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Pseudouridylation goes regulatory

U Thomas Meier

Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY, USA
Correspondence to: tom.meier@einstein.yu.edu

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RNA modifications are omnipresent among non-coding RNAs, so much so that pseudouridine (an isomer of uridine) was originally designated the 'fifth nucleoside'. Their role ranges from fine-tuning to being functionally essential to their target RNAs. Ever since their discovery some 60 years ago, RNA modifications were considered constitutive. In this issue of *The EMBO Journal*, however, Wu *et al* demonstrate that environmental stimuli induce pseudouridylation of spliceosomal small nuclear (sn) RNA U2 at novel sites impacting pre-mRNA splicing. After the regulatory modification of protein and of DNA, that of RNA now adds another level of complexity to the cellular signaling landscape.

Well over 100 different modifications decorate nucleotides in cellular RNA (Czerwoniec *et al*, 2009). Such modifications are particularly prominent in non-coding RNAs such as tRNA, ribosomal RNA, and snRNAs. Pseudouridylation (Figure 1, inset) and 2′-*O*-methylation far outnumber the other modifications. For example, mammalian ribosomal RNA contains about 100 (and U2 snRNA 13) pseudouridines at specific sites where they are important for proper biogenesis and function of the ribonucleoproteins (RNPs) defined by these RNAs (Yu *et al*, 1998; King *et al*, 2003). Relative to uridine, pseudouridine provides an additional opportunity for hydrogen bonding (Figure 1, inset, green) and renders the RNA backbone more rigid (Charette and Gray, 2000). Modifications are introduced right after synthesis at the polynucleotide level, co- or post-transcriptionally, and are believed to remain part of the RNA for its lifetime.

The cell uses two mechanisms for site-specific pseudouridylation, both relying on recognition of the nucleotides flanking the target uridine. In the first, single-polypeptide pseudouridine

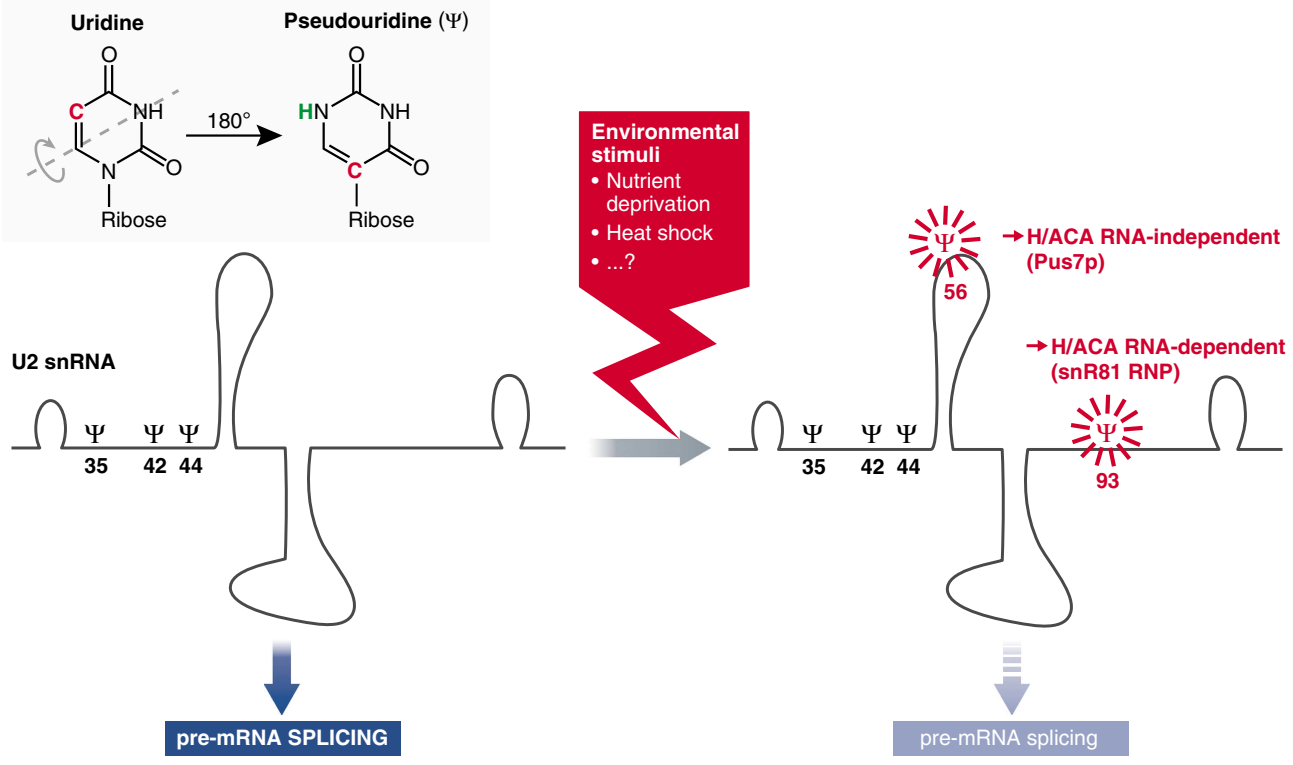

Figure 1 Inducible pseudouridylation of the spliceosomal snRNA U2 at novel sites 56 and 93 (red) by H/ACA RNA-dependent and -independent mechanisms impairs its function in pre-mRNA splicing. Isomerization of uridine to pseudouridine (inset), previously believed to be constitutive, occurs through 180° rotation of the base, yielding a carbon- (red) instead of a nitrogen-glycosidic bond and thus providing an additional opportunity for hydrogen bonding (green).

synthases specify the uridine and convert it to pseudouridine (Nurse *et al*, 1995); for tRNA this is the preferred modification mechanism. In the second, small nucleolar or Cajal body RNAs (collectively known as H/ACA RNAs) specify the target uridine by base pairing with its flanking nucleotides, while the conversion is catalysed by an associated pseudouridine synthase that—together with the guide RNA and three additional proteins—forms an H/ACA RNP (Kiss *et al*, 2010). Surprisingly, Wu *et al* (2011) now document that extracellular stimuli trigger the introduction of additional pseudouridines in yeast U2 snRNA, at novel sites and via both mechanisms (Figure 1).

The authors start out by using a semi-quantitative primer extension-based assay (Bakin and Ofengand, 1993) to identify at least two novel pseudouridines at positions 56 and 93 in yeast U2 snRNA isolated from nutrient-deprived cells. A quantitative, site-specific assay (Zhao and Yu, 2004) shows close to half of all uridines at position 93 being converted to pseudouridine under those conditions. In addition, pseudouridylation of uridine 56 can also be induced by heat shock.

What are the mechanisms for these inducible modifications? An inspection of the nucleotides flanking the novel pseudouridylation sites 56 and 93 in U2 snRNA revealed imperfect matches to those surrounding pseudouridine 35 in U2 snRNA and pseudouridine 1051 in 25S ribosomal RNA, which are isomerized by the protein-only Pus7p and the H/ACA-type snR81 RNP enzymes, respectively. Employing a decisive set of deletion and mutation experiments, the Yu group demonstrates these RNA-independent and -dependent enzymes indeed to be responsible for the inducible modifications at the novel sites (Figure 1). Moreover, the inducible modification is independent of the constitutive pseudouridylation mediated by these enzymes, indicating that the modification is direct and that one and the same enzyme isomerizes uridines flanked by perfectly and by imperfectly matching nucleotides. In fact, a series of mutations in the complementary sequences of snR81 RNA and/or U2 snRNA shows the requirement for imperfect base pairing (with two mismatches) for inducible modification. While necessary, such an imperfect match is, however, not sufficient, as targeting another uridine through an imperfect match does not result in its pseudouridylation upon nutrient deprivation.

It is remarkable that both RNA-dependent and -independent mechanisms rely on imperfectly matching flanking sequences—perhaps pointing towards a common evolutionary ancestor—and raising the question as to what makes these sites inducible—perhaps a common cofactor? With regard to the RNA-guided mechanism, future comparison with that of short inhibitory RNAs (which match their target perfectly) and of the related microRNAs (with an imperfect match) may reveal interesting parallels. Finally, the identification of imperfect guide sequences will make it even more challenging to develop reliable algorithms for the *in silico* identification of novel H/ACA RNAs and/or their target sites; on the other hand, it may allow matching up some of the many orphan H/ACA RNAs lacking recognizable targets with an actual target uridine.

What is the consequence of inducible pseudouridylation? To study the effect of pseudouridine at position 93 in U2 snRNA, the investigators used an artificial H/ACA RNA that guided pseudouridylation irrespective of growth conditions, i.e. constitutively, and combined it with a reporter pre-mRNA that when spliced conferred copper resistance (Lesser and Guthrie, 1993). Although pseudouridine 93 in U2 exhibited no significant impact on splicing of the wild-type reporter, it impaired splicing of a second-step mutant pre-mRNA (in which the consensus 3′ splice site was mutated), causing a growth defect on copper-containing medium. Even though the underlying mechanism remains to be uncovered, these data point to a role for inducible pseudouridylation in splicing regulation.

This initial discovery of inducible pseudouridylation, like many other discoveries, raises more questions than it answers. Are we just looking at the tip of the iceberg, i.e., is inducible RNA modification widespread, perhaps extending to pseudouridylation of mRNAs? Is it a general mechanism of fine-tuning cellular responses? Will it turn out to be reversible (akin to other long-considered irreversible modifications such as histone methylation)? Does it extend to other RNA modifications and organisms? Does it even contribute to the complex phenotype of the inherited bone marrow failure syndrome dyskeratosis congenita, which is characterized by mutations in H/ACA RNP components? Certainly, follow-up of this paradigm-shifting finding will keep researchers busy for years to come.

Conflict of interest

The author declares that he has no conflict of interest.

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