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REVIEW PAPER

Progress of Antisense Technology Applied in Metabolic Regulation of Bacteria

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Abstract: With the rapid development of genetic engineering and metabolic regulation, antisense technology displays its fascination to the world as a mild regulation genetic tool. Compared with other loss-of-function research methods (e.g. gene knockout), antisense methods have advantages such as low cost, short period, and easy operation. Hence, they have been increasingly used in bacterial metabolic regulation as a powerful genetic tool. This review briefly summarized the latest progresses and challenges in antisense methods that are recently used in metabolic engineering of bacteria and compared the advantages and disadvantages of these approaches.

Keywords: antisense technology; metabolic regulation; antisense RNA; antisense oligonucleotides; ribozyme

Introduction

Scientists were fascinated by bacteria because of their easy-to-culture characteristic, great diversity of enzymes, and abundant metabolic products. With the rapid development of genetic engineering, bacteria, as an excellent expression and gene manipulation host, metabolic regulation of bacteria was considered to be hot focus of metabolic engineering. Recently, researchers found that metabolic reconstruction and gene knockout would lead to metabolic turbulence, slow growth rate, and instability of metabolic product[1-3]. As a result, scientists pay great attention to antisense technology for it is a mild genetic regulation tool.

During the last 3 decades, antisense oligonucleotides, antisense RNA, and ribozyme have been successfully used in interference of gene expression of bacteria. Antisense methods are found to have increasing application in bacterial metabolic operation as powerful genetic tools. Compared with other loss-of-function research methods such as gene knockout, antisense technology can be performed with low cost, in short period, and with easy operation. For it will not cut off metabolic pathway, antisense methods cause less effect on growth and metabolism in bacteria. These merits attracted more and more metabolic engineering researchers to focus on the technology. As a result, antisense technology is increasingly applied in metabolic regulation of bacteria. Also, many splendid results have come out.

The first antisense method appeared in 1978, when Zamecnik constructed a 13nt antisense oligodeoxynucleotides to inhibit replication and cell transformation of Rous sarcoma virus[4]. Subsequently, with the boom of molecule biology, antisense methods were widely applied in the bacteriology. Gene knockout methods appear to be highly efficient in metabolic regulation of *Escherichia coli* and several common bacteria. People would like to take up antisense methods when the gene is difficult to knock out; therefore, antisense methods were almost used to be applied

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in interfering gene expression of gram-positive bacteria and several species of gram-negative bacteria. When the gene knockout methods were found deficient in application, gradually, antisense methods were used in gene inhibition of *E. coli* and other well-known bacteria.

1 Antisense methods applied in bacterial metabolic regulation

There are antisense RNA, antisense oligonucleotides, ribozyme, and small interfering RNA in antisense technology. Methods that can be applied in bacteria are antisense RNA, antisense oligonucleotides, and ribozyme. All antisense methods are based on the blockade and/or degradation of a target mRNA as a result of the binding of nucleic acids complementary to a subsequence of the mRNA, and various means to produce this specific inhibition of gene expression exist (Fig).

1.1 Antisense RNA

As a great discovery in 1980s, antisense RNA had great impact on metabolic engineering. Antisense RNA is a natural metabolic regulation mode of bacteria. The first antisense RNA in bacteria was found in replication regulation of natural plasmid ColE1 in *E. coli* [3]. The earlier reports of antisense RNA are focused on functional regulation of plasmid of bacteria. Antisense RNA strategy is to introduce a recombinant expression plasmid harboring gene encoding antisense RNA into bacteria cells, which interferes expression of the gene from which it is derived. Then, in this process, a duplex form of the antisense RNA transcript and complementary mRNA block translation (Fig., route A1) or target mRNA was degraded by RNase H (Fig., route A2). As a result, artificially introduced antisense RNA interfered with target gene expression and metabolism of the bacterium.

For antisense regulation is natural phenomena in bacteria, there are many merits in antisense RNA-mediated regulation of gene expression. 1) Compared with gene knockout, antisense RNA can perform minimal effect on metabolism of bacteria[5]. 2) Antisense RNA could be induced when the biomass get to a fine point. Thus, antisense RNA will not interfere with the growth of host bacteria, whereas it can inhibit target gene expression. As a result, we can efficiently regulate gene expression at any moment. 3) The expression vector can continuously and stably express antisense RNA. 4) Antisense RNA could selectively inhibit gene expression. The efficiency of antisense RNA can be regulated by length of antisense RNA, operation site, interaction of cellular factor, and space structure. Therefore, we can design different length and different kinds of antisense RNA to form a series of complementary base pairing and regulate gene expression by interfering with target mRNA in different extent.

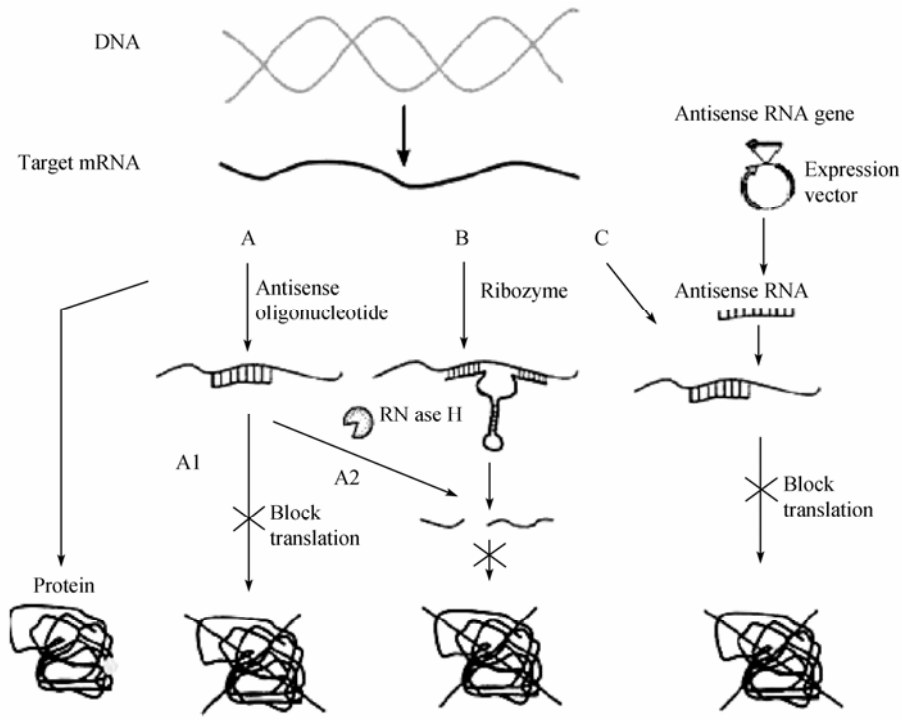

Fig. Mechanism of antisense technology applied in bacterial metabolic regulation

Route A: a fragment of antisense oligonucleotide possessing a sequence complementary to that of the target mRNA is transferred to the host cell, forms a duplex complex with the complementary mRNA sequences, and blocks mRNA translation via (A1) translational arrest or (A2) mRNA cleavage by RNase H. Route B: ribozyme is delivered to the host cell, forms specific complementary structure, hybridizes with target mRNA, and cleaves mRNA to fragments. Route C: antisense RNA strategy is to endogenously express antisense RNA through recombinant plasmids harboring antisense RNA gene. Then the antisense RNA forms a duplex complex with the complementary mRNA sequence and blocks translation by the ribosome