Molecular biology

Phosphorylation found inside RNA

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In an impressively thorough study, phosphorylation in the core of a transfer RNA molecule has been described for the first time, and the enzymes that add and remove the phosphate group have been characterized. See p.372

The addition or removal of a phosphate group is a well-established means of regulating the activity of protein molecules. Until now, enzymatic phosphorylation of RNA had been observed only at the molecule's ends. On page 372, Ohira *et al.*[1] report the internal phosphorylation of transfer RNA (tRNA) – a nucleic acid responsible for translating messenger RNA into protein. The authors' comprehensive study reveals that the properties of tRNA change after internal phosphorylation, improving its ability to participate in protein synthesis at high temperatures.

The backbone of RNA is composed of chains of alternating phosphate and ribose groups – the latter comprise cyclic sugars whose five carbon atoms are labelled 1′ to 5′. One of four bases attaches to the 1′ carbon of each ribose, forming a unit called a nucleoside (adenosine, guanosine, uridine or cytidine, depending on the base). The nucleosides are connected by phosphodiester bonds that link the hydroxyl (OH) group of the 3′ carbon atom of one ribose to the 5′-OH of the next. The occurrence of a 5′–3′ linkage is thought to have evolved because its formation is kinetically favourable compared with the alternative 5′–2′ linkage[2].

"The discovery of a writer and an eraser of RNA phosphorylation is a major advance."

Whereas phosphate groups are part of the RNA backbone, they are not common on the 2′-OH. Notable exceptions occur in temporary intermediates that form when mRNA (ref. 3) and tRNA (ref. 4) undergo a process called splicing, through which unwanted portions of the RNA sequence are removed. Notably, the 2′ phosphates in these intermediates result from molecular rearrangements, rather than from active placement by enzymes. They are then removed during the subsequent reaction steps and are not considered RNA modifications as such.

Ohira and colleagues set out to examine tRNA modifications in a group of microorganisms called archaea – specifically, in a species called *Sulfurisphaera tokodaii*, which grows at high temperatures. The authors analysed isolated *S. tokodaii* tRNA using mass spectrometry, and discovered a uridine with an unusual mass on its 47th nucleoside. The excess mass corresponded exactly to that of one phosphate group (Fig. 1). Chemical-structure analysis confirmed that this uridine is phosphorylated at its 2′-OH – a modification named $U^{p}47$. The authors demonstrated that the modification's presence stabilizes tRNA at high temperatures and increases the melting point of the tRNA molecule.

The authors also generated crystal structures of *S. tokodaii* tRNA with and without $U^{p}47$. The production of tRNA structures in both states is a remarkable feat, given the difficulties of RNA crystallization, which are brought about by RNA's highly negative charge and high instability under crystallization conditions. Comparing the two structures enabled Ohira and colleagues to glean details about the increased stability of the phosphorylated tRNA. Although moderate, the structural differences between the two molecules recall the structural rearrangements caused by phosphorylation in proteins – a widespread mechanism for regulating enzymatic activity known as allostery.

Phosphate groups are added to nucleic acids or proteins by enzymes called kinases and are removed by phosphatases. Ohira *et al.* identified a previously unknown kinase–phosphatase pair that acts as a writer and an eraser of the $U^{p}47$ modification. The discovery of these enzymes provides strong evidence that the 2′-phosphate has a biological effect (unlike the internal RNA phosphorylation that occurs as a by-product of splicing), and thus that it is a newly discovered post-transcriptional modification. The authors show that the kinase, named Ark1, acquires the phosphate group from the breakdown (hydrolysis) of the energy-carrying molecule ATP.

Ohira and colleagues' impressive study has a wide range of implications that span many fields of research. First, the discovery of a writer and an eraser of RNA phosphorylation is a major advance in the young field of epitranscriptomics, which focuses on RNA

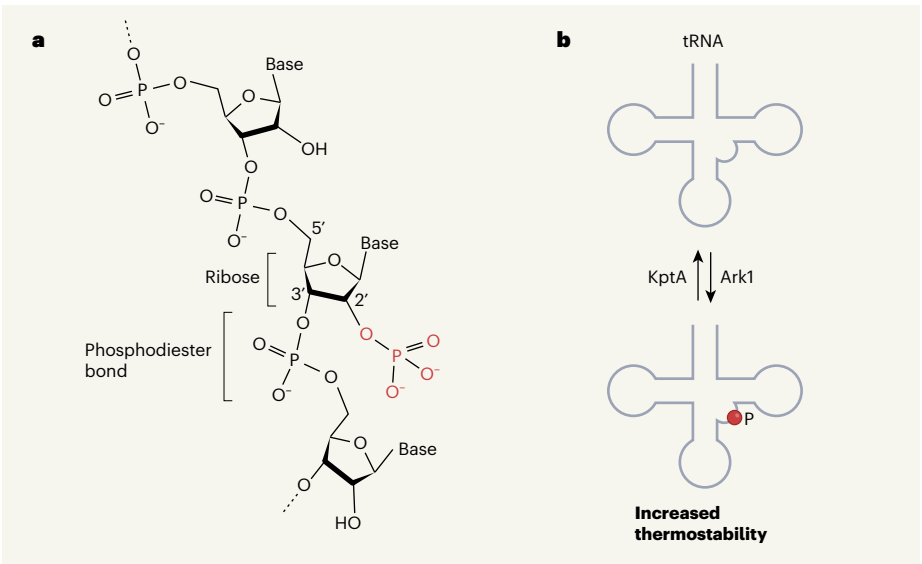

Figure 1 | Reversible internal phosphorylation of transfer RNA. a, The backbone of RNA is composed of chains of five-carbon ribose sugars linked by phosphodiester bonds. A base is attached to the 1′ carbon of each ribose. Studying the microbe *Sulfurisphaera tokodaii*, Ohira *et al.*[1] now find that tRNA can be modified by a phosphate group (P) on the hydroxyl group (OH) of the 2′ carbon of the 47th ribose (modification in red). **b**, The authors show that the phosphate group is added by the kinase enzyme Ark1, and can be removed by the phosphatase enzyme KptA. The modification alters the molecule's structure and biophysical properties, improving its stability at high temperatures.

modifications that do not modulate the underlying RNA sequence. The identification of these enzymes adds to a growing understanding of how RNA modification can help the cell respond to stressors such as extreme temperatures, antibiotics or low oxygen or nutrient levels. The central role of tRNA in protein synthesis makes its modification a particularly effective way to mediate stress responses. Stressful stimuli have already been shown to alter certain tRNA modification patterns in other types of organism — modification by methyl groups in eukaryotes (organisms whose cells have a nucleus)[5], and by methyl and acetyl groups in bacteria[6,7].The fact that tRNA phosphorylation has now been shown to mediate heat responses in archaea indicates the universality of epitranscriptome-mediated stress responses throughout the kingdoms of life.

The way in which Ark1 exerts its enzymatic activity by transferring a phosphate group from ATP to tRNA mirrors that of protein kinases, highlighting parallels between the post-transcriptional modification of RNA and that of proteins. However, although the concept of repeated writing and erasing on the same protein is essentially uncontested, it has not been confirmed for RNA, possibly because most work has been focused on mRNA, which has a short half-life. Is any RNA modification ever written and removed more than once on a given molecule? The fact that tRNAs are generally longer-lived than other RNA species makes multiple cycles of writing and erasing plausible — something that could be studied in future.

Finally, the discovery of a new type of reversible RNA modification, driven by a class of enzyme that is well understood and amenable to molecular engineering, offers tremendous potential for the field of synthetic biology. Researchers who currently use RNA-based enzymes (ribozymes) and molecular switches (riboswitches) to modulate cell behaviours can now use ATP hydrolysis to add another layer of regulation to their systems.

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M.H. declares competing interests. See go.nature.com/3dnzc57 for details.
This article was published online on 27 April 2022.