Max-Planck-Innovation

Technology Offer

Generation of RNA therapeutics by conjugating NAD-capped RNAs to proteins

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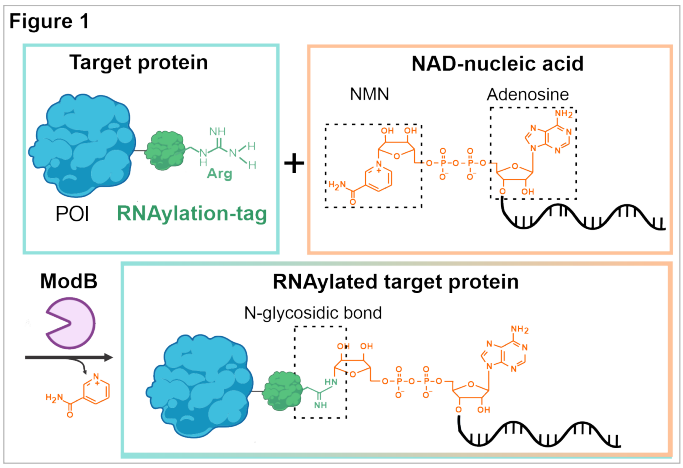
Background

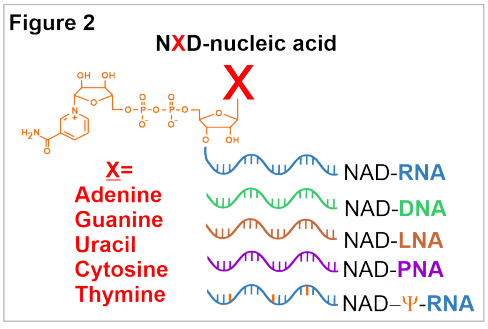
RNA-based vaccines and therapeutics are promosing new tools to combat a wide range of incurable diseases and expand the range of druggable targets. Its translation into clinics, however, requires optimal RNA delivery with high RNA stability, efficient cellular internalisation and precise target affinity. Bioconjugation opened up new ways to engineer biomolecules with improved properties. However, chemical synthesis strategies to link a protein to a nucleic acid are manufacturing intensive and the non-natural bond is mostly non-hydrolysable *in vivo* and can cause increased cytotoxicity.

Figure 1



Recently, the cofactor NAD was identified as a novel 5'-modification of cellular RNAs in various bacteria, archaea, eukaryotes, and viruses. The NAD modification has been shown to modulate RNA stability. In addition, scientists from the Max Planck Institute for Terrestrial Microbiology have discovered that the NAD-capped RNAs can be covalently attached to specific target proteins by the phage T4 ADP-ribosyltransferase (ART) ModB, which is termed *RNAylation* (Figure 1). This finding reveals a distinct biological role of NAD-RNA, namely activation of RNA for enzymatic transfer and a novel way how RNAs can interact with proteins in nature. RNA-substrate and protein-target specificity of ModB have been investigated. It has been shown that ModB accepts various NAD-capped nucleic acids (Figure 2) and attaches them covalently to defined arginine residues of proteins carrying an oligonucleotide-binding fold – called RNAylation tag (Figure 1).

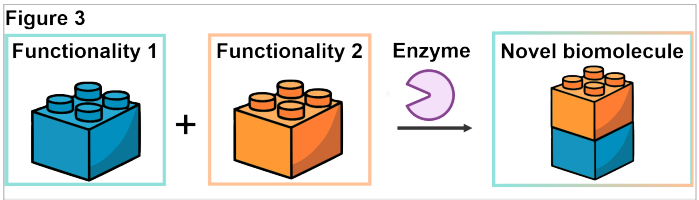
Figure 2



Technology

Conjugating a NAD-capped nucleic acid to a protein catalysed by an ART generates a novel biomolecule – the RNAylated protein – with two functionalities derived from the nucleic acid and the protein (Figure 3): i) the covalent attachment of a protein to the 5'-terminus of a nucleic acid via a naturally occurring bond decreases the nucleolytic processing of nucleases and Nudix hydrolases, thereby increasing the stability of the biomolecules, ii) the negative charge of the nucleic acid can change the properties of the protein or vice versa, and iii) the covalent linkage of RNAs

Figure 3



RNAylation
covalent, enzymatic conjuagtion of proteins (Functionality 1) and nucleic acids (Functionality 2) to generate novel biomolecules

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and proteins prevents the diffusion of the biomolecules into different cellular directions and might allow for specific localisation of RNAylated proteins in the cell, where both, the protein and the nucleic acid, can become functionally active.

In addition, scientists from the Max Planck Institute for Terrestrial Microbiology have developed a chemoenzymatic approach to synthesise 5′-NAD-capped nucleic acids independent of length, structure, and nucleotide sequence in high yields and purity under mild conditions. This technology allows for the preparation of NXD-capped RNA (Figure 2). Here the adenosine in the NAD-cap can be exchanged to guanosine (NGD), uracil (NUD) or cytosine (NCD), which allows for the modulation of the stability of RNAylated proteins in the presence of deconjugation enzymes (e.g. ADP-ribose hydrolases) *in vivo*.

Objectives for the RNA therapeutic industry

- **Bioconjugation**: The RNAylation reaction represents a novel and sustainable conjugation method which creates a naturally occurring N-glycosidic bond with the potential to reduce immune responses and cytotoxicity *in vivo* compared to chemically synthesised RNA-protein conjugates.
- **RNA therapeutics**: The RNAylated proteins possess two functionalities derived from the nucleic acid AND the protein which (i) increase stability of the biomolecules, (ii) can change the properties of the protein due to the negatively charged RNA or by recruiting RNA-binding proteins and (iii) might allow for targeted delivery into cells to regulate cellular processes. RNAylated proteins may provide a platform to engineer the genome with high precision and represent a starting point for developing next generation therapeutics.

Patent Information

An international patent application was filed in April, 2022: WO2023006264A1.

Publication

Maik Wolfram-Schauerte, Nadiia Pozhydaieva, Julia Grawenhoff, Luisa M. Welp, Ivan Silbern, Alexander Wulf, Franziska A. Billau, Timo Glatter, Henning Urlaub, Andres Jäschke, Katharina Höfer: A viral ADP-ribosyltransferase attaches RNA chains to host proteins. bioRxiv 2021.06.04.446905. DOI: https://doi.org/10.1101/2021.06.04.446905

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