5. TRANSLOCATION

5.1 Introduction

To study translocation across the ER membrane, one need simply supplement the cell-free translation system with microsomal membranes, typically from dog pancreas. The membranes are prepared (see biological reagents) and stored in small aliquots frozen first in liquid nitrogen and stored at -80øC. IT IS ABSOLUTELY CRITICAL TO REFREEZE IN LIQUID NITROGEN AFTER EACH USE. SIMPLY PUTTING THEM BACK AT -80øC WILL LEAD TO RAPID LOSS OF TRANSLOCATION ACTIVITY. ALSO, ONE ALIQUOT SHOULD BE FROZEN/THAWED NO MORE THAN 4 TIMES. Membranes can be prepared from just about any eukaryotic source, but few tissues seem to be as good as dog pancreas.

Since translocation normally proceeds coupled to translation, it is important to have the membranes present from the onset of incubation of the translation reaction. I set up the translation reaction exactly as before, except that I decrease the amount of water by the volume of membranes I intend to use, and after adding and spinning down all components, I add the desired volume of membranes directly to the reaction, one tube at a time, changing pipette person tips after each addition, on ice. Then gently mix by vortexing and put to incubate at 24øC for 1 hr.

The amount of membranes needed varies with the system being used (WG vs RRL), the protein being translated, and the particular batch of membranes. RRL appears to support a given percentage of translocation at a lower membrane concentration than WG (by about 50%), all else being equal. Different signal sequences translocating the same passenger and different passengers being translocated by the same signal sequence, can require different membrane concentrations to achieve the same percentage of translocation. Finally, different batches of membranes will vary as to their translocation competence, as well as in their ability to glycosylate chains. One complicating factor is that membranes can result in generalized inhibition of protein synthesis (apart from inhibition as a result of SRP mediated elongation arrest of signal bearing chains). Thus, the comparison of two membrane batches can be misleading if effort is not taken to use them at concentrations that result in similar levels of protein synthesis.