



What is RNA?

RNA is a biological macromolecule that serves a number of different functions. Messenger RNA (mRNA), transcribed from DNA, serves as a template for synthesis of proteins. Protein synthesis is carried out by ribosomes, which consist of ribosomal RNA (rRNA) and proteins. Amino acids for protein synthesis are delivered to the ribosome on transfer RNA (tRNA) molecules. RNAs are also part of riboproteins involved in RNA processing.

In addition, many viruses contain RNA as their genome instead of DNA, and RNA species called ribozymes catalyze biochemical reactions, similar to enzymes. Since RNA is able to perform functions usually associated with DNA and proteins, it has been suggested that RNA was the original biological molecule, with subsequent evolution of DNA and proteins.

A typical mammalian cell contains 10–30 pg total RNA. The majority of RNA molecules are tRNAs and rRNAs. mRNA accounts for only 1–5% of the total cellular RNA although the actual amount depends on the cell type and physiological state. Approximately 360,000 mRNA molecules are present in a single mammalian cell, made up of approximately 12,000 different transcripts with a typical length of approximately 2 kb (Tables 1 and 2, page 46). Some mRNAs comprise as much as 3% of the mRNA pool whereas others account for less than 0.01%. These “rare” or “low abundance” messages may have a copy number of only 5–15 molecules per cell. However, these rare species may account for as much as 11,000 different mRNA species, comprising 45% of the mRNA population (Table 3, page 46.)*

* For more information, see reference 1.

While the genes of an organism are relatively fixed, the mRNA population represents how genes are expressed under any given set of conditions. Analysis of RNA by hybridization technologies, including northern blotting and microarray analysis, or by RT-PCR can provide a good reflection of an organism’s gene-expression profile.

Compared to DNA, however, RNA is relatively unstable. This is largely due to the presence of ribonucleases (RNases), which break down RNA molecules.

RNases are very stable, do not require cofactors, are effective in very small quantities, and are difficult to inactivate. RNase contamination can come from human skin and dust particles, which can carry bacteria and molds. Isolation and analysis of RNA therefore requires specialized techniques.

This chapter describes procedures for successful stabilization, purification, and analysis of RNA.

**Table 1.** RNA content of a typical human cell

Total RNA per cell	~10–30 pg
Proportion of total RNA in nucleus	~14%
DNA:RNA in nucleus	~2:1
mRNA molecules	$2 \times 10^5 - 1 \times 10^6$
Typical mRNA size	1900 nt

Table 2. RNA distribution in a typical mammalian cell

RNA species	Relative amount
rRNA (28S, 18S, 5S)	80–85%
tRNAs, snRNAs, low MW species	15–20%
mRNAs	1–5%

Table 3. mRNA classification based on abundance

Abundance class	Copies/cell	Number of different messages/cell	Abundance of each message
Low	5–15	11,000	<0.004%
Intermediate	200–400	500	<0.1%
High	12,000	<10	3%

Table 4. RNA content in various cells and tissues

Source		Total RNA (μg)	mRNA (μg)
Cell cultures*	–	30–500	0.3–25
	NIH/3T3	120	3
	HeLa	150	3
	COS-7	350	5
Mouse — developmental stages†	Unfertilized egg	0.43 ng	nd
	Oocyte	0.35 ng	nd
	2-cell	0.24 ng	nd
	8–16-cell	0.69 ng	nd
	32-cell	1.47 ng	nd
	13-day-old embryo	450	13
Mouse tissue‡	Brain	120	5
	Heart	120	6
	Intestine	150	2
	Kidney	350	9
	Liver	400	14
	Lung	130	6
	Spleen	350	7

* 10^7 cells † Per organism ‡ 100 mg nd = not determined