

A Conversation with Lynne Maquat

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Lynne Maquat is the J. Lowell Orbison Endowed Chair, Professor of Biochemistry & Biophysics, Founding Director of the Center for RNA Biology, and Founding Chair of Graduate Women in Science at the University of Rochester School of Medicine and Dentistry.

Richard Sever: You've been working on nonsense-mediated decay [NMD], which was something you discovered.

Dr. Maquat: It was toward the end of my postdoc—and also starting my own lab—when we discovered NMD in mammalian cells and began working out mechanism by studying mRNA half-lives using nucleated bone marrow cells from patients with β^0 -thalassemia.

Richard Sever: For those who have forgotten molecular biology, what is the problem that's being solved by NMD?

Dr. Maquat: In human diseases of the type that we studied, translation of an mRNA terminates prematurely. The disease-associated mutation is either a frameshift or a nonsense mutation that generates a premature termination codon, which, when recognized by a ribosome, triggers decay of the mRNA. This is a good thing in the sense that if a cell were to make truncated proteins, these proteins could be toxic. The open reading frame of the mutated mRNA is abnormally short, and therefore the encoded protein would be truncated, with the potential to gum up the cellular machine it works in. We figured out the rules for how a cell differentiates a normal termination codon, which generally doesn't trigger mRNA decay, from a premature termination codon, which generally does.

Richard Sever: How does the cell decide it's too short?

Dr. Maquat: Splicing an mRNA precursor, a pre-mRNA, in the nucleus deposits what we called a "mark" that persists until the first round of translation of the mRNA in the cytoplasm. We figured this out not only by studying β^0 -thalassemia and other human diseases, but also by generating reporters so we didn't have to rely on patient samples. Using the reporters, we could move introns around, and we could move premature termination codons around. We realized that there must be a mark that persists during that first round of translation, because we knew NMD, at least the type that depends on a mark, depends on the position of the premature termination codon relative to

where introns reside within nuclear pre-mRNA, is largely restricted to newly synthesized RNA, and depends on cytoplasmic translation.

Richard Sever: This is a protein complex that sits on a splice site?

Dr. Maquat: That's right. It resides on newly made mRNAs slightly upstream of where splicing occurred to generate the mRNA. When I collaborated with Melissa Moore on our hypothesis of a mark, working together with postdoc, Hervé Le Hir, who started in my lab and then moved to Melissa's lab, we renamed the mark the "exon junction complex" [EJC]. Hervé figured out that this complex of proteins is deposited on newly spliced mRNAs ~20–24 nt upstream of exon–exon junctions generated by pre-mRNA splicing. We figured out—and Joan Steitz's lab also figured out—that this complex consists of some of the NMD factors that we named UPF [up-frame-shift] proteins, after their orthologs in *Saccharomyces cerevisiae*.

So, depending on how many introns are in a pre-mRNA, you can expect to have that many exon junction complexes, one at each exon–exon junction. During the pioneer round of translation, if there is a termination event—it can be premature, or it can be at the normal termination codon—where one or more downstream EJCs remain, then the mRNA will be targeted for NMD.

Richard Sever: Basically, the cell's ordering the "stop" site, and it says "Wait a second, there's one behind where it should be."

Dr. Maquat: That's right. In the process of translation, the ribosome can remove EJCs. We figured out a rule before we even knew where the EJC was deposited. This rule said if translation terminates 50–55 nt or more upstream of an exon–exon junction, then the mRNA will be targeted for NMD. Now that we know where the EJC is deposited, and we know where the leading edge of the terminating ribosome would be situated relative to the termination codon, this rule makes sense.

As I've said, this happens during a pioneer round of translation. But that was another surprise, in the sense that when people at the time were thinking about mRNA translation, they were thinking about mRNA that had eukaryotic initiation factor [eIF]4E at the cap. It is true that mRNAs bound by eIF4E at the cap produce the bulk of cellular proteins. But because we knew NMD is largely restricted to newly synthesized RNA, and we knew that there is a different cap-binding protein that is acquired by the pre-mRNA, I asked postdoc Yasu Ishigaki to test if it was mRNA that has this different cap-binding protein, which is acquired very early on in mRNA biogenesis, that's still there during the pioneer round of translation. The answer turned out to be yes. So we define the pioneer round during which EJC-dependent NMD largely occurs as the translation of mRNA that's bound at the cap not by eIF4E, but by CBP [cap-binding protein] 80, and it's binding partner, CBP20.

It's a matter of timing, because we showed there's a precursor-product relationship between the pioneer round and subsequent steady state rounds of translation in mammals. We think of it as a completely different mRNA in the sense of the proteins that are associated with it when compared to the steady state translation initiation complex. The pioneer translation initiation complex not only has CBP80 and 20 at the cap but it also has the EJCs.

Richard Sever: You mentioned a group of UPFs in those EJCs. What are the proteins there, and how is this coordinated with degradation of the RNA?

Dr. Maquat: We've done a lot of work on understanding how proteins rearrange on an NMD target during the pioneer round of translation and the consequential decay steps. The key NMD factor is an ATP-dependent RNA helicase called UPF1. We find it either transiently or weakly associates with CBP80 at the 5' cap of the mRNA. To detect that interaction, we have to cross-link proteins before we lyse cells. We found that CBP80 escorts UPF1 to the translation termination complex together with a kinase—SMG1—that phosphorylates UPF1 not as a part of the termination complex, but later when CBP80 escorts UPF1 and its kinase to the EJC. The activation of NMD is when the kinase becomes free to function—that is, it's not inhibited—and it can then phosphorylate UPF1 at the EJC. That's critical.

Then there's a step of translational repression that we found to be important for decay—a feedback to stop more translation initiation once translation terminates and UPF1 becomes phosphorylated. We know the mechanism for that. While ribosomes continue to translate the open reading frame of the mRNA, there's an inhibition of further translation initiation events. Phosphorylated UPF1 goes back and prevents the 60S ribosomal subunit from coming in and joining any 43S translation initiation complex, which includes the 40S ribosome, poised at the translation initiation codon. If you don't have this prevention, you don't get decay.

Richard Sever: Right, and then you get these peptides.

Dr. Maquat: Then you get decay of the mRNA, and we know that decay can be from the 5' end, from the 3' end, and/or, endonucleolytic.

Richard Sever: You mentioned that NMD is not the only pathway for this. There are several parallel pathways?

Dr. Maquat: Proteins can multitask in cells, and the way that we discovered other unexpected pathways is by looking to see what else in the cell the RNA helicase, UPF1, interacts with. By yeast two-hybrid analysis, postdoc Yoon Ki Kim found that Staufien interacts with UPF1, and that there is a pathway we named Staufien-mediated mRNA decay (SMD) that's also dependent on UPF1 and translation. The way that UPF1 is recruited to an SMD target at a position situated downstream of what is normally the termination codon is via a Staufien-binding site. When translation terminates—usually normally, but it doesn't have to be—and Staufien isn't removed by the terminating ribosome, it is at that point that I think the SMD and NMD pathways converge. SMD is not restricted to newly synthesized RNA since there is no need for an EJC. SMD targets both the pioneer round and steady state rounds of translation.

Richard Sever: What's it being used for during the steady state rounds of translation?

Dr. Maquat: It is being used to regulate differentiation processes. To make it even more complicated, graduate student Chenguang Gong showed that NMD and SMD are in competition. They're in competition because UPF1 functions in both pathways in a mutually exclusive way. UPF1 can bind to either UPF2, which is important for NMD, or Staufien, which is important for SMD. During myogenesis, the efficiency of SMD goes up. That's important. For example, there's a natural SMD target encoding a protein that keeps myoblasts in an undifferentiated state, so myoblasts eliminate that mRNA by SMD, and thus its encoded protein, to differentiate to myotubes. And by having SMD be more efficient, NMD becomes less efficient. That's important because the expression of myogenin, which derives from a natural NMD target, promotes the differentiation process. The cell has evolved to use these pathways as a way to regulate not only myogenesis but other differentiation processes (e.g., adipogenesis).

Richard Sever: You've also worked on the regulation of apoptosis and the way in which this pathway was used to control that, but it didn't sound like decay was the aim. This wasn't an NMD process, but it was using the same machines. Is that correct?

Dr. Maquat: It is NMD. For apoptosis, postdoc Max Popp found that the cell reduces the efficiency of NMD by the caspase-mediated cleavage of UPF1, which up-regulates natural NMD targets that include those encoding proapoptotic proteins. But, we can ask, why do we have NMD? We constantly make mistakes when we process our pre-mRNAs. We have a lot of alternative splicing, we have a lot of alternative 3'-end formation, And, because of the

flexibility in those processes, we make mistakes that are eliminated by NMD. On top of that, I've been talking about natural NMD targets when mentioning differentiation processes and apoptosis. There are ~5%–10% of our messages that are naturally degraded by NMD. In some of these cases, there is an upstream open reading frame; in others there is a splicing event downstream from the normal termination codon, as two possibilities.

Richard Sever: So it's not really nonsense then?

Dr. Maquat: Well, yes it is. I wanted to call Staufen-mediated mRNA decay (Staufen-mediated NMD) and people said "No, 'nonsense' means 'PTC' [premature termination codon]." But actually, of course, it doesn't exclusively. It means "no sense," which applies to both PTCs and normal termination codons, which as we've just discussed can trigger NMD. It's a semantic issue.

Among those mRNAs that are up-regulated during severe DNA damage where it behooves the cell to trigger apoptosis are those that terminate normally. NMD is used as a rheostat by cells to adapt. Now, are all the natural NMD targets proapoptotic? No, but they, too, are up-regulated and translated. They are producing proteins, but it's background gene expression. It's not pertinent to the key process, which also has a lot of other apoptotic caspase-mediated events ongoing that are pertinent. Another way to think about NMD is that it maintains cellular homeostasis until a stress or another change in environment becomes sufficiently severe so that a response should be triggered. In these cases, often the efficiency of NMD is then decreased by one of many possible mechanisms, depending on the cell type and stressor, so as to promote the appropriate response.