

Uracil in DNA: error or signal?

Uracil is well known as one of the bases used in RNA, but why is it not used in DNA – or is it? **Angéla Békési** and **Beáta G Vértessy** investigate.

Endopterygotes such as ants lack the enzyme capable of removing uracil from their DNA

Image courtesy of spXChrome / iStockphoto and Nicola Graf

Thymine versus uracil

Our genetic information is stored in the form of DNA, using a four-letter alphabet. The four 'letters' correspond to the four chemical bases that each building block of DNA – called a nucleotide – can have: adenine (A), thymine (T), cytosine (C) and guanine (G; see Figure 1 on page 28). As James Watson and Francis Crick famously

discovered, DNA forms a double helix in which the four bases always pair up the same way, through specific hydrogen bonds: adenine binds to thymine, and guanine to cytosine (see Figures 2 and 3).

There is an alternative fifth letter, though: uracil (U), which forms the same pattern of hydrogen bonds with adenine (see Figure 4). But although



- ✓ Biology
- ✓ Genetics
- ✓ Immune system
- ✓ Insect development
- ✓ Cell proliferation
- ✓ General cytology
- ✓ Enzyme pathways
- ✓ Cancer research
- ✓ Ages 16+

This article demonstrates that science never sleeps, shaking up the dogma that uracil only exists in RNA. As the article explains, this is not always the case. And even when it is, why should that be?

To help the students understand the article, guiding questions could be:

1. Describe the bonding structures between the two complementary base pairs in DNA.
2. Which of the bases is replaced in RNA?
3. Describe and draw a graph of the repair enzyme pathway triggered when uracil is found in DNA.
4. The ratio of which molecules could be adjusted to stop cancerous cells from growing and dividing?
5. Why is uracil 'tolerated' in RNA?
6. Which living organisms use uracil DNA and how?

*Friedlinde Krotscheck,
Austria*

REVIEW

Figure 1

The key components of a nucleotide, the basic building block of DNA. The sugar deoxyribose and the phosphate group are invariant, whereas the organic base can be of one of four types: A, T, G and C

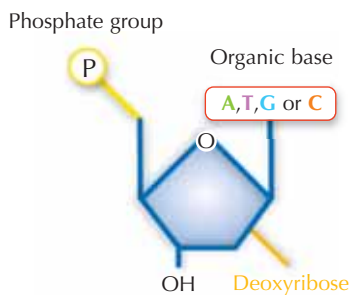


Image courtesy of Nicola Graf

Figure 2

The chemical structure of DNA, showing the base-pairings A-T and G-C. The hydrogen-bonded bases link together the two sugar-phosphate backbones

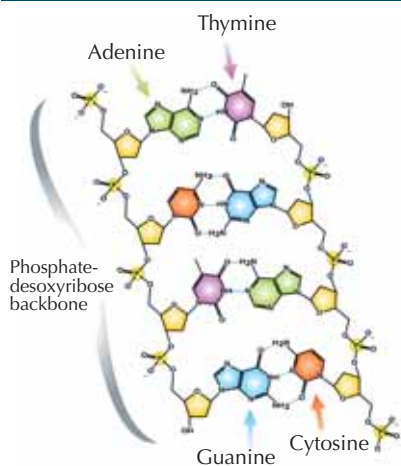


Image courtesy of Madeline Price Ball; image source: Wikimedia Commons

Figure 3

The double helix structure of DNA

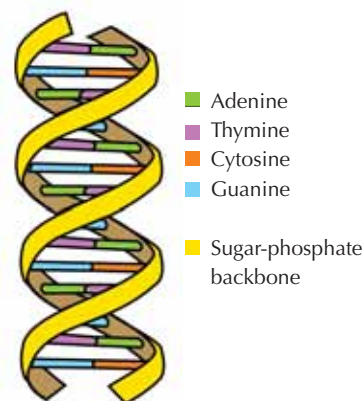


Image courtesy of Forlvovf; image source: Wikimedia Commons

uracil is commonly used in RNA, this is not the case in DNA, where thymine is used instead. Why might this be?

Chemically, thymine is a uracil molecule with an extra methyl group attached. What would be the advantage, in evolutionary terms, of using this more complex building block in DNA? The answer may lie in how cells correct damage to DNA.

Cytosine can spontaneously turn into uracil, through a process called

hydrolytic deamination (see Figure 4). When this happens, the guanine that was initially bound to that cytosine molecule is left opposite uracil instead (remember that uracil normally binds to adenine). When the cell next replicates its DNA, the position opposite this uracil molecule would be taken up by an adenine instead of the guanine that should be there, altering the message that this section of DNA encodes (see Figure 5). This process of cytosine deamination is

one of the most common types of DNA damage, but is normally corrected effectively. How does the cell do this?

Cells have a repair system that can detect when a uracil is sitting where a cytosine should be, and correct the mistake before it is replicated and passed on. The complex machinery to do that consists of several enzymes: first uracil-DNA glycosylases recognise the uracil, and cut it out of the DNA. Then several enzymes contribute to the elimination and re-synthesis of the damaged part of DNA, during which the abasic ('empty') site in the DNA is replaced with a cytosine (see Figure 6).

However, the most common form of uracil-DNA glycosylase cannot tell which base the uracil is paired with, i.e. whether the uracil was intended to be there (if bound with adenine) or if it is a mutated cytosine (and is opposite guanine); instead, it would recognise and cut out both types of uracil. Clearly, this would cause prob-

Figure 4

Guanine and cytosine form a base pair stabilised by three hydrogen bonds, whereas adenine and thymine bind to each other through two hydrogen bonds. The red frames highlight the functional groups of cytosine and thymine that are responsible for forming the hydrogen bonds. Cytosine can spontaneously undergo hydrolytic deamination, resulting in a uracil base with the same capability for hydrogen bond formation as thymine

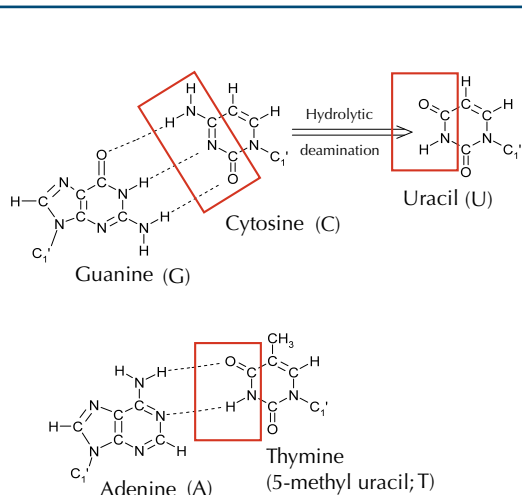


Image courtesy of Angéla Bekési



Image courtesy of taramol / iStockphoto

Figure 5

Hydrolytic deamination of cytosine can change the amino acids encoded by the sequence

Image courtesy of Nicola Graf

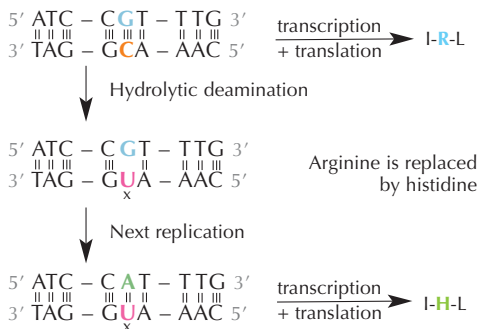
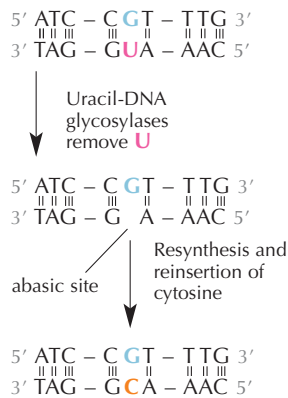


Figure 6

Repair of hydrolytic deamination



Thymine-less cell death

When DNA is synthesised, the DNA polymerase enzymes (which catalyse the synthesis) cannot discriminate between thymine and uracil. They only check whether the hydrogen bonds form correctly, i.e. whether the base pairs are matched properly. To these enzymes, it does not matter whether thymine or uracil binds to adenine. Normally, the amounts of deoxyuridine triphosphate (dUTP, a source of uracil) in the cell are kept very low compared to levels of deoxythymidine triphosphate (dTTP, a thymine source), preventing uracil incorporation during DNA synthesis.

If this strict regulation is perturbed and the ratio of dUTP to dTTP rises, the amount of uracil that is incorrectly incorporated into DNA also increases. The repair system – which, unlike DNA polymerases, can distinguish uracil from thymine – then attempts to cut out the uracil with the help of uracil-DNA glycosylase and to re-synthesise the DNA, which involves temporarily cleaving (cutting) the DNA backbone. However, if the ratio of dUTP to dTTP is still elevated, this re-synthesis may again incorporate uracil instead of thymine. This cycle eventually leads to DNA strand breaks and chromosome fragmentation, when these temporary cuts in the DNA happen one after the other and too close to each other (see Figure 7). This results in a specific type of programmed cell death, called thymine-less cell death.

The process of thymine-less cell death can be deliberately exploited in the treatment of cancer. Because cancer cells proliferate at such a high rate compared to normal cells, they synthesise a greater amount of DNA per given time period and therefore require large amounts of dUTP. By raising the ratio of dUTP to dTTP, these cancer cells can be selectively targeted and eliminated.

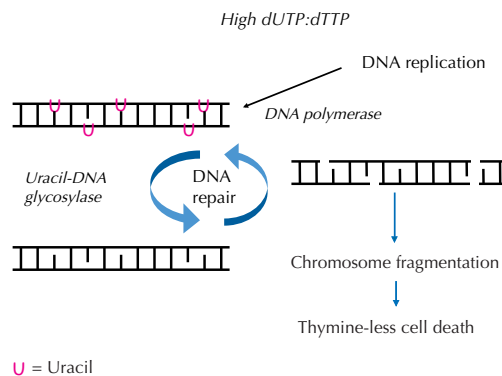
lems. The solution to this potential problem is thought to have been the evolution of a mechanism in which 'correct' uracils (paired with adenine) were labelled with a methyl group – resulting in thymine. This way, if the cell machinery found a uracil, it cut it out and repaired it, but if it found a uracil with a methyl label – a thymine (see Figure 4) – it left it. Over time, therefore, thymine in DNA became the standard instead of uracil, and most cells now use uracil only in RNA.

Why was uracil retained in RNA? RNA is more short-lived than DNA and – with a few exceptions – is not the repository for long-term storage of genetic information, so cytosine molecules that spontaneously turn into uracils in RNA do not present a great threat to the cell. Thus, there was probably no evolutionary pressure to replace uracil with the more complex (and presumably more costly) thymine in RNA.

Figure 7

Image courtesy of Angéla Békési

If dUTP:dTTP increases, DNA polymerase frequently incorporates uracil instead of thymine during both replication and repair. Uracil-DNA glycosylase removes the uracil and initiates further repair involving DNA strand breaks in an intermediate step. Repair synthesis, however, may reintroduce uracil, leading to a futile DNA repair cycle. Eventually, the system is overloaded and chromosome fragmentation occurs, leading to cell death



Uracil DNA still exists

Although most cells use uracil for RNA and thymine for DNA, there are exceptions. Some organisms have uracil instead of thymine in all their DNA, and other organisms have uracil in only some of their DNA. What could be the evolutionary advantage of that? Let's take a look at some examples.

Uracil in viral DNA

Two species of phage (viruses that infect bacteria) are known to have DNA genomes with only uracil and no thymine. We do not yet know whether these phages are representatives of an ancient life form that never evolved thymine DNA, or whether their uracil-substituted genomes are a newly evolved strategy. Nor do we know why these phages use uracil instead of thymine, but it may play an essential role in the life cycle of these viruses. If that is the case, it would make sense for the viruses to ensure that the uracil in their DNA is not replaced with thymine. And one of these phages has in fact been shown to have a gene that encodes a specific protein to inhibit the host's uracil-DNA glycosylase, thus preventing the viral genome from having its uracil 'repaired' by the host enzymes.

Programmed cell death in insect life cycles

Uracil-DNA also appears to play a role in the development of endopterygotes – insects that undergo pupation during their life cycle (ants and butterflies do;

grasshoppers and termites do not).

These insects lack the main gene for uracil-DNA glycosylase, which would otherwise remove uracil from their DNA.

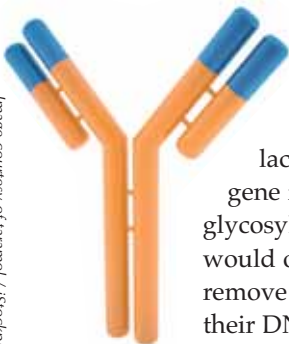
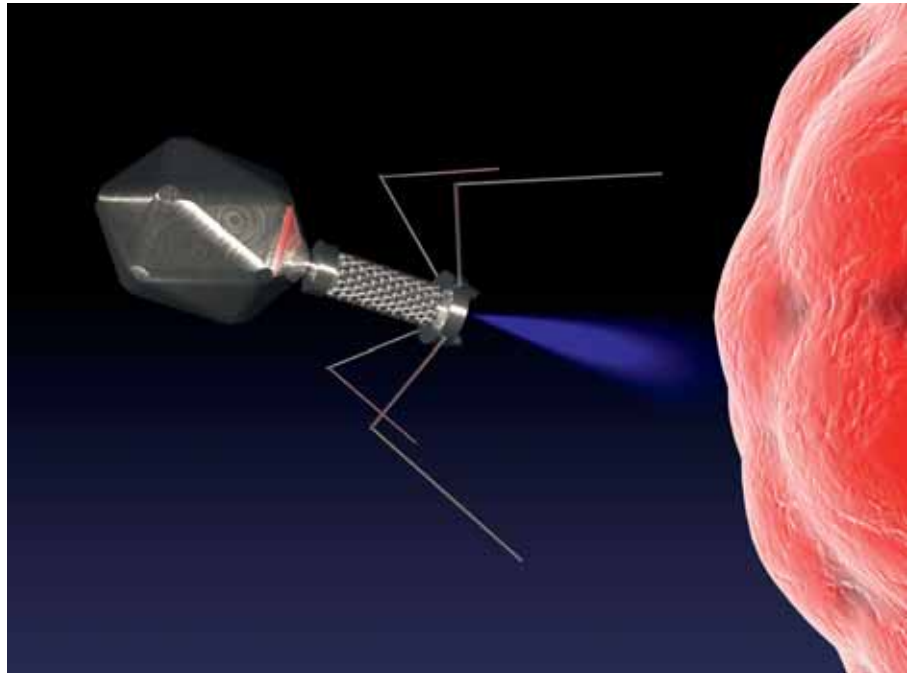


Image courtesy of iananol / iStockphoto

Image courtesy of cdascher / iStockphoto



Artist's impression of a phage virus infecting a bacterial cell

Moreover, our own research has shown that, in larvae of the fruit fly *Drosophila melanogaster*, the ratio of dUTP to dTTP is regulated in an unusual manner: in all tissues that will not be needed in the adult insect, there are much lower levels of the enzyme that breaks down dUTP and generates a precursor for dTTP production. Consequently, significant amounts of uracil are incorporated into these tissues during DNA synthesis.

So during the larval stages, uracil-DNA is produced and seems not to be corrected in tissues that are to be degraded during the pupal stage. As these insects lack the main uracil-DNA glycosylase enzyme, at the pupal stage, additional uracil-DNA-specific factors may recognise this accumulated uracil as a signal to initiate cell death. We have already identified an insect-specific protein that seems to be capable of degrading uracil-DNA, and we are investigating whether this enzyme is used to initiate programmed cell death.

Beneficial errors: the vertebrate immune system

Uracil in DNA, however, can also be found closer to home – in the immune system of vertebrates like us. Part of our immune system, the adaptive immune system, produces a large number of different antibodies that are trained to protect us from specific pathogens. To increase the number of different antibodies that can be created, we shuffle the DNA sequence in the regions that code for them, not only by recombining the existing sequences in the cells but also by creating new ones through vastly increased mutation rates, known as hypermutation.

Hypermutation starts with a specific enzyme (an activation-induced deaminase) that changes cytosine into uracil (see Figure 4) at specific DNA loci, eliciting an error-prone repair response, which the organism uses to its advantage: 'errors' generate new sequences that can be used to make different antibodies. This system is very strictly regulated, however, as if it got out of hand, it would lead to cancer.

When considering the question of why uracil or why thymine, we need to consider the evolutionary context. Living organisms have evolved in a continuously changing environment, facing a dynamic set of challenges. Thus, a solution that avoids mistakes being incorporated into DNA is advantageous to most organisms and most cells, which explains why thymine-DNA became the norm. Under certain circumstances, however, 'mistakes' themselves can be beneficial, which is why some cells still use uracil in their DNA.

Resources

To find out more about the work of Beáta Vértessy's research group, see: www.enzim.hu/~vertessy

To download a summary of Villő Muha's PhD thesis, which was written under Beáta Vértessy's supervision and focuses on uracil-DNA in *Drosophila melanogaster*, use the link: http://teo.elte.hu/minosites/tezis2010_angol/v_muha.pdf

The complete thesis is available here: http://teo.elte.hu/minosites/ertekezes2010/muha_v.pdf

If you enjoyed reading this article, why not take a look at the full collection of biology-related articles published in *Science in School*? See: www.scienceinschool.org/biology

Angéla Békési was born 1977 in Kaposvár, Hungary. In 2001, she graduated in chemistry from the Eötvös Loránd University of Sciences, and in theology from the Pázmány Péter Catholic University (both in Budapest, Hungary), having joined the lab of Beáta Vértessy in 2000 as an undergraduate student. In 2001, she began a PhD on the regulation of uracil-DNA repair and uracil processing in pupating insects. She identified a new protein candidate for a novel type of uracil-DNA sensor and

received her PhD in structural biochemistry from Eötvös Loránd University of Sciences in 2007. She is continuing her work as a postdoctoral scientist, and was a school ambassador in the SET-Routes programme (www.set-routes.org/school/profiles/bekesi_en.html).

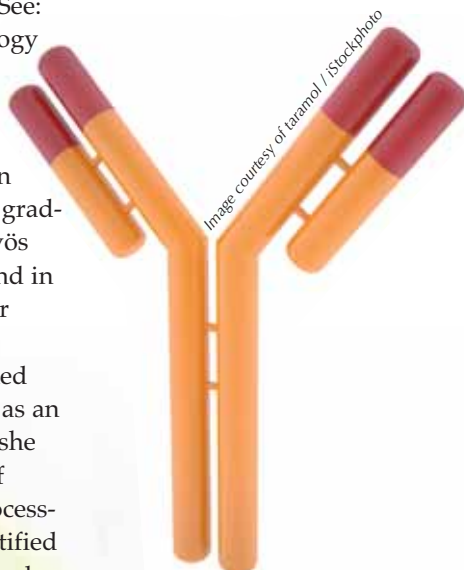
Beáta G Vértessy was born in Budapest, Hungary and was trained in the biological sciences. She has an MSc from the University of Chicago, USA, a PhD / CSs from the Eötvös Loránd University in Budapest, Hungary, and the Hungarian Academy of Sciences, and a DSc from the Hungarian Academy of Sciences.

Since 2000, she has been the head of a laboratory focusing on genome metabolism and repair at the Institute of Enzymology, Budapest, Hungary. The lab's current research aims to understand the prevention, recognition and repair of uracil in DNA from the perspectives of structural and cell biology.



To learn how to use this code, see page 1.

The presence of uracil in antibody gene sequences elicits a DNA repair response, which has the effect of increasing antibody protein diversity. An extensive antibody pool increases the chance of the immune system recognising unwanted invaders



EIROforum Teachers School 2011 9-12 October 2011



EIROforum invites European science teachers to attend a three-day course on the 'Physics and chemistry of life' at the European Photon and Neutron Science Campus in Grenoble (France).

The course lectures, tutorials and practicals will be given by top scientists from EMBL, ESRF, ILL and the European XFEL.

Information and application on <http://www.epn-campus.eu/eiro-teachers-school>

