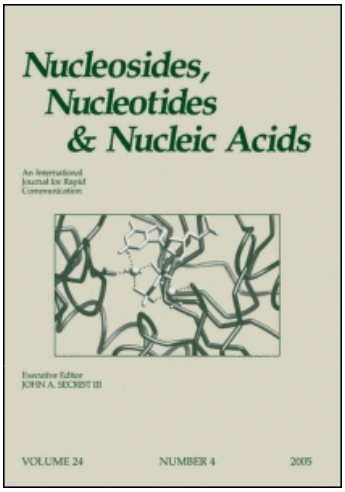

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A Role for Oligonucleotide-Based RNA-Knock Down Technologies in Functional Genomics

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ABSTRACT

Functional genomics is inundating the pharmaceutical industry with large numbers of potential gene targets from several sources such as gene expression profiling experiments (DNA microchips, proteomics) or database mining. Oligonucleotide-based RNA-knock down technologies such as antisense or RNA interference can aid in the filtering and prioritization of target candidates in the drug discovery process.

Key Words: Antisense; siRNA; Gene-knock down; High-throughput oligonucleotide synthesis.

At the core of functional genomics research lies a fundamental problem: how to apply the available experimental techniques in the right balance to effectively filter large numbers of candidate genes for those that represent valid targets. Oligonucleotide-based technologies such as antisense or small interfering RNAs (siRNA) can be usefully applied in this process to highlight genes as new potential drug targets (for a

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recent review see Ref.[1]). Knowledge solely of part of the target mRNA sequence enables the rapid design of an inhibitor, specific for the given protein, even when the protein sequence is not known. The specificity of oligonucleotide-based mRNA knock-down reagents that allow for the discrimination among even closely related gene family members and the fact that these gene inhibitors can be designed with minimal sequence information such as an EST make them attractive candidates as research tools for the elucidation of gene function. Downregulation of a gene through use of oligonucleotide inhibitors in a suitable functional assay may reveal whether the gene in question contributes causally to a particular phenotype. If positive, this marks the gene as worthy of further investigation leading possibly to its validation as a pharmaceutical drug target.

Thus three main processes lie at the heart of the approach:

1. The preliminary selection of candidate genes for investigation.
2. The identification of effective antisense/siRNA inhibitors for each of these genes.
3. The evaluation of the consequences of inhibiting expression of these genes in a variety of functional assays.

The identification of a potent oligonucleotide reagent for target validation against a particular target gene has remained an issue. Due to RNA secondary and tertiary structure that determine the accessibility of the RNA target site to an oligonucleotide the synthesis of 8–10 different antisense oligonucleotides is usually required in order to identify a gene inhibitor with reasonable activity.[2] An advantage of siRNAs is that compared to antisense oligonucleotides lower concentrations are needed to achieve similar levels of mRNA downregulation. However, recent studies suggest that the potency of siRNA inhibitors is also likely determined by RNA structure.[3] A reporter gene based assay has been designed that allows for screening of large numbers of oligonucleotides thereby enhancing the selection process of oligonucleotide sequences against a given target. Automation of reporter-based oligonucleotide screening has been successfully applied in combination with expression profiling to aid in the validation of novel drug target candidates in the field of chronic neuropathic pain. Using the purinergic receptor $P2X_3$ as a proof-of-concept[4] a template process for the use of antisense in validation of chronic pain targets in vivo has been established. The approach starts with the isolation of total RNA derived from the dorsal root ganglia (DRG) from various rat pain models. The RNA is assayed on DNA chips and the corresponding gene expression profiles are compared to expression patterns profiles generated from normal rats. The genes that are found to be modulated in the disease state are further prioritized according to criteria such as novelty or "druggability". Oligonucleotide-based knock-down technologies can then be used to further validate these genes as potential drug targets for the treatment of chronic pain (Fig. 1).

A high-throughput mRNA knock-down based target validation platform has been developed and implemented allowing for screening of prioritized genes such as "druggable" gene family members (e.g., enzymes or receptors) or candidates that are highlighted by genomics approaches such as database mining, differential display technologies or model organism studies. Oligonucleotide sequences are selected

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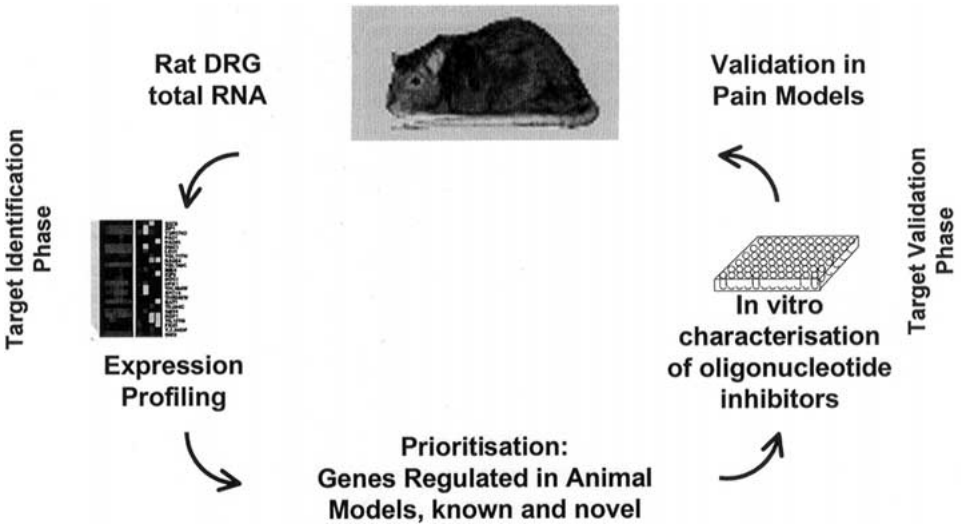

Figure 1. Identification of drug targets for the treatment of chronic pain.

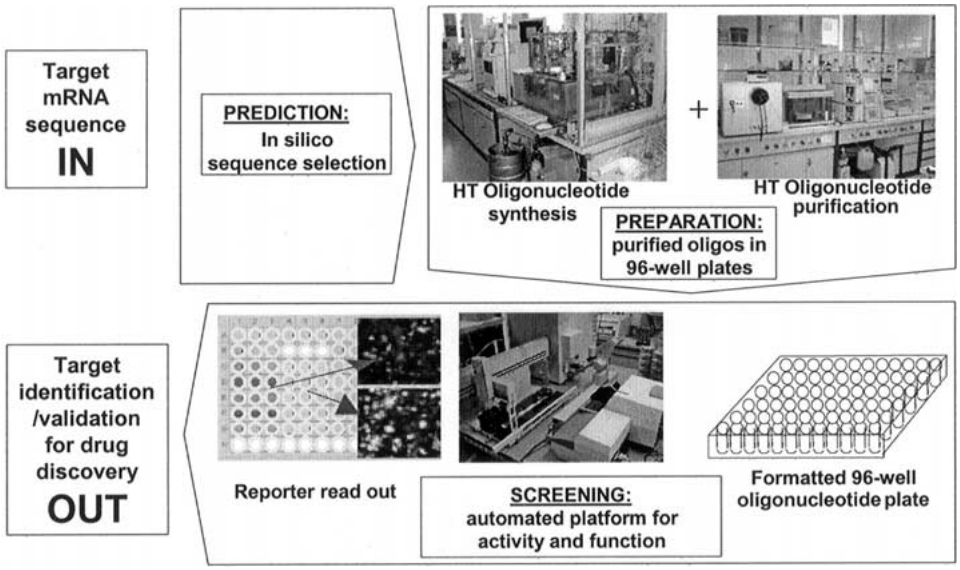

Figure 2. Target validation using high-throughput oligonucleotide synthesis in combination with automated reporter assay screening.

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starting from raw sequence information and the corresponding gene inhibitors are synthesised and purified in an automated process using a 96-well plate synthesiser (Bioautomation, Plano, Texas, USA) coupled to an LC/MS (Waters, Paris, France) device. The resulting gene inhibitors are subsequently screened in automated reporter-based functional assays in order to "functionalise" the prioritized target gene candidates (Fig. 2).

The integration of high-throughput oligonucleotide synthesis and automated assays allows for rapid screening of a large number of oligonucleotides for their application as antisense or siRNA gene knock-down reagents. Combination of these tools will aid in accelerating the process of associating genes with disease pathways and in combination with other functional genomics technologies will ultimately support the discovery of novel targets suitable for therapeutic intervention.

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