6 TRANSLATION PAUSING

6.1 Introduction

The rate of polypeptide chain elongation by ribosomes is not constant, in vitro and in vivo. In certain cases, ribosomes will consistently pause at discrete sites on the mRNA. Pausing may be caused by a rare codon on the mRNA for which the appropriate tRNA is in low abundance, by secondary structure in the mRNA (this is controversial), by SRP-mediated translation arrest, by ribosomal frameshifting or by other unknown mechanisms. There are also pauses at the initiation and termination events of translation. The original assays have been modified only slightly in the following procedures and should be easily adaptable to a variety of applications.

The pausing assay reveals the distribution of ribosomes on a population of mRNA molecules in a cell-free translation reaction. The translation reaction is allowed to proceed until it has approximately reached steady state. At this point, translation is terminated with cycloheximide to "freeze" the ribosomes in position on the mRNA. The reaction is then digested with nuclease, leaving only ribosome protected mRNA fragments (rpfs). The ribosomes are purified by centrifugation and the ribosomal protein components destroyed with protease. The remaining rpfs are isolated by phenol extraction and ethanol precipitation. The original location of the rpfs within the mRNA can be mapped by annealing the rpfs in addition to an end labelled primer onto a single stranded DNA template and performing a T4 polymerase primer extension reaction. T4 polymerase will not displace the annealed rpfs but will terminate. The size of the terminated products run on a sequencing gel can be determined by comparison with a sequencing ladder generated from the same primer. The distribution of rpfs and by extrapolation the ribosomes are represented by the pattern of primer extension "toeprint" bands. Note that this assay is not quantitative. It cannot distinguish between a large or small number of evenly distributed ribosomes and therefore provides only a relative measure of translation pausing. Furthermore, a large number of toeprint terminations close to the primer will reduce the intensity of toeprints farther away, and varying amounts of rpfs should be tested. Finally, only the 5' ends of the rpfs are mapped (hence toeprinting not footprinting) and the size of the rpfs should be checked to ensure they were derived from monosomes.

References:

Wolin SL & Walter P, EMBO J 7(11):3559-3569, 1988.

Wolin SL & Walter P, J Cell Biol 109(6):2617-2622, 1989.

Wolin SL & Walter P, J Cell Biol 121(6):1211-1219, 1993.