2 GENERAL INTRODUCTION TO CELL-FREE TRANSLATION

2.1 What it is and when its useful: native RNA vs cloned gene transcripts

This is a general method by which the protein(s) encoded in a mRNA can be synthesized and studied in a test tube system. It involves preparation of (i) an appropriate RNA (either extracted from tissue or transcribed in vitro from a cloned gene), (ii) a cell extract capable of supporting protein synthesis (containing ribosomal subunits, initiation, elongation and termination factors and amino acyl-tRNA synthetases), (iii) necessary buffers including a cocktail of amino acids and an energy generating system to provide the ATP and GTP that drive protein synthesis. You also require a method by which to analyze your newly synthesized proteins. Typically, one of the amino acids is provided in radiolabelled form. Thus, newly synthesized proteins will be radioactive upon incorporation of the radiolabelled amino acid. If the products of the reaction are subjected to electrophoresis on polyacrylamide gels in sodium dodecyl sulfate followed by autoradiography, it is possible to analyse by size the newly synthesized products.

Provided appropriate additional reagents are available (e.g. a good antibody against the protein of interest), cell-free translation systems can be an excellent method of determining whether the mRNA for a particular protein is present in a particular tissue and whether a particular cloned cDNA encodes the protein of interest. Quantitative determinations and comparisons of levels of mRNAs are also possible, but must be interpreted with caution since different mRNAs even from the same tissue have been documented to require different optimal conditions for expression in cell-free systems. Thus cell-free translation is a powerful adjunct technology for simple expression of gene products.

Another important use of cell-free translation systems is in reconstitution of events that take place in a fashion coupled to protein synthesis in living cells. The best example is, of course, the transport of proteins into and across the membrane of the endoplasmic reticulum (ER). The supplementation of cell-free systems with microsomal membranes derived from the ER allowed the establishment of powerful assays for nascent chain translocation. Again, such analysis can be performed with either native mRNA from tissues or using cell-free transcripts from cloned genes.