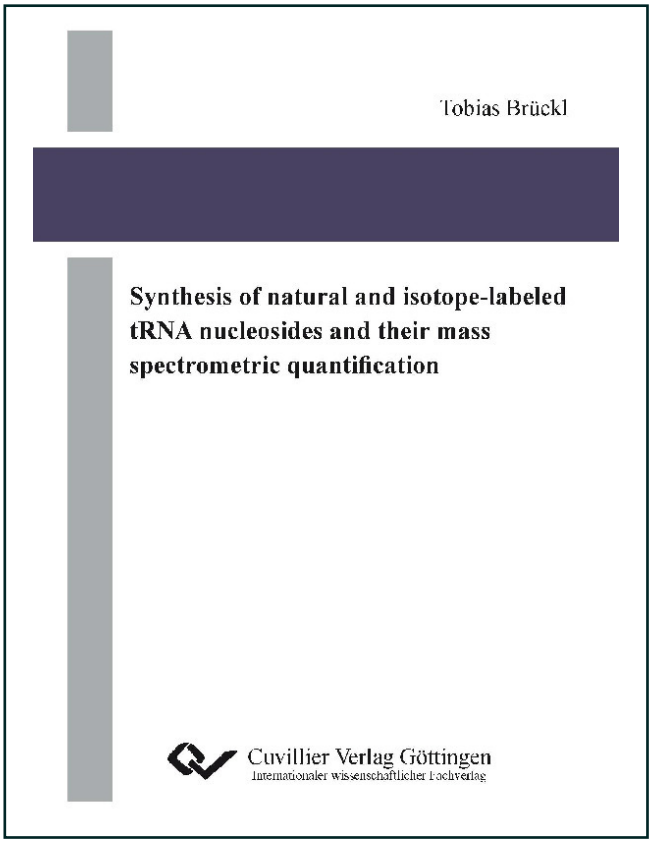
Tobias Brückl (Autor)

Synthesis of natural and isotope-labeled tRNA nucleosides and their mass spectrometric quantification



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3 Introduction

3.1 Natural modified nucleosides in DNA and RNA

Nucleic acids are the central "devices" in biological "information technology". Present textbooks usually content themselves with four nucleosides for deoxyribonucleic acids (DNA) and ribonucleic acids (RNA) each: deoxyadenosine (dA), deoxycytidine (dC), deoxyguanosine (dG), and deoxythymidine (dT) for DNA and adenosine (A), cytidine (C), guanosine (G), and uridine (U) for RNA. This general statement, although sufficient for an understanding of the biological functions and processes involving nucleic acids, is all but exhaustive. Both DNA and RNA contain so called modified nucleosides. These are derived from the canonical ones but feature further substituents. Their additional groups, which are attached either to the heterocycle or to the C2'OH, range from simple methyl groups to advanced ring systems. Modified nucleosides are involved in the most fundamental processes in cells: inheritance, transcription, and translation.

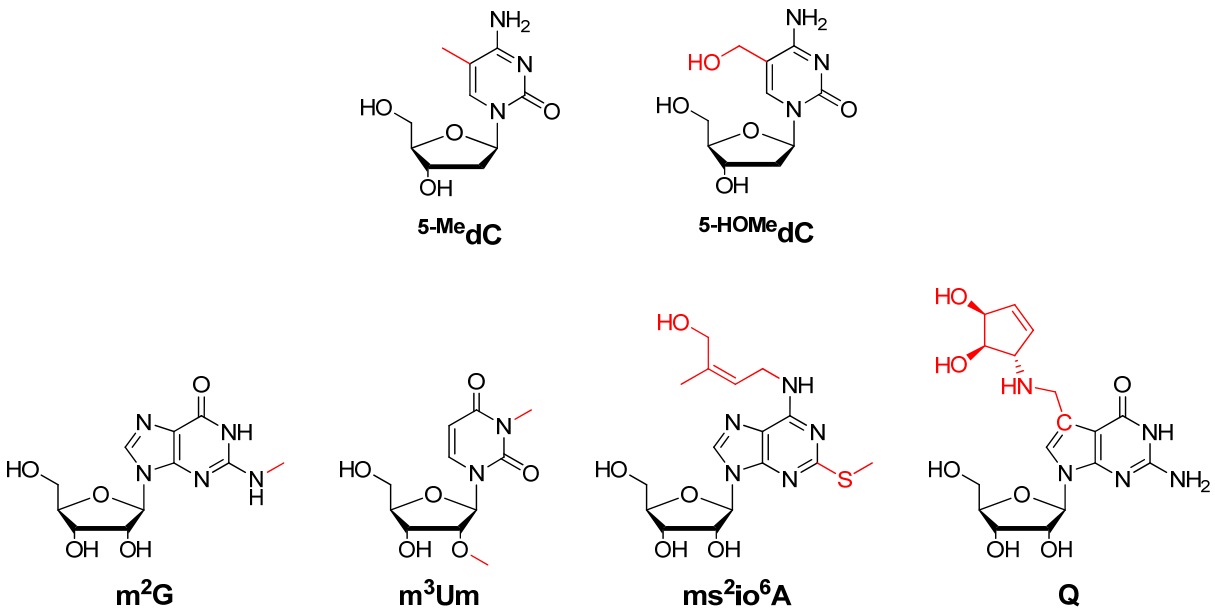


Figure 4: Structures of exemplary DNA and RNA modifications.
DNA modifications: $^{5\text{-Me}}$dC and $^{5\text{-HOMe}}$dC, upper row; RNA modifications: m^2G, m^3Um, ms^2io^6A, and queuosine (Q); bottom row. Alterations relative to the canonical nucleoside are indicated in red.

To date two modified nucleosides are known in DNA: 5-methyl deoxycytidine ($^{5\text{-Me}}$dC) and 5-hydroxymethyl deoxycytidine ($^{5\text{-HOMe}}$dC). The modification $^{5\text{-Me}}$dC is one of the key players in epigenetics and thus involved in the control of transcriptional activity.[1] Special

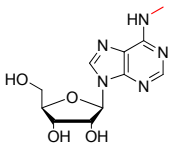
methyltransferases replace the H-atom at position C5 by a methyl group to form $^{5\text{-Me}}$dC.[2] Methylation occurs in CpG sequences and is mostly responsible for the silencing of genes.

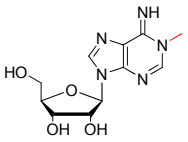
The modification $^{5\text{-HOMe}}$dC was established as a new post-replicatively formed DNA nucleoside in neuronal cells and stem cells in two recent back-to-back reports.[3] Nevertheless, the biosynthesis and the functions of $^{5\text{-HOMe}}$dC are still unknown.

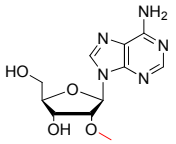
Modifications in RNA are by far more numerous. More than 100 different modified RNA nucleosides have been identified to date.[4] The highest modification level is found in tRNA. More than 80 tRNA modifications are presently known making this RNA class the first choice for the investigation of modified nucleosides. They form about 12 % of the tRNA nucleosides with a median of eight modifications per tRNA.[5] Correspondingly, numerous functional groups are used by nature to establish such a high number of different yet related molecules. To implement the various modifications in tRNA a broad range of chemistry has to be performed and numerous enzymes are involved. The structural diversity of tRNA modifications is also reflected by a myriad of functions they mediate. For instance they are involved in cellular processes such as mRNA decoding, cell development, translation control, development of diseases, occurrence of virulence, adaption to the environment, proliferation, and metabolism. An exhaustive discussion of all biological pathways and functions related to modified nucleosides thus by far exceeds the scope of this thesis. For the modifications synthesized during this thesis work an overview about their biosyntheses and functions is given as a short introduction preceding the discussion of their syntheses (chapter 7). In addition, they are discussed in numerous review articles.[5-6]

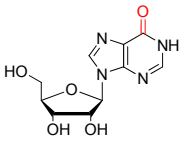
In general, all modified tRNA nucleosides effect the translation of genetic information from the messenger-RNA (mRNA) to proteins either by directly interacting with the mRNA and the ribosome or indirectly by influencing the three-dimensional structure of the tRNA. An impaired modification pattern of the tRNAs can thus severely impact the viability of the affected organism. In unicellular organisms this can lead to a reduced fitness or death.[7] In higher organisms it was shown that an altered tRNA modification pattern can cause severe diseases[8] and might be associated with progression and malignancy of cancer in humans.[9] As a foundation for the following discussion of this important topic all modified nucleosides in animals according to this work and Sprinzl and coworkers[4a, 4b] are summarized in Figure 5.

Adenosines


m^6A


m^1A


Am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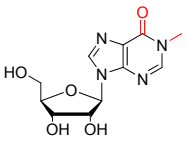
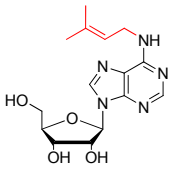

m^1I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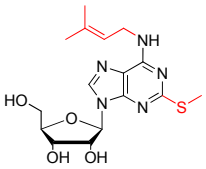
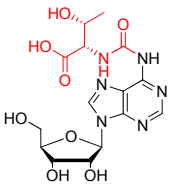
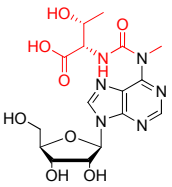
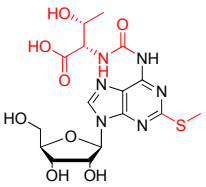
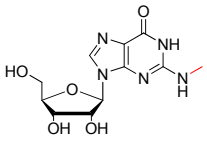

ms^2i^6A

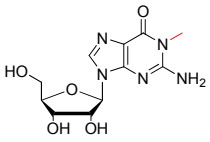

t^6A


m^6t^6A


ms^2t^6A

Guanosines


m^2G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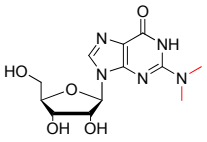
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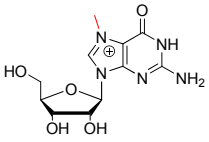
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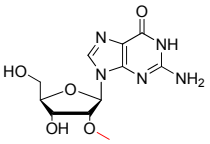
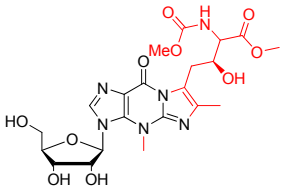
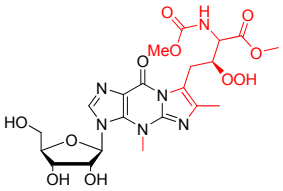
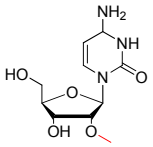

Gm


OHyW


O_2yW

Cytidines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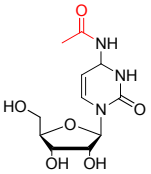
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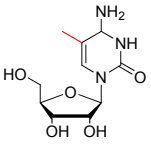
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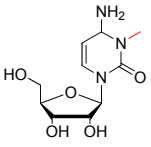
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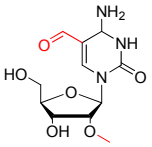
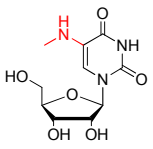
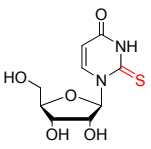
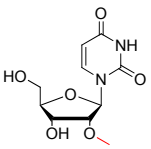

f^5Cm

Uridines


mnm^5U


s^2U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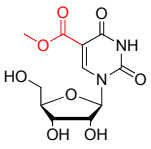
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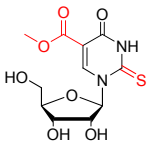
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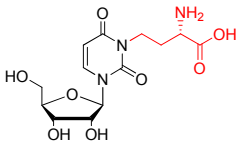
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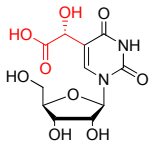
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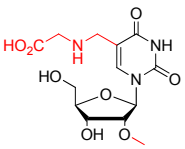
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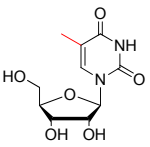
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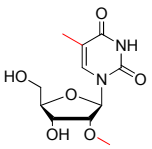
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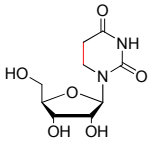
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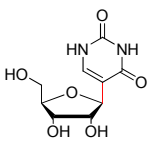
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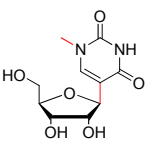
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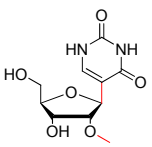
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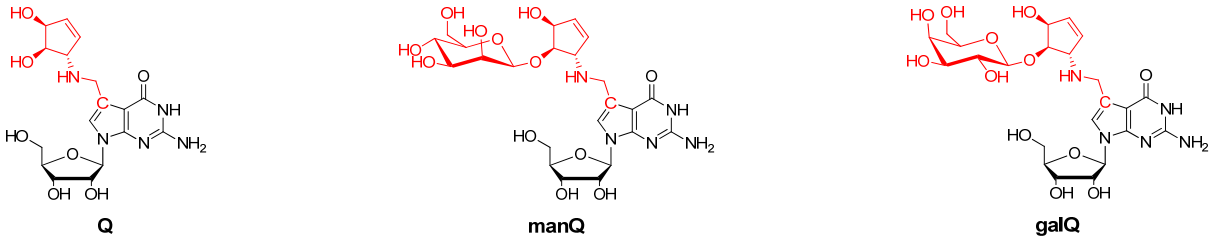

Figure 5: Summary of the modified nucleosides in animals according to this work and Sprinzl and coworkers.[4a, 4b] Alterations relative to the canonical nucleosides are indicated in red.

3.2 Modified tRNA nucleosides in cancer

3.2.1 tRNA methylation levels in tumor tissues

Neoplastic tissues exhibit an altered methylation pattern in comparison to their nearest healthy tissues.[10] Both hyper- and hypomodification have been observed, while the activity of tRNA methylating enzymes is generally increased in neoplastic tissues. The direction and the extent of the tRNA methylation level changes vary between tissues. Similar alterations have also been detected for other modified nucleosides like wybutosine.

When methylated bases were first observed in tRNA, they were suspected to control translation and thus to participate in differentiation including the development of cancer.[11] This assumption was supported by the finding that administration of carcinogenic dimethylnitrosamine to healthy animals induced development of tumors and, simultaneously, formation of methylated nucleosides not only in DNA but also in RNA.[12]

Inspired by these observations various groups investigated the activity of methyltransferases in healthy and in tumor tissues. The general approach included the isolation of tRNA from one species (generally bacteria or yeast) and collection of the methyltransferases from the tissue of interest. As different species contain different methylation patterns, methyltransferases from one species can further methylate tRNA from another species. In a large number of investigations preparations of methyltransferases from neoplastic tissues introduced two to ten times more methyl groups in tRNA samples than identical preparations from the closest healthy tissue.[10-11, 13] Similar results were obtained for studies with cell cultures. Comparison of mouse embryo and Syrian hamster kidney cell line BHK21 with their respective transformed cell lines revealed an increased methylation capacity of the transformed cells.[14] In addition, analysis of cells transformed by DNA viruses, which cause cancer, led to the same results.[15]