

which constructs a protein in the cytoplasm. The protein levels of the cells are adjusted through the enzymatic breakdown of existing proteins and the RNA transcripts.

For a eukaryote, the protein breakdown cycle is categorized into several phases. Gene regulation is very important for the expression, so that the expression occurs only upon the requirement of the product. Gene regulation is affected by the environmental changes and nutrient availability. Gene expression control for eukaryotes is more complex as compared to the prokaryotes as a larger number of regulatory proteins are involved.

6.4 ANALYTICAL TECHNIQUES FOR GENE EXPRESSION

The information available on the human genome sequence can be applied to treat diseases like cancer. The gene expression pattern reflects many cellular functions like survival, growth, and differentiation. Sequence alternations, DNA methylation, and viral infections are the main factors which affect cell regulation. When these changes affect the gene controlling cell growth, cell division, cell repair, and apoptosis, cancer might develop. Hence, the gene expression and quantization of transcription levels for specific genes has been the core area of research (Bustin, 2000; Adams and Kelly, 1994; Bieche et al., 1999; Chiocchia and Smith, 1997; Bustin and Dorudi, 1998; Ståhlberg et al., 2005; Freeman et al., 1996; Ferre, 1992). To quantify levels of RNA in clinical diagnostics, different procedures have been designed to quantify and measure the transcription for gene-encoded therapeutics along with the detection of viral and bacterial pathogens (Bustin, 2000). For the quantification of transcription, five different methods are used, as illustrated in Fig. 6.5.

The gene expression measurement has been revolutionized by the invention of real-time PCR (polymerase chain reaction) and real-time reverse transcription PCR (real-time RT-PCR). The real-time RT-PCR method possesses many advantages over other methods of gene expression:

- Does not require postamplification manipulation.
- Can produce quantitative data with a range of almost 7–8 orders of magnitude.
- Detection of even a single copy of a specific transcript.
- Almost 1000-fold more sensitivity compared to the dot blot hybridization.
- Up to 100,000-fold more sensitivity compared to RNase protection assays.
- Capability of discriminating mRNAs with almost the same sequences.
- Ability to detect gene expression differences ~23% between samples.
- Possess lower coefficients of variation than end point assays.

In the following section, we will be discussing only real-time PCR due to its advancement over other gene expression techniques.