Polish National Centre for Research and Development grant winners

Help for Forty Million

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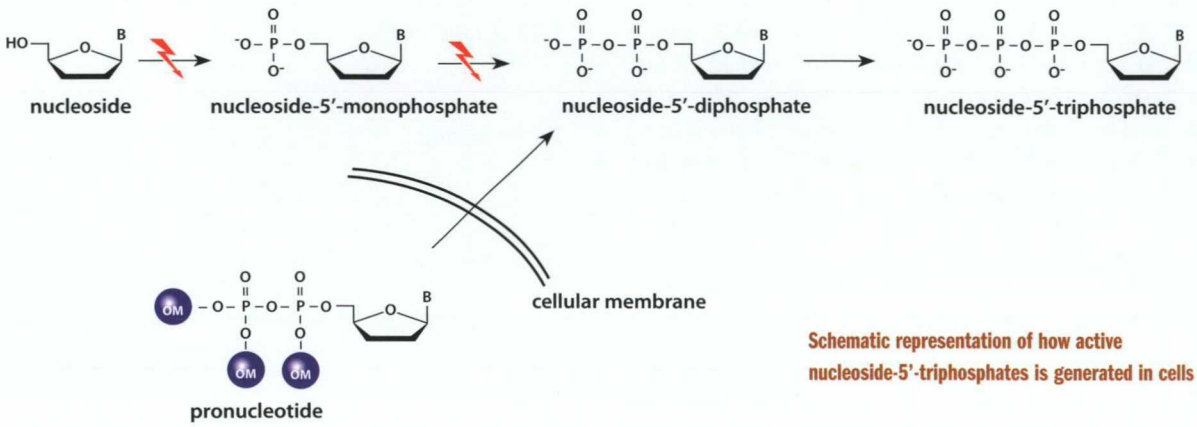
Dr. Joanna Romanowska works at the Institute of Nucleic Acid Chemistry. She has received a grant from the National Centre for Research and Development for her project "Nucleoside diphosphates: an innovative concept for anti-HIV pronucleotides."

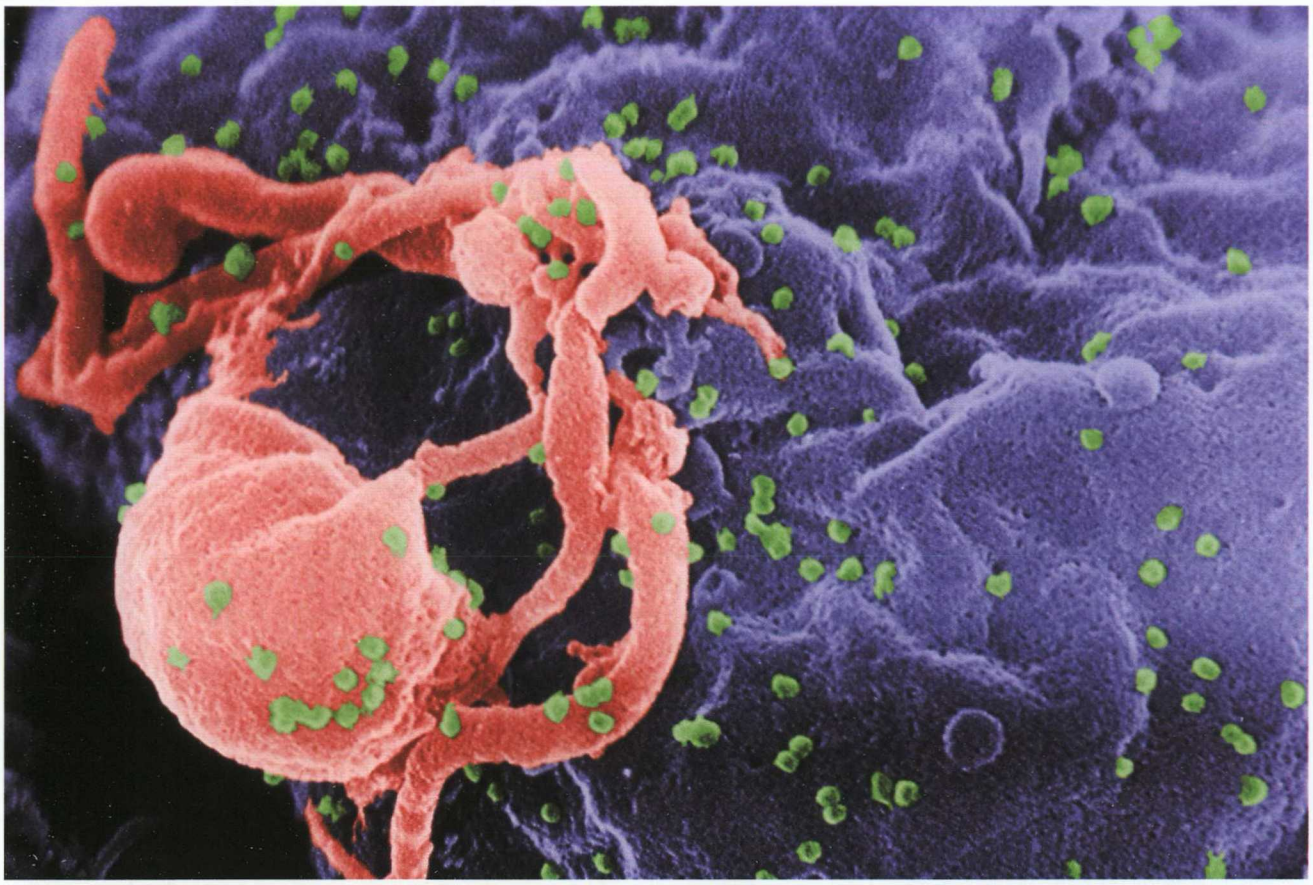
Contemporary medicine is able to keep HIV in check: patients taking a carefully selected combination of drugs are prevented from developing AIDS. And yet the number of new infections continues to grow, with up to 40 million people living with HIV around the world. New research into pronucleotides, conducted at the PAS Institute of Bioorganic Chemistry, offers hope of potential new treatments

One of the main reasons why HIV is so difficult to combat is its extraordinary rate of replication (109 virions per person per day), accompanied by very high genetic variation (108 nucleotides per day) and growing numbers of mutations resistant to existing drugs. As such, the therapeutic approach requires a cocktail of multiple drugs, which means new ones need to be developed all the time. An understanding of molecular mechanisms is necessary to design new drugs targeting individual mechanisms of viral replication. In one such mechanism, driven by reverse transcriptase, the genetic material is RNA (ribonucleic acid); the enzyme transcriptase is responsible for the transcription of viral RNA into cDNA (complementary DNA), which becomes a matrix for new copies of the viral RNA following integration with the host's DNA. An interruption of the transcription process blocks the replication process, thus preventing the onset of AIDS. The strategy has been employed to develop nucleoside analogues, corresponding to elements required for cDNA synthesis; a few, such as AZT (azidothymidine, a nucleoside reverse transcriptase inhibitor), are currently used in the management of AIDS. Nucleoside analogues are in fact prodrugs; this means that after penetrating cells, they must be phosphorylated by cellular kinases into mono-, di- and triphosphates, which are the actual inhibitors of viral RNA synthesis. However, some of the compounds (e.g. ddU) are weak substrates for enzymes of this type, limiting their therapeutic potential.

The concept of using pronucleotides originally arose as a result of limitations on using analogue nucleosides in HIV therapy. Nucleotides are also components of the DNA chain; however, they are more complex than nucleosides, which means it is possible to avoid the difficult first step of phosphorylating the nucleoside analogue within cells. Following this concept, a prodrug would be a protected nucleotide – a pronucleotide with protective phosphate groups removed within the cell as a result of chemical hydrolysis and/or using cellular enzymes, freeing nucleoside-5'-monophosphate, which



Schematic representation of how active nucleoside-5'-triphosphates is generated in cells



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Scanning electron microscope photograph showing viruses (green) emerging from a lymphocyte

is phosphorylated into diphosphate and finally into active triphosphate. Pronucleotides must also meet a few criteria that are often contradictory: they must be highly soluble in lipids (lipophilic), highly soluble in water, and stable in physiological media. They should also be able to penetrate target cells, where they are converted into corresponding nucleotides and then into active 5'-triphosphates. Unfortunately this concept also faces certain limitations: the phosphorylation process of some analogue nucleosides is inhibited at the diphosphate formation stage, which prevents the formation of the desired nucleoside-5'-triphosphate.

Our project, conducted as part of the LIDER Programme, will involve comprehensive research into nucleoside-5'-diphosphates, innovative pronucleotides which may be used in the fight against HIV. My team will focus on the synthesis of nucleoside derivatives, which frequently present problems with phosphorylation into mono- and diphosphates. This should improve the formation of active triphosphate in cells, which in practice should accelerate the desired therapeutic effect. Our team aims to develop a method of obtaining these anti-HIV prodrugs, which remain poorly understood. This innovative concept will require new methods of synthesis of the desired compounds. We aim to apply the chemistry of H-phosphates, something our laboratory has extensive experience with. We will design and study several types of nucleoside-5'-diphosphates whose structures comprise various nucleosides with known antiviral properties (ddU, d4T, AZT). The compounds will be modified in their phosphate chain; at least one phosphate group will be masked in order to introduce a degree of lipophilicity. An important part of the project will involve physicochemical studies of obtained compounds. We aim to use calculation methods to mark the partition coefficient logP, which we will compare against the same parameters obtained experimentally (standard measurement methods in an octanol/water system). We believe that the results will allow us to predict the ability of the obtained pronucleotides to permeate cellular membranes and enter into cells. Cytotoxicity (CC50 and CC90) and anti-HIV (EC50 and EC90) activity studies will be key in defining the biological parameters of the obtained compounds; they will be marked using CEM-T4 cell lines. We will also conduct measurements on TK- cells (with a deficit of cellular kinase), with the aim of obtaining data on the compounds' mode of activity.

Another essential element of our work will be our close collaboration with chemistry and microbiology research groups. We hope that our participation in this interdisciplinary project will provide a valuable contribution to reaching the final goal of obtaining nucleoside diphosphates as a new, effective, and highly necessary HIV treatment. ■