The research is scheduled to be published online March 21 in Nature Structural and Molecular Biology.

Since its Nobel Prize-winning discovery in 1977, gene splicing has been studied in a number of organisms, including yeast and human cells, using both genetic and biochemical approaches. While these methods can yield snapshots, they can't monitor the ongoing process. The new study, which utilizes a technique called fluorescence resonance energy transfer (FRET) and a sophisticated microscope that watches single molecules in action, allows researchers to observe in real time the contortions involved in spliceosome assembly and operation.

By molecular-scale standards, the spliceosome is a monster of a machine, made up of five RNA and 100 or more protein subunits that agilely assemble, step-by-step, into the giant complex when it's time to carry out its work.

True to the movie director analogy, the spliceosome not only wields the scissors, it's also "the brain that decides where to cut," Walter said. The "footage" it works on is the genetic material contained in RNA molecules.RNA carries coded instructions for producing the proteins our body needs for building and repairing tissues, regulating body processes and many other functions, but interspersed among stretches of code (called exons) are extraneous non-coding sections called introns. The spliceosome's task is to recognize and excise introns. Once the introns are removed, the spliceosome can stitch together exons in various combinations. Thanks to this mixing and matching of exons, a relatively small number of genes (a little over 20,000 in humans) can serve as blueprints for a great variety of proteins.

Walter and colleagues spied on the splicing process by attaching fluorescent tags to exons on either side of

an intron in a short section of RNA they designed specifically for such studies. When laser light is shined on the tags, FRET can detect how close together or far apart the exons are. Repeated observations over time result in a molecular-scale "movie" that reveals how parts of the RNA molecule wiggle around, both before and during splicing.

The researchers first studied the RNA in the absence of the spliceosome. "Conventional wisdom has been that the spliceosome directs the whole splicing process, that the RNA itself has little influence on it," Walter said. "But we saw the RNA molecule flexing on its own, with the intron folding and unfolding in a way that brings the exons closer together, suggesting a more active role for introns."

When the team added an extract containing spliceosome components, along with ATP—the energy currency that fuels spliceosome assembly—the distance between exons first increased, then decreased even more, and splicing occurred. Interestingly, the series of contortions that RNA went through during splicing was not a one-way path; the steps were reversible.

"Imagine the movie director having doubts about what scenes to cut and continuously going back and forth in holding different pieces of footage together before actually making a decision and splicing the film. That's what we saw happening at the molecular level," Walter said. "To our knowledge, our data provide the first direct glimpse of such reversible conformational changes during the splicing process."

Next, the researchers plan to attach fluorescent tags to different parts of the system to see how the various parts relate to one another in space and time during splicing. The eventual goal is to construct a comprehensive model showing how RNA and the spliceosome can so faithfully interact throughout the splicing process to avoid the onset of disease.

Walter's coauthors on the paper are U-M graduate students Mario Blanco, Franklin Fuller and Pavithra Aravamudhan; former graduate student Mark Ditzler; former undergraduate student Mona Wood; and Abelson, Guthrie, Tommaso Villa, Daniel Ryan, Jeffrey Pleiss and Corina Maeder of the University of California, San Francisco.

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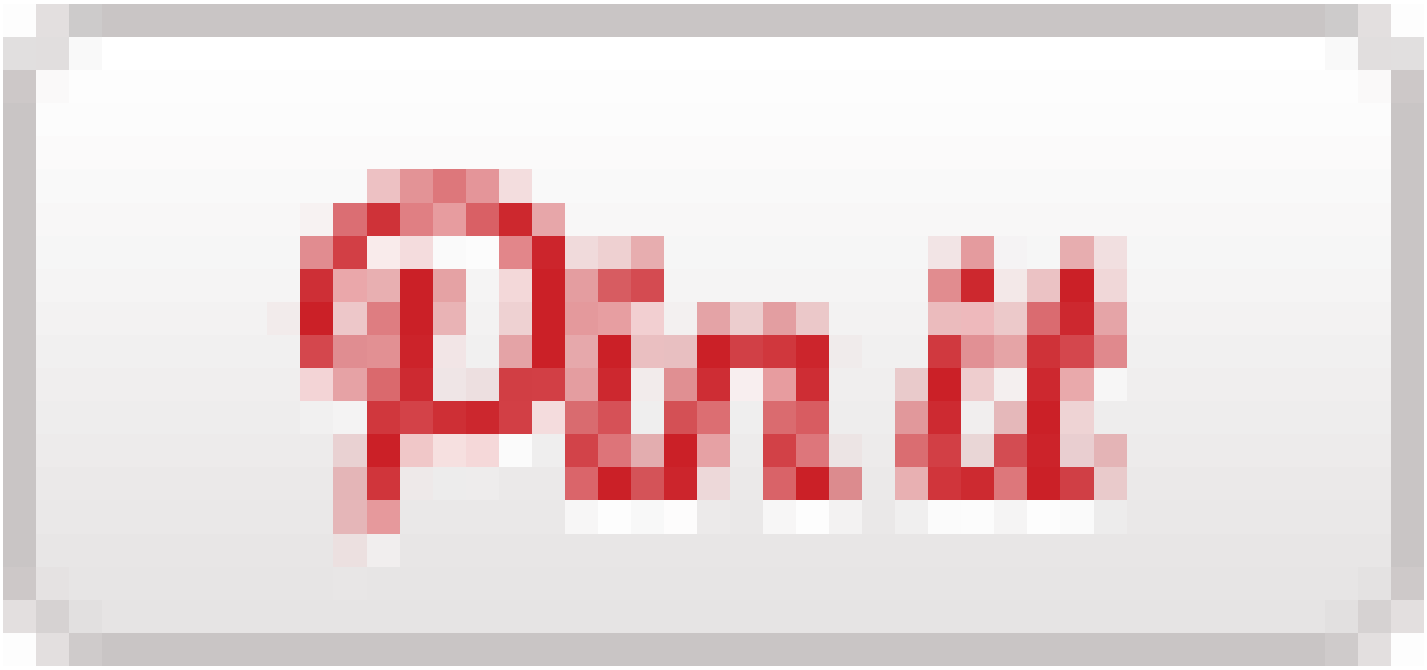
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