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SERIES: BASIC SCIENCE RESEARCH IN RELATION TO THE LUNG

# Basic molecular biology

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**Summary** Rapid advances in molecular biology over the past 20 years have and will continue to impact on the practice of medicine. Advances in molecular biology are having an immense impact in determining the underlying aetiology of lung disease and its treatment. In this review, basic molecular biology techniques will be discussed with examples of how these techniques are used in clinical practice.


## INTRODUCTION

Molecular biology is a broad area of study aimed at the common goal of understanding the mechanisms of basic cellular function. This review has been written as an introduction to the basic molecular biology techniques used to study DNA, RNA and proteins. The techniques utilised in basic and clinical research as well as the strengths and weaknesses of each technique are described. In addition, a bibliographical list of reference materials for an in-depth description of molecular biology techniques has been included.

## NUCLEIC ACIDS

DNA encodes the molecular template for all molecules necessary for cell function and viability. The nucleotide is the most basic structural unit of DNA and is comprised of deoxyribose sugar, a phosphate group and a purine (adenine and guanine) or pyrimidine (thymine and cytosine) nitrogenous base. DNA is a double helical molecule with two sugar–phosphate strands serving as the backbone of the molecule with the nitrogenous bases projecting toward the centre. The two strands are held together by hydrogen bonding, a non-covalent interaction, between the purine and pyrimidine bases. The interaction between the bases occurs with high fidelity (adenine always pairs with thymine and guanine with cytosine). Importantly, the two strands of the DNA molecule can be disassociated and then re-associated with the addition or removal of energy to break the forces of hydrogen bonding. Together these two physical features make DNA a dynamic molecule that allows for its replication and for transcription of encoded genes.

The gene is the smallest functional unit of DNA and encodes for proteins. In general, genes are made up of a promoter region and a coding region (Fig. 1). The promoter region of the gene contains short, conserved, defined nucleotide sequences that positively or negatively regulate gene transcription. Gene transcription is a tightly regulated process that is cell-type specific and greatly influenced by the physiological stress placed upon the cell. Gene transcription produces a ribonucleic acid (RNA) template from which a protein is produced.

RNA, like DNA, contains a sugar–phosphate backbone and nitrogenous bases. The major differences are that the sugar is ribose, the base uracil is incorporated in place of thymine and the molecule is single-stranded. Total cellular RNA is a mixture of three types: transfer (tRNA), ribosomal (rRNA) and messenger (mRNA), which is the

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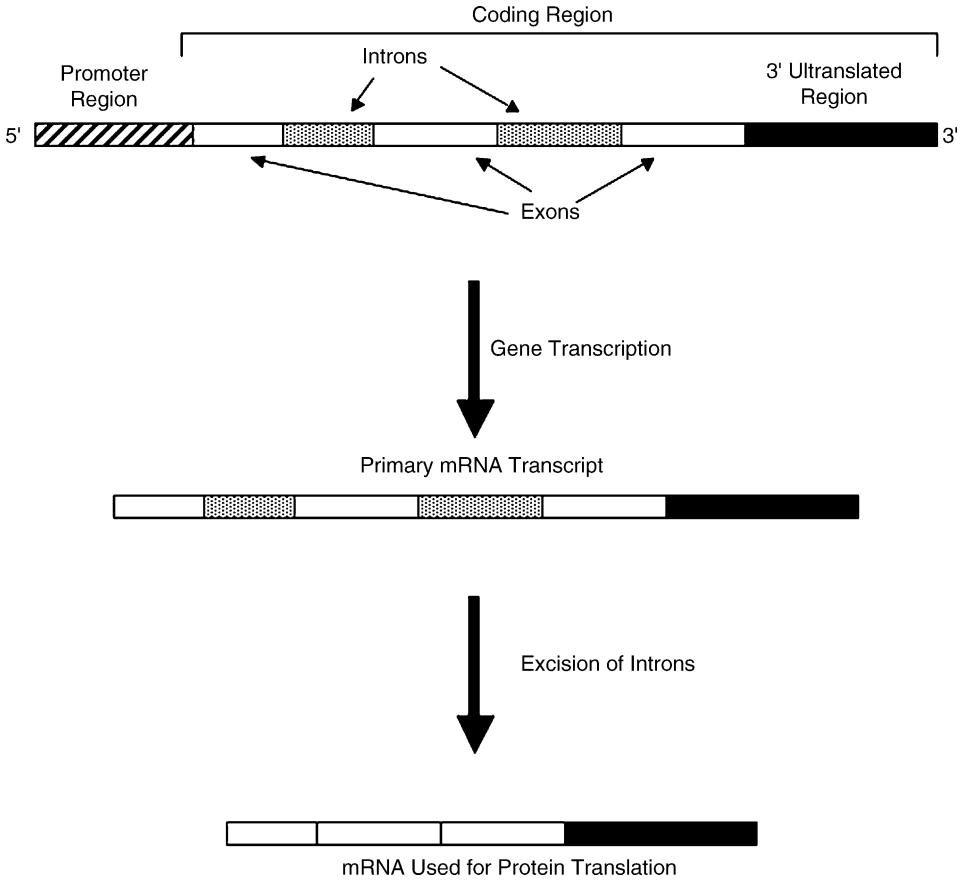


**Figure 1** A hypothetical gene, primary transcript and final mRNA.

template for protein production. Since mRNA encodes for proteins, this will be the focus of the remaining discussion of RNA. Initial transcription results in the formation of a primary transcript that includes introns, exons and the 3′ untranslated region (3′ UTR) (Fig. 1). Exons are segments that encode the amino acid sequence of the protein. Introns are intervening sequences that do not encode a protein but guide splicing of the mature mRNA and allow alternative combinations of exons to produce variations of similar proteins. Excision of introns and splicing of exons must occur before mRNA protein translation can occur.

## Methods for the Analysis of Nucleic Acids

### *Polymerase Chain Reaction*

Polymerase chain reaction (PCR) is used to amplify specific DNA sequences from limited biological samples and has revolutionised biomedical research. The technique is based on the following: DNA can be denatured from a double-stranded molecule to a single-stranded molecule by heat. When the single-stranded DNA molecules are cooled they anneal to a double-strand, the process of two single-stranded DNA molecules annealing occurs with high fidelity (i.e. A always pairs with T and C with G). The polymerase chain reaction is illustrated in Fig. 2. Sequentially, the template DNA is denatured by heating. Specific primers designed for the sequence of interest anneal on the corresponding DNA sequence of the template DNA when the reaction is cooled. DNA polymerase extends the DNA from 5′ to 3′ building a mirror image of the template strand. At the end of one cycle, two exact copies of template DNA have been formed. Using this technique, DNA can be amplified exponentially. This allows minute amounts of DNA from biological samples to be assayed, making the technique extremely powerful for basic science and clinical research.

PCR is a common approach used for the diagnosis of clinical diseases. For example, PCR can be used for the detection of herpes simplex virus in cerebral spinal fluid[1] and enterovirus in myocardial biopsy samples.[2]

### *Northern blot analysis*

Northern blot analysis is a sensitive technique for detecting mRNA expression levels. RNA is separated by agarose gel electrophoresis. Since, RNA has a negative charge it