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GroEL-related molecular chaperones are present in the cytosol of oat cells

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In eukaryotic cells GroEL-related molecular chaperones (cpn 60) are considered to be restricted to plastids and mitochondria. Re-evaluation of the intracellular localization of chaperonins by electron microscopy, using two different anti-chaperonin antisera, revealed additionally their presence in the cytosol of oat primary leaf and coleoptile cells. The distribution of cpn 60 is not influenced by heat or light treatments.

GroEL; Molecular chaperone; Cytosol; Oat

1. INTRODUCTION

Molecular chaperones influence the folding kinetics of a range of proteins by binding to non-native structures that are possibly folding intermediates [1–10]. This folding arrest by molecular chaperones has an important cellular role in, for example, maintaining polypeptides in non-native states for translocation [1,3,6], or mediating the folding pathway of other proteins by preventing incorrect molecular interactions [2,7–11].

Among the most abundant molecular chaperones are the 'chaperonins' [12], a class of oligomeric proteins found in bacteria [12], chloroplasts [12–14] and mitochondria [15]. The essential role of chaperonins in bacterial growth [16] and mitochondrial biogenesis [17,18], together with their demonstrated influence on the folding and assembly of proteins [1–11], has led to speculation that chaperonins may also exist in the cytosol of eukaryotic cells to perform similar vital functions [19].

Polyclonal antisera raised against the purified *E. coli* GroEL chaperonin protein, and against the plastid homologue RUBISCO subunit binding protein (RBP), were used to study the intracellular localization of chaperonin proteins in the higher plant oat (*Avena sativa*).

We now show by electron microscopy that proteins related to the bacterial GroEL chaperonin are not only localized in plastids and mitochondria, but are also distributed throughout the cytosol of oat coleoptile and primary leaf cells, and that this distribution is not influenced by heat or light treatments.

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2. MATERIALS AND METHODS

2.1. *Plant material*

Oat seedlings were grown on moist vermiculite in complete darkness for 4 days at 26°C or treated with white light (8.5 $W \cdot m^{-2}$) for 2, 4, 6, 12 and 24 h after growth in darkness. A 2 h treatment at 42°C was given for all tissues in parallel.

2.2. *Electron microscopy*

Preparation of the plant material for electron microscopy and immunogold-labeling were carried out as described in [18]. Control experiments were performed by: (i) omission of the primary antiserum; (ii) dilution series of primary antiserum; and (iii) presence of 0.5 M NaCl in all buffers used. Purification of GroEL and RUBISCO subunit binding protein (RBP) and raising of antisera were carried out essentially as described elsewhere [2,7].

2.3. *Protein extraction*

Whole oat seedlings, except roots, were harvested except roots and homogenized with 2 ml extraction buffer per gram fresh weight. Extraction buffer was 100 mM sodium phosphate, pH 7.5, 5 mM sodium ethylene tetraacetic acid, 28 mM 2-mercaptoethanol, 4 mM phenylmethylsulphonylfluoride, 5 mM ϵ-aminocaproic acid and 1 mM benzamidine. Crude extracts were clarified by squeezing through two layers of miracloth (Calbiochem, La Jolla, USA) and centrifugation at 20000 × *g* for 20 min.

2.4. *Protein determination, SDS-PAGE and Western blotting*

Total protein was determined according to the method of Bradford [21]. Equal amounts of total protein per lane were separated by denaturing gel electrophoresis on 10% polyacrylamide gels according to Laemmli [22]. Processing of Western blots was as described in [23] except all buffers containing 0.5 M NaCl instead of 0.15 M NaCl. Staining of the blots was performed using the ECL-detection system (Amersham-Buchler, Braunschweig, Germany) according to the manufacturers protocol.

Fig. 1. An electron micrograph of primary leaf cells from oat seedlings grown 4 days in darkness, labeled with anti-RBP antiserum. Bar = 1 μm. E, etioplast; CW, cell wall. Preparation of the plant material for electron microscopy and immunogold-labeling were carried out as described in section 2.

3. RESULTS AND DISCUSSION

As shown in Fig. 1, the anti-RBP antiserum selectively recognizes immunoreactive material in the stroma of etioplasts, and also in maturing and mature plastids (data not shown). Immunogold labeling was not observed for mitochondria, nuclei or the cytosol. This distribution of immunoreactive material does not change following heat treatment or exposure to light, although the actual amount of RBP has been observed by others to increase following light treatment [24]. Interestingly, the amount of immunoreactive proteins is significantly higher in etioplasts than in mature plastids (data not shown).

In contrast, a more complex staining pattern of cells is observed when anti-GroEL antiserum is used (Fig. 2a–d). Not only is immunoreactive material present in etioplasts, developing and mature plastids (cf. Fig. 1), there is also staining in mitochondria (although very weak), nuclei (Grimm and Speth, in prep.) and throughout the cytosol. The antigenically reactive proteins in the cytosol are concentrated in localized regions, but do not appear to be associated with either polysomes, rough endoplasmic reticulum, membranes or membrane structures, or with the cytoskeleton. This distribution pattern is found in all tissues from seedlings grown completely in the dark, as well as from seedlings during the process of greening (Fig. 2a–d). Again, heat treatment for 2 h at 42°C with different light regimes did not influence the staining pattern in cells, and did not result in a significant increase of total amount of immunoreactive 60 kDa chaperonin homologues in extracts of oat cells (Fig. 3). The cytosolic GroEL-related proteins are therefore constitutively expressed. Unpublished data cited by Hemmingsen [25] indicate that the plastid chaperonin is only slightly increased by heat shock, although the plant mitochondrial chaperonin exhibits a 2–3-fold increase following heat shock [26].

Interestingly, in all of the tissues we examined, there was considerable variation in the amount of immunoreactive GroEL-related proteins found in the cytosol of different cells (Fig. 2a–d). This could simply represent a stochastic distribution of the protein within cells, or may have physiological significance. For example, cells of young tissues show very high rates of protein synthesis, and a higher concentration of chaperonins may be required to facilitate the correct folding of proteins, not only in mitochondria and plastids, but also in the cytosol. Cells of different tissues and ages may also show a different sensitivity or competence to deal with stress conditions, and therefore have different cytosolic levels of GroEL-related proteins.

Two of the best studied molecular chaperones are those of the hsp70 class and chaperonins. Examples of these proteins exist in prokaryotic organisms, where the hsp70-related protein is DnaK [27] and the chaperonin is GroEL [12]. Both are heat shock proteins in *E. coli*. For full biological activity of GroEL an additional protein (GroES) is required [9,16,28]. Until recently, the reported cellular distribution of the hsp70 and chaperonin classes of proteins in eukaryotic organisms was quite distinct, with hsp70-related proteins localized in the cytosol, endoplasmic reticulum and nucleus (reviewed in [29]), and chaperonins present in mitochondria [15,26] and plastids [12–14]. However, hsp70 homologues have now been identified in chloroplasts [30] and mitochondria [31], and a GroES homologue is also present in mitochondria [32] and possibly in chloroplasts [32]. These organelles, therefore, contain representatives of the three most abundant molecular chaperones (DnaK, GroEL and GroES) found in bacteria, and thus have the components considered necessary for successful folding and assembly of proteins in organello [33].

In this report we demonstrate that GroEL-related proteins are not confined to only chloroplasts and mitochondria, but are also localized throughout the cytosol of plant cells. It is also probable that homologues of both GroEL and GroES exist in the cytosol of other eukaryotic organisms. Some sequence homology has already been identified between chaperonins and the mouse cytosolic t-complex polypeptide 1 (TCP-1) [34], and in unpublished data cited by Ellis [19] a monoclonal antibody against TCP-1 can recognize a pea cytosolic protein.

GroEL-related chaperonins appear to have a ubiquitous distribution in plant cells, which is consistent with their essential role in cell viability [16,17], and involvement in an apparently disparate range of cellular processes such as protein folding, assembly, transport, and in some organisms a stress response.

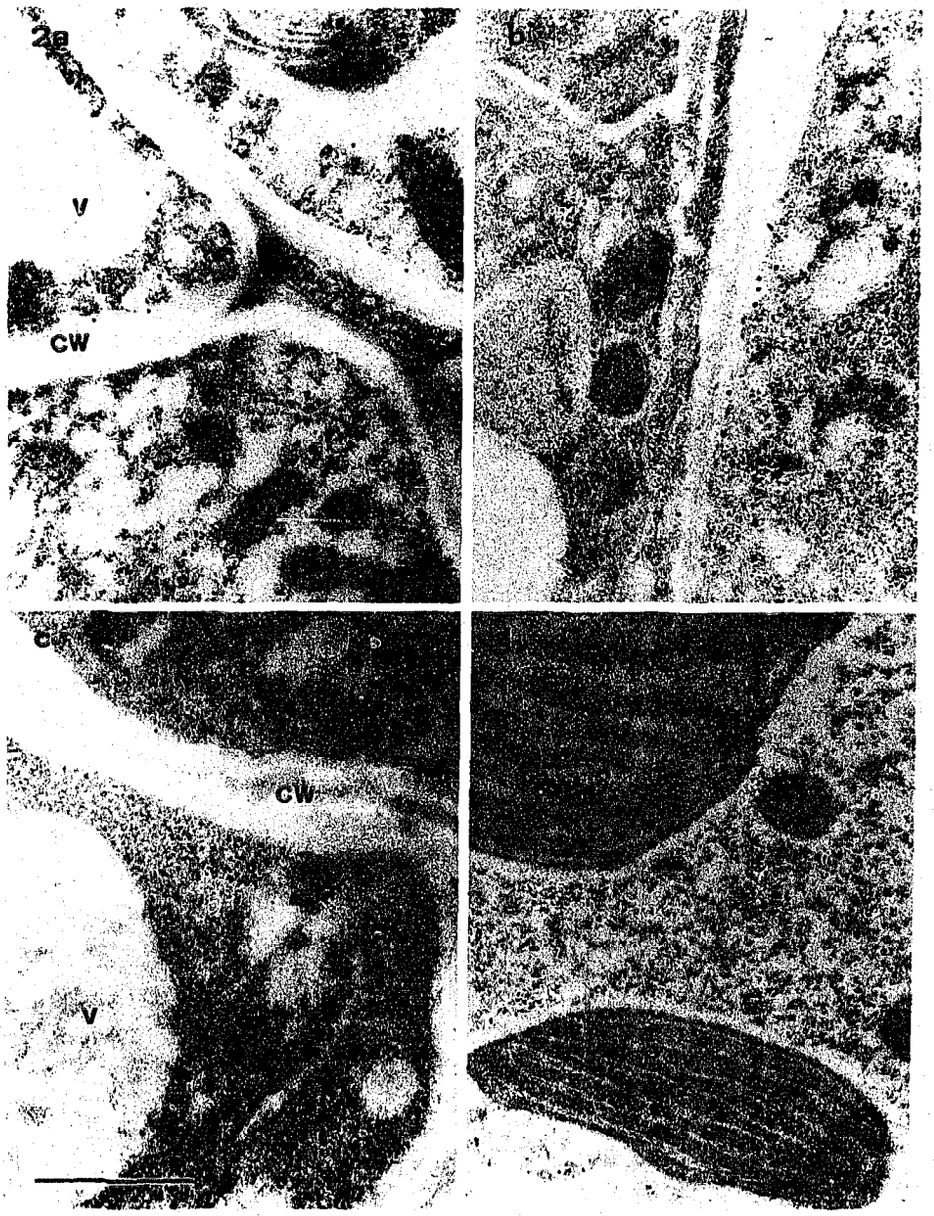

Fig. 2. (a–d) Electron micrographs of oat coleoptile and primary leaf cells (a) grown in darkness for 4 days (b) with additional 2 h light treatment (c) corresponding to (b) with 2 h heat treatment at 42°C (d) with additional 12 h light treatment. Immunolabeling was performed with anti-GroEL antiserum. E, etioplast; P, plastid; V, vacuole; CW, cell wall. Bar = 1 μm. Preparation of plant material for electron microscopy, immunogold-labeling and control experiments were performed as described in section 2.

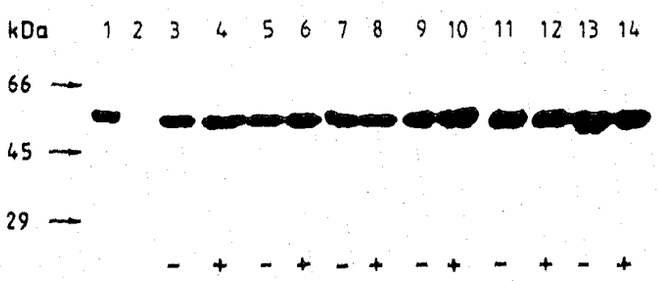

Fig. 3. Western blot analysis of dark-grown oat tissue and of tissue during the process of greening using the anti-GroEL antiserum. Arrows indicate molecular masses in kilodalton (kDa). (Lane 1) Purified bacterial GroEL; (lane 2) molecular weight marker proteins (not visible on processed blots); (lanes 3–14) crude extracts from oat seedlings grown under the following conditions: either completely in darkness at 26°C for 4 days (lanes 3, 4) or treated with white light (8.5 $W \cdot m^{-2}$) for 2 h (lanes 5, 6), 4 h (lanes 7, 8), 6 h (lanes 9, 10), 12 h (lanes 11, 12) and 24 h (lanes 13, 14) after growth in darkness. A 2 h treatment at 42°C was given for all tissues in parallel. (−) Without 2 h heat treatment; (+) with 2 h heat treatment at 42°C.

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REFERENCES

[1] Bochkareva, E.S., Lissen, N.M. and Girshovich, A.S. (1988) Nature 336, 254–257.
[2] Goloubinoff, P., Christeller, J.T., Gatenby, A.A. and Lorimer, G.H. (1989) Nature 342, 884–889.
[3] Lecker, S., Lill, R., Ziegelhoffer, T., Georgopoulos, C., Bassford, P.J., Kumamoto, C.A. and Wickner, W. (1989) EMBO J. 8, 2703–2709.
[4] Ostermann, J., Horwich, A.L., Neupert, W. and Hartl, F.-U. (1989) Nature 341, 125–130.
[5] Laminet, A.A., Ziegelhoffer, T., Georgopoulos, C. and Plückthun, A. (1990) EMBO J. 9, 2315–2319.
[6] Hardy, S.J.S. and Randall, L.L. (1991) Science 251, 439–443.
[7] Viitanen, P.V., Lubben, T.H., Reed, J., Goloubinoff, P., O'Keefe, D.P. and Lorimer, G.H. (1990) Biochemistry 29, 5665–5671.
[8] Buchner, J., Schmidt, M., Fuchs, M., Jaenicke, R., Rudolph, R., Schmid, F.X. and Kiefhaber, T. (1991) Biochemistry 30, 1586–1591.
[9] Fischer, G. and Schmid, F.X. (1990) Biochemistry 29, 2205–2212.
[10] Morimoto, R.I., Tissieres, A. and Georgopoulos, C. (eds.) (1990) Stress Proteins in Biology and Medicine, CSH, New York, USA.
[11] Goloubinoff, P., Gatenby, A.A. and Lorimer, G.H. (1989) Nature 337, 44–47.
[12] Hemmingsen, S.M., Woolford, C., Van der Vies, S.M., Tilly, K., Dennis, D.T., Georgopoulos, C.P., Hendrix, R.W. and Ellis, R.J. (1988) Nature 333, 330–334.
[13] Barraclough, R. and Ellis, R.J. (1980) Biochim. Biophys. Acta 608, 19–31.
[14] Bloom, M.V., Milos, P. and Roy, H. (1983) Proc. Natl. Acad. Sci. USA 80, 1013–1017.
[15] McMullin, T.W. and Hallberg, R.L. (1988) Mol. Cell. Biol. 8, 371–380.
[16] Fayet, O., Ziegelhoffer, T. and Georgopoulos, C. (1989) J. Bacteriol. 171, 1379–1385.
[17] Reading, D.S., Hallberg, R.L. and Myers, A.M. (1989) Nature 337, 655–659.
[18] Cheng, M.Y., Hartl, F.-U., Martin, J., Pollock, R.A., Kalousek, F., Neupert, W., Hallberg, E.M., Hallberg, R.I. and Horwich, A.L. (1989) Nature 337, 620–625.
[19] Ellis, R.J. (1990) Science 250, 954–959.
[20] Speth, V., Otto, V. and Schäfer, E. (1986) Planta 168, 299–304.
[21] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[22] Laemmli, U.K. (1970) Nature 227, 680–685.
[23] Grimm, R., Lottspeich, F., Schneider-Poetsch, H.J. and Rüdiger, W. (1986) Z. Naturforsch. 41c, 993–1000.
[24] Lennox, C.R. and Ellis, R.J. (1986) Biochem. Soc. Trans. 14, 9–11.
[25] Hemmingsen, S.M. (1990) Semin. Cell Biol. 1, 47–54.
[26] Prasad, T.K. and Hallberg, R.L. (1989) Plant Mol. Biol. 12, 609–618.
[27] Bardwell, J.C.A. and Craig, E.A. (1984) Proc. Natl. Acad. Sci. USA 81, 848–852.
[28] Tilly, K., Murialdo, H. and Georgopoulos, C. (1981) Proc. Natl. Acad. Sci. USA 78, 1629–1633.
[29] Pelham, H.R.B. (1986) Cell 46, 959–961.
[30] Marshall, J.S., DeRocher, A.E., Keegstra, K. and Vierling, E. (1990) Proc. Natl. Acad. Sci. USA 87, 374–378.
[31] Leustek, T., Dalie, B., Amir-Shapira, D., Brot, N. and Weissbach, H. (1990) Proc. Natl. Acad. Sci. USA 86, 7805–7808.
[32] Lubben, T.H., Gatenby, A.A., Donaldson, G.K., Lorimer, G.H. and Viitanen, P.V. (1990) Proc. Natl. Acad. Sci. USA 87, 7683–7687.
[33] Kang, P.-J., Ostermann, J., Shilling, J., Neupert, W., Craig, E.A. and Pfanner, N. (1990) Nature 348, 137–143.
[34] Gupta, R.S. (1990) Biochem. Int. 20, 833–841.