Abstract

Endoplasmic reticulum (ER) of eukaryotic cells is an organelle with a remarkably narrow specialization for the biosynthesis, folding and posttranslational modification of the proteins destined for secretion, plasma membrane and secretory and endocytic organelles. The correct conformation and modifications are essential for the recruitment of secretory proteins into the transport vesicles heading to the Golgi and further along the exocytic pathway. Quality control of the potential cargo molecules in the ER is accomplished by the tight network of accessory proteins, molecular chaperones and folding enzymes that assist nascent polypeptides in achieving their secretion-competent conformations.

We have isolated and characterized a novel member of this team, an endoplasmic reticulum protein, ERp29. **Primary structure, homology and localization.** The ORF of ERp29 precursor encodes a putative protein of 260 amino acids, containing a cleavable signal sequence and a variant of the ER-retrieval signal, C-terminal tetrapeptide KEEL. The ER residency of ERp29 was confirmed by the immunofluorescence microscopy, protease protection and in vitro translation experiments. Weak similarities are found between ERp29 and P5-like protein disulfide isomerases (PDI) from different sources (human, plants, amoebae). In contrast to these proteins, ERp29 does not contain the active site double cysteine motif, which is a hallmark of redox-active PDIs. In addition, the windbeutel gene product from Drosophila is highly similar to ERp29 and lacks the double cysteine motif at the canonical location. The Windbeutel protein has been shown to act as an escort chaperone. **Gene structure and expression.** Comparison with the murine and human genes and phylogenetic analysis demonstrated common origin and close ortholog relationships of the ERp29 gene in rodents and human. CpG island of the 5'-flanking region, absence of canonical TATA-box, multiple transcription sites speak in favor of the house-keeping properties of ERp29. In line with this, we have found the ubiquitous expression of the ERp29 gene with exceptionally high levels in the secretory tissues. A 337 bp fragment of the promoter was identified as a core promoter region. The observed interaction between the predicted GC and E boxes with the Sp1/Sp3 and USF1/USF2 transcription factors respectively, suggest their key role in the basal expression of the gene. Stress induction. Concomitantly with other ER stress proteins (BiP, CRP94), ERp29 is induced in the rat hepatoma cells in response to ER stress, a variety of diverse impacts causing accumulation of misfolded proteins. Similar up-regulation was also demonstrated under the physiological stimulation of the synthesis of the thyroid prohormone, thyroglobulin (Tg) in the thyroid cells. **Interactions in the ER.** Cross-linking followed by the cell lysis and immunoprecipitation of ERp29 or Tg revealed association of ERp29, with Tg, BiP and GRP94, which was further confirmed by the sucrose density gradient analysis and immunofluorescent microscopy. Affinity chromatography approach using Tg as an affinity ligand demonstrated that ERp29 might be selectively isolated from the thyrocyte lysate along with other ER chaperones. Preferential association with the urea-denatured Tg-Sepharose was indicative of either
direct or circuitous ERp29/Tg interactions in a chaperone-like manner. Despite the presence of the C-terminal ER-retrieval signal, significant amounts of ERp29 were recovered from the culture medium of stimulated thyrocytes suggesting its secretion. Based on these data we hypothesize that the function of ERp29 in the thyroid cells is most likely connected with the folding and/or secretion of thyroglobulin. Quaternary structure. Using size exclusion chromatography and chemical cross-linking it was shown that at the micromolar concentrations in solution, as well as in the intact cells, ERp29 has a well-defined quaternary structure with two ERp29 molecules assembled in a non-covalently linked homodimer. However, at high concentrations ERp29 may also form large aggregates. 3D structure. Three-dimensional structures of the individually expressed domains of ERp29 were determined by NMR spectroscopy. Our results confirmed the thioredoxin fold for the N-terminal domain and yielded a novel all-helical fold for the C-terminal domain. Studies of the full-length protein revealed a short, flexible linker between the two domains, homodimerization by the N-terminal domain, and the presence of interaction sites for the formation of higher molecular weight oligomers. These multimerization sites associate with the flexible linker region between the two domains of ERp29 in a non-specific manner and might thus serve as a binding locus also for unfolded polypeptides, which is characteristic for many other chaperones. Phylogenetic analysis and structural similarity with PDIs suggests that ERp29 is a product of divergent evolution, in course of which the redox motif was lost, whereas the protein interaction features, characteristic for the alternative chaperone function of PDI, were preserved. Apparently, the ERp29-like genes emerged relatively late in course of evolution as they are present only in the genomes of multicellular eukaryots. Taken together with the high expression in the secretory tissues, association with nascent polypeptides (thyroglobulin), this suggests that ERp29 is an essential component of the folding/secretory machinery of the higher eukaryots.