Targeting of RNAs to ER Subdomains and its Relationship to Protein Localization

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Abstract The targeting of proteins to specific subcellular regions is directed by a variety of signal elements. Many of these signals consist of amino acid residues (peptide sorting signals) arranged contiguously or in a three-dimensional array. In addition to posttranslational processes, proteins can also be localized to specific regions of the cell by the targeting of their cognate RNA. Ongoing studies in developing rice endosperm have shown that the RNAs that code for the major storage proteins are localized to specific subdomains of the cortical endoplasmic reticulum (ER), and that there is a tight correlation between the initial site of RNA localization and the final destination of the encoded protein in the endomembrane system. The segregation of RNA onto distinct ER subdomains may be a necessary and sufficient step for the localization of the coded protein in the cell.

1 Introduction

The endoplasmic reticulum (ER) serves as the entry site for proteins that are to be secreted or located at one or more locations in the endomembrane system in eukaryotic cells. Proteins are targeted to the secretory system with the emergence of the signal peptide during initial protein synthesis, which is recognized by the signal recognition particle. The resulting translationally arrested complex (mRNA–ribosomes–translation factors) is then mobilized to the ER, which enables the translocation of the growing polypeptide chain to continue through the ER membrane to the lumen. Within this organelle the polypeptide is folded and assembled to a correct conformational maturation state aided by a plethora of resident molecular chaperones. The presence of additional peptide sorting signals or interacting domains may enable the protein to be either retained within the ER or transported to other destinations within the endomembrane system (Vitale and Denecke 1999; Vitale and Ceriotti 2004). In the latter instance, proteins can be exported from the ER by two distinct pathways, one involving the Golgi apparatus.
and a second that is suggested to be Golgi-independent (Hara-Nishimura et al. 1998).

An ideal system for studying the biochemical and cellular events of ER-dependent translation is developing seeds. During seed development, one or more organs synthesize vast quantities of reserve protein, which assemble within the ER lumen itself or within protein storage vacuoles to form discrete organelles termed protein bodies. One question that immediately arises is why some plants utilize the ER lumen whereas others develop protein storage vacuoles. Although the exact cellular basis for this is not known, it is clear that the nature of the storage protein does not dictate its ultimate storage site. For example, members of the prolamine superfamily that share common structural domains are not stored in the same compartment. The 2S albumins of several dicotyledonous plants and prolamins of wheat and barley are transported to the protein storage vacuole, whereas the maize prolamins (zeins) assemble within the ER lumen to form intracisternal inclusions (Shewry et al. 1995). Although peptide sorting signals as well as peptide interacting domains are ultimately responsible for the final destination of proteins, evidence is beginning to emerge that the intracellular location of a protein may also be influenced by where it is being translated on the ER (Crofts et al. 2004). In this chapter we elaborate on this hypothesis by discussing recent advances in the analysis of the relationship between RNA targeting to specific ER subdomains and protein localization.

2 RNA Localization in Animals and the Role of the ER

RNA localization is recognized as an important process in controlling the synthesis of proteins at specific sites within the cell. More than 100 messenger RNAs are now known to be targeted in a wide variety of eukaryotic cells. This process is essential for cell fate determination in yeast (Chartrand et al. 2001), during early vertebrate development (Bashurullar et al. 1998; Palacios and Johnston 2001), in polar cell growth of somatic cells (Ainger et al. 1997; Carson et al. 1998; Shestakova et al. 2001), and in mediating cell motility (Kloc et al. 2002). The use of this mechanism for several different developmental and cellular processes suggests that the process of RNA localization is common to all eukaryotes.

Several studies have implicated a role for the ER in the localization of RNAs and, in turn, for localized protein secretion during oogenesis. In *Xenopus laevis*, many maternal RNAs are found concentrated in the animal or vegetal pole of the oocyte and early embryo. One well-characterized protein is Vg1, which codes for a transforming growth factor-β and is required for mesoderm induction and right-left symmetry of the embryo (Kloc et al. 2001). In early stage oocytes, Vg1 is initially distributed throughout the cytoplasm but, as