N6-methyldeoxyadenine (6mA) is a rare beast - in animals at least (retrospective on DOI 10.1002/bies.201500076)

The year 2015 brought a big surprise. Genomes of creatures composed of more than one cell were reported to carry N6-methyldeoxyadenine (6mA), a modified nucleobase hitherto thought to be present for the most part only in bacterial DNA. If true, how would 6mA come to be in DNA of reputable model organisms, including mice? And what would 6mA be doing there?

These were the basic questions that Sun and colleagues explored in a paper published in BioEssays with the title "*N6-methyladenine functions as a potential epigenetic mark in eukaryotes*" [1]. The *Insights & Perspectives* paper compared 6mA with the well-understood modified base 5-methyldeoxycytidine (5mC). The authors went on to speculate how a methyl group could be added to and removed from adenines and suggested where in the genome such processes might occur. Attention was called to genes that could be involved in 6mA-mediated epigenetic regulation.

The premise of the paper's hypothesis was "that DNA methylation plays conserved epigenetic roles in a wide array of organisms from bacteria to mammals". Importantly, the authors continued, "if the DNA is not modified at cytosine, animals likely use other types of methylated bases, such as 6mA, to fulfill the related function of 5mC in mammals".

Ideas abound, but do they stand the test of time? Two years have gone by since they were put forward. 6mA certainly contributed to an 'epigenetics gold rush' to discover novel base modifications in nucleic acids. Yet, little primary literature has been published about this particular chemical tag in animal DNA and this is puzzling.

It is puzzling, because two widely used model organisms – the worm (*Caenorhabditis elegans)* and the fruit fly (*Drosophila melanogaster)* lack cytosine methylation and therefore would be ideally suited to test the hypothesis that 6mA steps in to do what 5mC normally does. It is puzzling, because both of these model organisms have short generation intervals, which permit quick genetic screens. Sophisticated tools and techniques are available that would allow genetic dissection of pathways associated with establishing, maintaining and erasing 6mA and to observe what happens when this epigenetic mark is not present in the genome.

The scarcity of 6mA in DNA and the difficulty in detecting it could be one of the reasons for the sluggish output of follow-up research papers.

Sun and colleagues suggested that 6mA is also present in genomes of vertebrates, albeit at overall low concentrations. 6mA might have biological significance in a small number of lineage-specific cells and the authors showed an example of their on results, where a minute portion of heart cells in the mouse appeared 6mA-positive by immunostaining [1]. Indeed, three recent papers have since reported the presence of 6mA in DNA of frog *(Xenopus laevis),* zebrafish (*Danio rerio*) mouse, pig and human as well as in embryonic stem cells [2][3][4]. The prediction of Sun and colleagues that 6mA is present in many genomes of different phyla seems to hold up. It would establish 6mA as a universal epigenetic mark.

But doubts remain about the existence and significance of 6mA as a biologically relevant

epigenetic mark in animals - and for good reason. Using a novel ultrasensitive method it was not possible for Carell's research group to detect 6mA in DNA isolated from mouse ES cells, nor from differentiated adult tissues such as brain and liver [5]. Sensitivity of the detection level was so high that maximally 170 N-6 methylated adenosines could have escaped discovery from the entire mouse genome [5].

Will 6mA in animal DNA have a similar fate as Yeti, the legendary beast claimed to be dwelling in the Himalayan mountain range? Will 6mA become a scientific folklore that captured our imagination for a short period in the 21st century? It may still be too early to say if reports of 6mA in animal genomes fall into the category of data misinterpretations or if the rare sightings – similar to mysterious tracks in the snow - are founded on grains of truth.

References

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