FEBS 21812 FEBS Letters 448 (1999) 201–205

Minireview

Quality control: from molecules to organelles

Valentin N. Luzikov*

Belozersky Institute of Physico-Chemical Biology, Lomonosov State University, Moscow 119899, Russia

Received 3 February 1999

Abstract There is a vast body of literature on the quality control of protein folding and assembly into multisubunit complexes. Such control takes place everywhere in the cell. The correcting mechanisms involve cytosolic and organellar proteases; the result of such control is individual molecules with proper structure and individual complexes both with proper stoichiometry and proper structure. Obviously, the formation of organelles as such requires some additional criteria of correctness and some new mechanisms of their implementation. It is proposed in this article that the ability to carry out an integral (key) function may serve as a criterion of correct organelle assembly and that autophagy can be accepted as a mechanism eliminating the assembly mistakes.

Key words: Protein folding; Protein complex assembly; Organelle formation; Chaperon; Protease; Autophagy

1. Quality control for proteins and their complexes

May I start the topic with a trite statement that in the cell the fate of any nascent polypeptide chain is under control of numerous overseers. When a growing polypeptide chain comprises just about 20 amino acid residues, this part is played by a cytoplasmic protein complex referred to as NAC (nascent chain-associated complex). This complex prevents both incidental association of the nascent chain with cytosolic proteins and mistargeting the nascent chains into the endoplasmic reticulum [1]. Polypeptide chains of about 50 residues bind with the Hsp-70/Hsp-40 chaperones (DnaK/DnaJ for bacterial cells) that keep them from arbitrary folding and/or oligomerization. The growing polypeptides longer than 100–150 residues can enter into the channel of the TRiC chaperonine complex (GroEL/GroES in bacteria) where their folding takes place (see review in [1]). If the folding results in aberrant protein molecules, they are degraded in the cytosol by the ubiquitin-dependent proteolytic system including the proteasome as a component (see recent reviews [2,3]). This event is obviously the simplest case of quality control.

The latter term is applicable not only to the formation of individual protein molecules but also to the assembly of multisubunit protein complexes in the cytoplasm. In this case the quality control consists in the elimination of unassembled subunits, again under the action of proteasomes, although lysosomal/vacuolar proteases can be involved under some physiological conditions [4]. In the cytoplasm of bacterial cells, abnormal and truncated proteins are degraded by Lon and/or ClpA-ClpP ATP-dependent proteases, whereas the assembly of membrane complexes is supervised by the FtsH protease [5].

The control over polypeptide chain folding and multisubunit complex assembly takes place in the cell organelles as well. In the case of endoplasmic reticulum, unfolded or aberrant polypeptide chains are re-exported into the cytosol where they make the targets for proteasomes, the cytoplasmic chaperones and ubiquitin-conjugating enzymes being involved in this process [6–9].

We can not currently estimate the scale of the above processes. In fact, to this end it would be well to block the proteasome-mediated intracellular proteolysis, which is impossible since proteasomes are involved in many vitally important processes [5]. However, it is known that proteasome inhibition by selective agents causes the induction of ER chaperones, which in its turn reflects the accumulation of appreciable amounts of abnormal proteins [10]. As regards the role of proteolysis in the regulation of the stoichiometry of oligomeric protein complexes, may I just mention that up to 90% of some initially synthesized subunits of the T-cell antigen receptor are eliminated during its formation [11].

In mitochondria and chloroplasts, abnormal and unassembled proteins undergo degradation by the inherent organellar proteolytic systems [12–14]. As to yeast mitochondria, their soluble proteins and membrane-bound proteins facing the matrix are targets for Pim1p protease, whereas integral membrane proteins are degraded by the Yta10-12 or Yme1p protease complexes, which are members of the AAA protein family [12,13].

As mentioned above, in the cytosol and ER the folding of polypeptide chains and their proteolysis involve chaperones and proteasomes, respectively. In mitochondria the Pim1p and Yta10-12 proteases are capable of performing both functions [8]. It has been shown, in particular, that overproduction of a mutant form of Pim1p lacking proteolytic activity somehow promotes the formation of enzymic complexes in yeast mitochondria devoid of the Yta10-12 complex [15]. The aforesaid is likely to apply to the bacterial FtsH protease located in the cytoplasmic membrane, by virtue of the fact that the effects of *ftsH* mutations are counterbalanced by the overproduction of GroEL/GroES and HtpG chaperones [16].

2. Quality control for organelle formation

Thus, we can currently argue that the cytosol and the organelles contain proteolytic enzymes which, in alliance with chaperones, are essentially capable of controlling the protein folding and the assembly of proteins into multisubunit complexes. If so, one should reason that there must also be special

*Fax: +7 (95) 939-3181. E-mail: luzikov@biogen.genebee.msu.su

PII: S0014-5793(99)00344-0

mechanisms ensuring the control over organelle assembly in the cell. In such a case it is necessary to reinforce the above-mentioned quality criteria by some new ones, such as the arrangement of enzymic complexes in an organelle, their optimal relative contents, the state of organellar matrix and limiting membrane, etc. The correctness of organelle formation can be estimated relying either on a plethora of criteria, taking into account a great number of organelle constituents, or on a single universal criterion. Logically, an ability to carry out an integral function, which directly or indirectly involves all constituents of the organelle and is necessary for the maintenance of its integrity, should be taken as the universal criterion. For mitochondria such a function is likely to be oxidative phosphorylation of ADP, for chloroplasts the analogous functions are photophosphorylation of ADP and photoelectron transfer, for peroxisomes – oxidation of some substrates, etc.

2.1. Mitochondria

There are grounds for believing that the integrity of organelles depends on their ability to carry out their key functions. Mitochondria seem to be a good example to illustrate this statement. The fact is that the composition of these organelles by itself predetermines their self-elimination through the processes controlled by the organellar functional status (for review, see [17]). One of them is conditioned by the presence of easily oxidizable unsaturated fatty acid residues in the phospholipid molecules, on the one hand, and by the production of highly reactive oxygen species, on the other. Normally, lipid peroxidation is retarded owing to the action of glutathione peroxidase/glutathione reductase pair located in the matrix. Besides, the two isoenzymes of superoxide dismutase, which reside in the matrix and the intermembrane space, are involved in this process. However, such self-defence of mitochondria is possible only at high enough intramitochondrial levels of the antioxidative enzymes, which the organelles release upon their uncontrolled high amplitude swelling resulting in the membrane ruptures.

One more way of mitochondrial degradation is realized through the action of specific Ca^{2+}-dependent phospholipase A_2 located in the mitochondrial membranes (see [17] for review). Activation of phospholipase A_2 results in destructive changes in the lipid bilayer, which causes increased permeability of the inner membrane to H^+ ions and various metal cations, the collapse of transmembrane potential, and the release of matrix enzymes [18]. All these processes occur only after de-energization of mitochondria, e.g. under anaerobic conditions. They do not proceed to an essential extent until mitochondria lose the ability to import exogenous ADP and carry out oxidative phosphorylation. One should add therewith that a high enough level of intramitochondrial ATP ensures lysophosphatide reacylation and β-oxidation of free fatty acids [18]. Obviously, the prevention of mitochondrial degradation by the above mechanisms depends directly or indirectly on the functional state of these organelles.

Going back to the problem of quality control, one should note that in the case of mitochondria we are dealing with the degradation of the whole organelle or at least its substantial parts. Simple logic suggests that the structural and functional deterioration in any mitochondrial compartment can hardly occur without drastic consequences for other compartments (see for details pp. 108–131 in [17]).

A direct correlation between the integrity of mitochondria and their ability to carry out oxidative phosphorylation was demonstrated both in vitro and in the experiments with the facultatively anaerobic yeast *Saccharomyces cerevisiae*. In this organism anaerobic, respiratory inhibitors, and uncouplers caused disintegration of the respiratory chain; in parallel, one could observe degenerative changes in the mitochondrial morphology (for review, see [19,20]). Hence it follows that for the mitochondrial formation the quality control may imply elimination of whole organelles or vast regions thereof [21].

In support of this conjecture I can first mention that inhibition of vacuolar proteases in *S. cerevisiae* leads to intracellular accumulation of surplus cytochromes *b*, c_1+*c*, and aa_3 [22]. There were grounds for believing that these cytochromes form potentially active enzymic complexes taking for some reasons no part in the mitochondrial respiration. Besides, a portion of completely assembled mitochondrial enzyme complexes has been shown to undergo rapid degradation in the *Acantamoeba castellanii* cells growing in a synchronous culture [23].

Notice that the high rate of electron transfer in the respiratory chain most likely implies a uniform distribution of the respiratory complexes in the inner mitochondrial membrane. In fact, this process is known to be limited by the lateral diffusion of mobile electron carriers in the membrane [24]. Therefore, the closer are the partner complexes the faster is electron transfer between them. However, some complexes were shown to be distributed quite unevenly, being apparently absent from vast membrane regions [25]. Evidently, such areas cannot contribute much either to the mitochondrial respiration or to the generation of transmembrane potential.

These considerations are in accord with the data on the regional heterogeneity of transmembrane potential in giant mitochondria, and heterogeneity of individual mitochondria as regards the value of their potential in some cell lines [26]. In other words, the cells may contain locally or totally de-energized mitochondria. Such mitochondria are convenient targets for lysosomes [27].

In principle, we can currently suggest two essentially different ways of mitochondrial degradation. First, local defects in the arrangement of enzymic complexes in the inner membrane provoke swelling of adjacent parts of the matrix, which results in local membrane ruptures and release of membrane vesicles as well as matrix constituents into the medium. This material may be further digested by lysosomes and proteasomes. It should be specified that this concerns the cases when the release of cytochrome *c* (among other mitochondrial constituents) is not massive enough to trigger a cascade of apoptotic events in the cell.

Second, whole de-energized mitochondria are somehow recognized, captured, and digested by lysosome/vacuole. Although this event was repeatedly described in the literature [28], its mechanism still remains puzzling. The main question is how lysosomes can differentiate energized and de-energized mitochondria. A plausible idea is that de-energization provokes the release of special 'fusogenic' proteins into the medium. The existence of such soluble proteins in mitochondria was revealed recently [29]. However, it is still unknown how these proteins work and whether their leakage from mitochondria depends on the functional status of the latter. Interestingly, both types of mitochondrial degradation can be seen under the same conditions (for illustration, see Figs. 10b and 11d in [27]).

2.2. Chloroplasts

In many respects the degradation of chloroplasts is similar to that of mitochondria. It is well known that in the dark the detached leaves rapidly lose proteins and chlorophyll. The chlorophyll content decreases through the action of chlorophyllase, an enzyme located in the thylakoid membranes and activated upon degradative changes in them. Such changes take place, e.g. upon interruption of photophosphorylation followed by activation of galactolipase and accumulation of free fatty acids (reviewed in [30]).

Noteworthy is that intense illumination can also be the cause of chloroplast degradation, lipid peroxidation and photodestruction of Q_B (which is necessary for stabilization of quinone ion-radicals) being the primary processes in this case. All the light-induced degradative processes in chloroplasts are retarded upon functioning of the chloroplastal electron transfer system, whereas inhibition of electron transfer stimulates photodestruction (reviewed in [30]).

Thus, the integrity of chloroplasts depends on their ability to carry out their key functions, i.e. photophosphorylation and light-induced electron transfer. The loss of these functions entails grave consequences for the whole organelle. In particular, thylakoid swelling, occurring upon incubation of isolated chloroplasts or detached leaves in the dark, may cause disruption of the chloroplast envelope and the release of stromal proteins into the medium. Probably, just because of this chloroplasts lose ribulose-1,6-biphosphate carboxylase under such conditions [31].

Unfortunately, there are yet no data concerning the aberrations arising in the course of formation of chloroplasts under normal conditions. Although the involvement of endogenous proteases in the control over assembly of protein complexes in chloroplasts was widely covered in the literature (reviewed in [14], see also [32–34]), not much is known about selective autophagy of these organelles [35–37]. It has been shown that under certain conditions the chloroplast envelope can invaginate into the vacuole [38]. Such an observation suggests local (selective) degradation of some parts of chloroplasts, which in its turn may indicate local corrections.

2.3. Endoplasmic reticulum

The specificity of ER lies in the fact that this organelle carries out several key functions, which seem to be unconnected directly with each other. In fact, the rough ER is involved in the synthesis of definite proteins and their introduction into the exocytic pathway through the consecutive import/export processes. On the other hand, the smooth ER is responsible for oxidative hydroxylation of numerous xenobiotics and for hormone metabolism.

In the context of this article, the events taking place upon the induction of oxidative hydroxylation system by its substrates followed by the removal of inducers are of interest. A good example is that phenobarbital stimulates the synthesis of cytochrome P-450[PB] and NADPH: cytochrome P-450[PB] reductase, which leads to an essential proliferation of smooth ER in hepatocytes. Subsequent removal of the substrate/inducer results in the reduction of ER through the autophagic mechanism sequestering the ER segments with the above enzymes [39]. This event is likely to be associated not only with inductive but also with the stabilizing action of phenobarbital on cytochrome P-450[PB] and related enzymes [40].

In the steroid-secreting cells, the number of autophagosomes containing the ER elements was the higher the lower was hormone production [41], which suggested that non-functioning (or weakly functioning) regions of the smooth reticulum underwent selective degradation through autophagy.

On the other hand, there are the data testifying that stimulation of the protein synthesis in liver cells by increasing the amino acid content results in the decreased ratio of vacuoles containing rough relative to smooth reticulum, the degradation of RNA being inhibited therewith [42]. It looks as if rough ER can also be the target for degradation in autophagosomes, this process being dependent on the intensity of ER-associated protein synthesis.

Thus, there are grounds to believe that different parts of ER can be degraded selectively through autophagy and that this process is regulated by the functional status of the corresponding parts.

2.4. Peroxisomes

These organelles are known to undergo total (or large-scale) degradation under definite physiological conditions. This process was most extensively studied for yeast. Normally these organisms (e.g. *Hansenulla polymorpha*, *Candida utilis*) growing in a medium with methanol as a sole carbon source, are characterized by a high content of alcohol oxidase and catalase. When the yeast cells are transferred into a medium containing ethanol or glucose instead of methanol, peroxisomes are progressively degraded. This process begins with the inactivation of alcohol oxidase [43,44] and proceeds through a mechanism depending on the carbon source and the organism. In the glucose medium, non-functioning peroxisomes of *S. cerevisiae* and *H. polymorpha* are degraded by macroautophagy [43], whereas peroxisomes of the *Pichia pastoris* cells transferred into the ethanol-containing medium undergo microautophagic degradation [45,46] (see below).

Obviously, the integrity of peroxisomes is somehow associated with their functioning. In fact, the addition of a key substrate (methanol or long-chain fatty acids) into the medium serves well to maintain peroxisomes in yeasts and hepatocytes [43,47], whereas the decrease of the intracellular fatty acid content by antilipolytic agents stimulates degradation of peroxisomes [48]. Besides, peroxisomes undergo degradation even in the methanol-containing medium if alcohol oxidase is inactivated by cyanide [49].

It has been found that the specific structure of the peroxisomal matrix shows signs of disorganization if the medium contains a substrate for the key oxidase but is devoid of a substrate for coupled transamination [50]. These data imply that a complete set of peroxisomal enzymes is necessary for the formation of a 'standard' organelle, such stability being achievable only under the conditions of concerted action of the whole ensemble.

It particularly follows from the afore-said that the mutations in individual peroxisomal components might be pleiotropic. For example, in the case of the Zellweger syndrome the cells contain empty 'membrane ghosts' instead of normal peroxisomes [51]. This event is accounted for by the defects in the protein import system in the ghosts. Because of this, the bulk of peroxisomal proteins are left in the cytoplasm, where most of them (e.g. the enzymes responsible for β-oxidation) undergo rapid degradation [52]. However, the ghosts themselves are also degraded by autophagy [53]. Another simple case is that the lack of catalase, caused by its impaired targeting into

peroxisomes, results in multiple enzymic deficiencies in the organelles, accounted for by the H_2O_2 toxicity [54].

The mutations in the individual enzymes involved in β-oxidation do not result in the disappearance of morphologically detectable organelles. Sometimes such mutations are characterized by a reduced intracellular content and altered shape of peroxisomes [55,56]. To interpret these data, one should take into account that peroxisomes usually contain several isoforms of the enzymes with different substrate specificity. Besides, it is not necessary that the mutations result in complete loss or inactivation of these enzymes. In such cases, peroxisomes persist as individual organelles but undergo some changes in the form and number.

The mechanisms of discrimination of peroxisomes by their functional status seem to be different for the cases of microautophagy and macroautophagy of the organelles. For the microautophagic degradation, when the peroxisome is captured by a lysosome together with the adjacent cytoplasm, the changes in the structure and surface properties of the organelle itself can hardly be the initial cause of its degradation. Rather, the changes in yeast metabolism induce the synthesis of *PAG* gene products (Pag1p and Pag2p) which control two stages of peroxisome capture [57].

The macroautophagic degradation begins with covering the non-functioning peroxisome by a multilayer membrane. This membrane closely adjoining to the peroxisomal membrane is capable of fusing with the vacuolar membrane, thus making the peroxisomal contents accessible for hydrolytic enzymes. In *H. polymorpha* these two stages are under control of *PDD* genes (peroxisome degradation deficiency genes) [58]. Pdd1p makes the peroxisome committed for degradation, whereas Pdd2p ensures the fusion of peroxisome. Evidently, in this case the metabolically induced alterations in the composition and structure of peroxisomal membranes may trigger the macroautophagic mechanism. In particular, it is known that clofibrate or aspirin, as well as diabetes or starvation, induce the changes in surface charge and hydrophobic properties of peroxisomal membranes [59,60].

3. Conclusions

It follows from the afore-said that the quality control for organelle formation cannot be reduced to a set of partial controlling mechanisms that are realized upon folding of constituent proteins and their assembly into multisubunit complexes, although such control takes place indeed (at least in ER, mitochondria, and chloroplasts). Normal functioning of an organelle becomes possible only under the conditions when there is a complete set of its constituent parts in proper stoichiometry, when the constituents are arranged in a definite way, etc. In such a case, the only reasonable criterion of the proper organelle assembly is its ability or inability to carry out specific integral functions. In the earlier publications, the dependence of mitochondrial stability on the functional status of this organelle was considered in detail. It has been proposed that the assembly of mitochondria in the cell is controlled according to the principle of selection by a performance criterion. A distinct analogy between mitochondria and chloroplasts is seen in this respect. In the recent years, data were accumulated establishing the correlation between the stability of peroxisomes and ER compartments, on the one hand, and their functional status, on the other. The crucial question is how the elimination of non-functioning organelles is realized in the cell.

Unlike the improperly folded polypeptide chains, which are eliminated by specific proteolytic systems located in the cytoplasm or organelles, the preferable mechanism of organelle degradation is total or local autophagy involving lysosomes/vacuoles. This process may be preceded by the degradative processes occurring in the organelles and consisting in selective proteolysis, impairment of the lipid bilayer of limiting membrane, local organelle swelling followed by membrane rupture and discharge of organelle fragments into the cytoplasm.

Unfortunately, the mechanisms of selective autophagy are still unclear. It remains mysterious how the non-functioning organelles are recognized in the cell. In this respect, the nature and the composition of the autophagic limiting membrane, the triggering of such membrane formation, and the mechanism of fusion of the autophagosome with the lysosome/vacuole are of special interest. The changes in organellar membranes resulting from the impairment of their functions also deserve thorough study.

The stabilization of cell organelles by functioning may underlie not only the control over their 'quality', as I tried to show above, but their 'quantity' in the cell as well. The matter is that there must be an overall adjustment of the abundance of every type of organelle to a given metabolic status of the cell, characterized by definite steady-state concentrations of substrates for the organelles. It is tempting to predict that injection of some organelles into the cell will inevitably result in proportional degradation of 'sister-organelles'. Actually, something like this has been reported [61]. It may sound paradoxically, but the content of intracellular 'executors', which part is played by proteasomes and lysosomes/vacuoles, is controlled by the same principle. In fact, in the starving cells proteasomes are degraded by lysosomes [62]; the latter, as it has been known long ago [63], undergo self-digestion.

Acknowledgements: I am grateful to Prof. V.P. Skulachev and Dr. A.V. Galkin for careful reading the article and valuable criticism.

References

[1] Hartl, F.U. (1996) Nature 381, 571–580.
[2] Hilt, W. and Wolf, D.H. (1996) Trends Biochem. Sci. 21, 96–102.
[3] Varshavsky, A. (1997) Trends Biochem. Sci. 22, 383–387.
[4] Egner, R., Thrumm, M., Straub, M., Simeon, A., Schuller, H.J. and Wolf, D.H. (1993) J. Biol. Chem. 268, 27269–27276.
[5] Suzuki, C.K., Rep, M., van Dijl, J.M., Suda, K., Grivell, L.A. and Schatz, G. (1997) Trends Biochem. Sci. 22, 118–123.
[6] Wiertz, E.J.H.J., Tortorella, D., Bogyo, M., Yu, J., Mothes, W., Jones, T.R., Rapoport, T.A. and Ploegh, H.J. (1996) Nature 384, 432–438.
[7] Hiller, M., Finger, A., Schweiger, M. and Wolf, D.H. (1996) Science 273, 1725–1728.
[8] Pilon, M., Schekman, R. and Römisch, K. (1997) EMBO J. 16, 4540–4548.
[9] Brodsky, J.C. and McCracken, A.A. (1997) Trends Cell Biol. 7, 151–156.
[10] Bush, K.T., Goldberg, A.M. and Nigam, S.K. (1997) J. Biol. Chem. 272, 9086–9092.
[11] Minakami, Y., Wessman, A.M., Samelson, L.E. and Klausner, R.D. (1987) Proc. Natl. Acad. Sci. USA 84, 2688–2692.
[12] Rep, M. and Grivell, L. (1996) Curr. Genet. 30, 367–380.
[13] Langer, T. and Neupert, W. (1996) Experientia 52, 1069–1076.

[14] Cohen, Y., Yalovsky, S. and Nechushtai, R. (1995) Biochim. Biophys. Acta 1241, 1–30.
[15] Rep, M., van Dijl, J.M., Suda, K., Schatz, G., Grivell, L.A. and Suzuki, C.R. (1996) Science 274, 103–106.
[16] Shirai, Y., Akiyama, Y. and Ito, K. (1996) J. Bacteriol. 178, 1141–1145.
[17] Luzikov, V.N. (1985) Mitochondrial Biogenesis and Breakdown, Plenum Publ. Corp., New York.
[18] Parce, J.W., Spach, P.I. and Cunningham, C.C. (1980) Biochem. J. 188, 817–822.
[19] Luzikov, V.N. (1973) Sub-Cell. Biochem. 2, 1–31.
[20] Luzikov, V.N., Zubatov, A.S. and Rainina, E.I. (1973) J. Bioenerg. 5, 129–149.
[21] Luzikov, V.N. (1981) FEBS Lett. 125, 131–133.
[22] Galkin, A.V., Tsoi, T.V. and Luzikov, V.N. (1980) Biochem. J. 190, 145–156.
[23] Lloyd, D., Edwards, S.W., Williams, J.L. and Evans, B. (1983) FEMS Microbiol. Lett. 16, 307–312.
[24] Hackenbrock, C.R. (1981) Trends Biochem. Sci. 6, 151–154.
[25] Hackenbrock, C.R. and Miller Hammon, K. (1975) J. Biol. Chem. 250, 9185–9197.
[26] Smiley, S.T., Reers, M., Mottola-Hartshorn, C., Lin, M., Chen, A., Smith, T.W., Steele, G.D. and Chen, L.B. (1991) Proc. Natl. Acad. Sci. USA 88, 3671–3675.
[27] Mancini, M., Anderson, B.O., Caldwell, E., Sedhinasab, M., Paty, P.B. and Hockenbery, D. (1997) J. Cell Biol. 138, 449–469.
[28] Dunn Jr., W.A. (1994) Trends Cell Biol. 4, 139–143.
[29] Cortese, J.G., Voglino, L.A. and Hackenbrock, C.R. (1998) Biochim. Biophys. Acta 1371, 185–198.
[30] Luzikov, V.N. (1988) Biokhimiya (Engl. Transl.) 53, 1382–1383.
[31] Yoshida, T. and Minamikawa, T. (1996) Eur. J. Biochem. 238, 317–324.
[32] Ostersetzer, O., Tabak, S., Yarden, O., Shapira, R. and Adam, Z. (1996) Eur. J. Biochem. 236, 932–936.
[33] Ostersetzer, O. and Adam, Z. (1997) Plant Cell 9, 957–965.
[34] Itzhaki, H., Naveh, L., Lindhal, M., Cook, M. and Adam, Z. (1998) J. Biol. Chem. 273, 7094–7098.
[35] Pettitt, J.M. (1976) J. Cell Sci. 20, 671–685.
[36] Thorsness, P.E. and Weber, E.R. (1996) Int. Rev. Cytol. 165, 207–234.
[37] Wu, J., Lightner, J., Warwick, N. and Browse, J. (1997) Plant Physiol. 113, 347–356.
[38] Frank, T., Crevecoeur, M., Wuest, J., Greppin, H. and Gaspar, T. (1998) Biotech. Histochem. 73, 32–43.
[39] Masaki, R., Yamamoto, A. and Tashiro, Y. (1987) J. Cell Biol. 104, 1207–1215.
[40] Schuetz, E.G., Wrighton, S.A., Safe, S.H. and Guzelian, P.S. (1986) Biochemistry 25, 1124–1133.
[41] Yi, J. and Tang, X.M. (1995) Anatom. Rec. 242, 137–146.
[42] Lardeux, B.R. and Mortimore, G.E. (1987) J. Biol. Chem. 262, 14514–14519.
[43] Veenhuis, M., Douma, A., Harder, W. and Osumi, M. (1983) Arch. Microbiol. 134, 193–203.
[44] Hill, D.J., Hann, A.C. and Lloyd, D. (1985) Biochem. J. 232, 743–750.
[45] Tuttle, D.L. and Dunn Jr., W.A. (1995) J. Cell Sci. 108, 25–35.
[46] Sakai, Y., Koller, A., Rangell, L.K., Keller, G.A. and Subramani, S. (1998) J. Cell Biol. 141, 625–636.
[47] Luiken, J.J.F.P., van den Berg, M., Heikoop, J.C. and Meijer, A.J. (1992) FEBS Lett. 304, 93–97.
[48] Locci Cubeddu, T., Masiello, P., Pollera, M. and Bergamini, E. (1985) Biochim. Biophys. Acta 839, 96–104.
[49] Van der Klei, I.J., Veenhuis, M., Nicolay, K. and Harder, W. (1989) Arch. Microbiol. 151, 26–33.
[50] Heupel, R. and Heldt, H.W. (1994) Eur. J. Biochem. 220, 165–172.
[51] Santos, M.J., Imanaka, T., Shio, H., Small, G.M. and Lazarow, P.B. (1988) Science 239, 1536–1538.
[52] Schram, A.W., Stijland, A., Hashimoto, T., Wonders, R.J.A., Schutgens, R.B.H., van den Bosch, H. and Tager, J.M. (1986) Proc. Natl. Acad. Sci. USA 83, 6156–6158.
[53] Arias, J.A., Moser, A. and Goldfischer, S.L. (1985) J. Cell Biol. 100, 1789–1792.
[54] Sheikh, F.G., Pahan, K., Khan, M., Barbosa, E. and Singh, I. (1998) Proc. Natl. Acad. Sci. USA 95, 2961–2966.
[55] Poll-The, B.T., Roels, F., Ogier, H., Scotto, J., Vamecq, J. and Schutgens, R.B.H. et al. (1988) Am. J. Hum. Genet. 42, 422–434.
[56] Van Grunsven, E.G., van Berkel, E., Ijlst, L., Vreken, P., de Klerk, J.B.C., Adamski, J., Lemonde, H., Clayton, P., Cuebas, D.A. and Wanders, R.J.A. (1998) Proc. Natl. Acad. Sci. USA 95, 2128–2133.
[57] Sakai, Y., Koller, A., Rangell, L.K., Keller, G.A. and Subramani, S. (1998) J. Cell Biol. 141, 625–636.
[58] Titorenko, V.I., Keizer, I., Harder, W. and Veenhuis, M. (1995) J. Bacteriol. 177, 357–363.
[59] Horie, S., Ishii, H., Orii, H. and Suga, T. (1982) Biochim. Biophys. Acta 690, 74–80.
[60] Crane, D.I. and Masters, C.J. (1986) Biochim. Biophys. Acta 876, 256–263.
[61] Abramova, N.B., Korzh, V.P. and Neifakh, A.A. (1983) Biokhimiya (Russ.) 49, 1279–1286.
[62] Cuervo, A.M., Palmer, A., Rivett, A.J. and Knecht, E. (1995) Eur. J. Biochem. 227, 792–800.
[63] Cohn, Z.A. and Fedorko, M.E. (1969) in: J.T. Dingle and H.B. Fell (Eds.), Lysosomes in Biology and Pathology, Vol. 1, pp. 43–63.