RNA interference: confidence is returning

The 15-year history of RNA interference is not short on dramatic effects. It begins with the unexpected discovery and publication of the process of post-transcriptional gene silencing in 1998, for which the two Americans Andrew Fire and Craig Mello were awarded the Nobel Prize in Physiology or Medicine just eight years after their discovery. In 2001, Thomas Tuschl succeeded in switching off genes in human cells with small synthetic pieces of RNA (siRNA).

A speech praising the discoverers of RNAi, Craig Mello and Andrew Fire, at the awards ceremony for the Paul Ehrlich Ludwig Darmstaedter Prize in 2006, drew attention to "RNA interference as a comparatively simple and universal method used for gene silencing, in which messenger RNA is degraded in a complex mechanism involving small, double-stranded RNA molecules, thereby reducing the expression of proteins involved in pathological processes. RNAi has already made an invaluable contribution to our understanding of the molecular and medically relevant relationships" (Ärztezeitung, 2006). The regulation of gene expression after transcription by way of gene silencing also mediates resistance to pathogenic nucleic acids such as those of RNA viruses (see yellow box below).

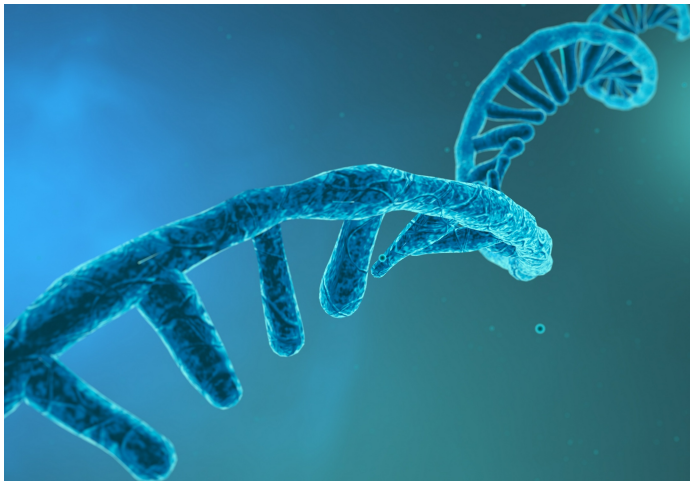
RNAi technologies have since become standard laboratory methods for the investigation of genes. Small double-stranded RNA molecules (small interfering RNAs, siRNAs) whose sequence is complementary to that of the gene to be silenced prevent the production of the respective proteins. In the biosciences, siRNA silencing can be used for any kind of RNA sequence and is an ideal tool for blocking genes, thereby enabling researchers to understand the function of the genes.

Use of RNAi for therapeutic purposes

RNAi is also studied intensively by researchers around the world for its potential use in drugs where siRNAs, shRNAs and miRNAs (see yellow box for more information) are used as active therapeutic substances. Around 20 RNAi-based therapeutics have reached the clinical testing phase (Kubowicz et al., 2013).

RNAi is is explored for its potential in treating macular degeneration, cancer, viral infections, asthma, hypercholesterolemia as well as genetic skin diseases and amyloidosis. Moreover, the development of RNAi-based therapeutics for the treatment of Duchenne muscular dystrophy, hepatitis C (HCV) and influenza are restoring the confidence of the RNAi community in the small synthetic pieces of RNA (Mulcahy and Carter, 2013).

Improving the controlled delivery of RNAi therapeutics



The emerging class of RNAi therapeutics is a completely novel approach to treating human disease by enabling the pursuit of molecular targets considered undruggable by small molecules and traditional therapeutic proteins (Barros and Gollob, 2012).

The clinical potential of nucleic acid based drugs is limited by their susceptibility to serum nuclease degradation, their rapid elimination through the kidneys and their accumulation in random tissue. In addition, due to their high molecular weight and polyanionic nature, synthetic siRNAs fail to cross biological membranes by passive diffusion. Improvements in the delivery of siRNAs into and within cells seem to be the key to success (González et al., 2013).

So far, no RNAi-based drug has been placed on the market. Experts are nevertheless sure that this will

Symbolic image of an RNA strand. RNA interference (RNAi) is an endogenous process that regulates the expression of genes. Short double-stranded RNA (dsRNA) fragments trigger an enzyme cascade that causes the degradation of homologous mRNA molecules, which in turn affects the synthesis rate of the target protein.

eventually happen and the press has noted the growing interest in effective RNA delivery technologies in view of the large number of corporate alliances and acquisitions. The return of optimism is related to the fact that the fundamental problems of RNAi technology (cellular uptake and stability) seem to be solvable by the chemical modification of the RNA molecules or by packaging the RNA molecules into nanoparticles, the feasibility of which has been demonstrated by numerous studies (Bouchie, 2012).

RNAi technology reaches new level of maturity

Meanwhile, researchers from the American company Life Technologies have succeeded in developing a method that involves the lipid-mediated transfection of RNAi into living organisms. With the company's principle of delivery, nucleic acids can be delivered into and analysed in small animals without the risk of degradation (Labant, 2013).

Researchers have recently successfully produced siRNA molecules in living cells (E. coli). Compared to chemical or in-vitro syntheses, the production in bacteria leads to fully functional siRNA molecules with a property profile (few side effects and high knock-down, low production costs) which experts (Blau and McManus, 2013) believe will lead to their broad application in academia and industry.

New RNAi function discovered in mammals

Although great importance is accorded to the therapeutic application of RNAi, basic researchers are not yet short of work to do as RNA inference is well, but not yet fully understood. Not all the functions and endogenous target structures of RNAi have yet been discovered. Something that has long been assumed has now been confirmed in mouse cells. A group of researchers led by Olivier Voinnet from the Swiss Federal Institute of Technology in Zürich (Switzerland) has demonstrated that not only plants and invertebrate organisms, but also mammals use RNA interference in order to protect themselves against viruses (Maillard et al., 2013). Moreover, unicellular organisms such as archaebacteria and bacteria also use this mechanism, which German scientists are currently studying as part of a DFG-funded research project.

New applications in agriculture

RNA interference is also being explored as a method for pest management in crops. Moreover, Monsanto and others are looking to RNA interference to protect honeybees against varroa mites. In 2011, Monsanto acquired the Israeli company Beeologics, which had developed an RNAi technology to control bee-killing varroa mites. The idea is to develop a mite-killing RNA product that prevents the expression of vital mite genes and is fed to bees through sugar water. Bee larvae then consume the sugar water and the RNA molecules diffuse from the intestines into their haemolymph. The treatment is harmless to the larvae, but varroa mites that suck the haemolymph ingest a self-destruct signal in the form of RNA (Young, 2013).

RNAi research also targets plant viruses. The botanist Holger Jeske from Stuttgart, for example, has developed transgenic beans that produce siRNA to protect them against geminivirus infections. He now plans to transfer the technique to other plants (Deutschlandfunk, 2013).

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RNAi – an overview

RNA interference (RNAi) is an endogenous process that regulates the expression of genes. Short double-stranded RNA (dsRNA) fragments trigger an enzyme cascade that causes the degradation of homologous mRNA molecules, which in turn affects the synthesis rate of the target protein. RNAi can be divided into an initiation and an effector phase, wherein initiation starts with the formation of dsRNA. RNAi can be triggered by foreign, i.e. viral dsRNA, or genomically encoded microRNAs (miRNAs).

During the initiation phase, dsRNA binds to an endonuclease that is referred to as Dicer. Dicer processes dsRNA into short interfering RNAs (siRNA) about 21-23 nucleotides long. The 5' end of the siRNA is subsequently phosphorylated by a kinase enzyme that is associated with Dicer, and transferred to the RNA-inducing silencing complex (RISC) during the effector phase. RISC unwinds the siRNAs and uses one of the strands as a template for complementary mRNAs, which are subsequently cleaved by the enzyme components of RISC.

An RNA-dependent RNA polymerase binds to some of these fragments and synthesises complementary strands. This leads to more dsRNAs, which are recognised by the Dicer complex and cleaved into siRNAs. This process leads to the amplification of RNA interference.

shRNAs: Small hairpin RNAs are RNA sequences that form hairpin structures and can be used to prevent target gene expression via RNA interference.

siRNAs: Short interfering RNAs are small double-stranded RNA molecules, (17-25 in length) involved in the RNAi pathway. They have phosphorylated 5' ends.

miRNAs: MicroRNAs are non-coding RNA molecules involved in the post-transcriptional regulation of gene expression during development by degrading complementary target mRNA.

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Dossier

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