

The synapse-like interaction between chloroplast, dictyosome, and other cell compartments during increased ethylene production in leaves of rye (*Secale cereale* L.)

T. SELGA* and M. SELGA**

Department of Plant Cell Biology, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, LV-1848 Riga, Latvia*
Department of Plant Physiology, Institute of Biology, University of Latvia, 3 Miera St, LV-2169 Salaspils, Latvia**

Abstract

Rye (*Secale cereale* L.) plants were treated with an ethylene releaser ethephon (2-chloroethylphosphonic acid) in concentration of 4×10^{-2} M. We studied electron microscopically, if and how chloroplasts interact with well-documented sites of ethylene production/binding, *i.e.*, with endoplasmic reticulum, dictyosomes, mitochondria, plasma membrane, and tonoplast. During the sharp increase of ethylene synthesis in mesophyll cells of rye leaves, the direct local contiguity of chloroplast envelope or envelope protrusions with the above mentioned cell compartments was typical. Moreover, a large number and diversity of versatile chloroplast-dictyosome associations were conspicuous, in which both the chloroplast and each cisterna of dictyosome were capable to exo/endocytosis. The dictyosomes were directed towards the chloroplasts, plasma membrane, or tonoplast both with cis-face, trans-face, or with the rim, they could change their direction or shut up the trans-face, developing simultaneously several flexible chains of vesicular dispatches among chloroplasts and some other cell compartments. This reflects interaction of protein/ethylene producing, photosynthesising, DNA containing compartments, and regulated action of lysosomal system. Structural contacts and vesicular transport among compartments of symplastic system equalises concentrations of H^+ , Ca^{2+} , *etc.* ions, as well as provide connection with an apoplast. We propose that ethylene functions in plant mesophyll cells are both as intra/intercellular signalling substance and as phytohormone that regulates gene expression in nuclei, chloroplasts, and mitochondria in a complicated synapse-like process and causes programmed death of leaves of the main stalks of rye for the sake of promoted growth of side shoots.

Additional key words: electron microscopy; endoplasmic reticulum; ethephon; Golgi apparatus; intracellular signalling; mitochondrion.

Introduction

The interaction of chloroplast with sites of ethylene production and binding—the endoplasmic reticulum (ER) (Goodwin 1983), dictyosomes (Goodwin and Mercer 1983, Crevecoeur *et al.* 1990), plasma membrane (Lieberman 1979, Mayne and Kende 1986, Crevecoeur *et al.* 1990), tonoplast (Mayne and Kende 1986, Bouzen *et al.* 1989, Crevecoeur *et al.* 1990), and mitochondria (Vinkler and Apelbaum 1983)—were studied in mesophyll cells of rye leaves by help of electron microscopy, during the intentionally activated ethylene production. The chloroplast envelope membranes also contain whole set of enzymes, such as hydroperoxide lyase, hydroperoxide dehydratase, and lipoxygenase (Joyard *et al.* 1995), which are components of ethylene forming enzyme EFE, regulating the conversion of

1-aminocyclopropane-1-carboxylic acid to ethylene (Ievins and Romanovskaya 1991).

The chloroplast as photosynthesising system has been explored by combined efforts of several modern methods. Studies of active and passive transports of substances among compartments of chloroplast as well as between the chloroplast and cytosol were supported by electron microscopic observation (Heber 1981, Selga 1983, Heldt 1987). The translocation of sucrose and amino acids from the donor, leaf mesophyll, into apoplast is under the control of several sinks, such as growing and storing organs (Kursanov 1976, Mengel and Kirby 1987). Both symplastic and apoplastic phloem loading in the majority of plant species was ascertained (Bel 1989). However, the interaction of chloroplast with other compartments of

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the cell, especially with dictyosomes, is not clear. There are only suggestions that an essential compound for photosynthesis, plastoquinone, is synthesised in dictyosomes and transported to chloroplasts (Joyard *et al.* 1995).

Laser microscopy shows that chloroplasts are connected *via* tubes through which they share proteins and exchange information similarly as photosynthesising bacteria exchange genes through thin tubules, called pili (Köhler *et al.* 1997). According to widely accepted hypothesis of endosymbiosis (Schwartz and Dayhoff 1978, Margulis 1981, Gray 1989), the outer membrane of chloroplast envelope originated as a deep invagination of the plasma membrane of an ancient eukaryotic cell and thereby it is related with ER. ER is continuous with the outer membrane of nuclear envelope (Fig. 1A). It is believed that the space between the outer and inner membrane of chloroplast envelope is continuous with ER that carries out a common symplastic intracellular and intercellular transport of substances through plasmodesmata (Turgeon and Hepler 1989, Gamalei 1995).

Materials and methods

The winter rye (*Secale cereale* L.) plants were cultivated in soil, in a vegetation chamber. The air temperature day/night was 17/15 °C, irradiance 150 W m⁻² for 16 h. At the tubulation stage the plants were sprinkled with 4×10⁻² M water solution of ethephon (2-chloroethylphosphonic acid). It induced sharp increase in ethylene production immediately after plant treatment and during next 10 d (Warner and Leopold 1969, Abeles 1973, Lieberman 1979, Ievinsh *et al.* 1990). The length of shoots, leaf area, dry matter, and other morphologic indices were considered. For electron microscopy, samples of the middle part of the mature upper (flag) leaf

Consequently, the envelopes of nuclei and chloroplasts are different specialised regions of ER. The products of ER migrate by vesicles to the Golgi apparatus, the asymmetric stack of flattened sacs called dictyosome, surrounded by exo/endocytotic vesicles and/or system of interconnected tubules. The dictyosome, consisting from *cis*-, medial-, and trans-cisternae, is a system of processing, sorting, and transport of proteins, saccharides, glycoproteins, and predecessors of lignin. This is necessary for the development of plant cell wall and accomplished by vesicles to the periphery of cytoplasm. The dictyosomes develop also lysosomes and the central vacuole, evolve slime, wax, gum, and/or secretor products (Whaley 1975, Fawcett 1981, Rothman *et al.* 1984, Kristen *et al.* 1989, Driouich *et al.* 1993). Hardly ever Golgi apparatus was observed in mature mesophyll cells under normal conditions, but its hypertrophied appearance and activity is one of the most typical plant responses to the stress (Selga M. *et al.* 1985, Selga M. and Selga T. 1994, Selga T. and Selga M. 1996, 1998).

Results

The ultrastructural images of the present study reflect metabolism in leaves of the basic shoot at the time of morphogenetic change in the rye plants, under the effect of ethephon. Their main stalk was 30 % shorter, the stalk dry matter was 25 % larger, and the total number of stalks was doubled in comparison with control plants.

The joining of nuclear envelope with plasma membrane by a wide cisterna of rough ER, contiguity of chloroplast and nuclear envelopes (Fig. 2A), of the tips of thylakoids with the inner membrane of chloroplast envelope and the outer membrane of chloroplast envelope with plasma membrane near the plasmodesmata (Fig. 2B), the elongation of chloroplast envelope

blade of rye plants at the 10th d after the treatment with ethephon were double fixed with 2 % glutaraldehyde and 4 % osmium tetroxide in sodium cacodylate buffer. Samples were dehydrated in ethanol (20-96 %, 30 min in each concentration), contrasted with 1 % phosphowolframic acid, and embedded in the mixture of epoxy resin (Epon: 812, MNA, HY964, DYO64). Ultrathin sections were cut with an ultramicrotome LKB 8800, contrasted with lead citrate (Geyer 1973), analysed, and photographed with an electron microscope Tesla BS 500 under magnifications of 20 000-50 000.

to discharge exosomes (Fig. 2C), approach of chloroplast envelope protrusion to tonoplast (Fig. 2D) as well as to plasma membrane (Fig. 2E) show disposition to brief, local, direct communication of cell membranes, perinuclear, periplastidal, and extracellular space avoiding stroma and cytosol (Fig. 1B). In addition, a great diversity of dictyosomes was observed. They were typical with 2-4 short cisternae and without well developed ER in thereabouts. The associations of chloroplasts and dictyosomes were chosen to demonstrate some main directions of conjoined polar processes (Fig. 1C).

The chloroplast-dictyosome associations related with plasma membrane point to several activities. Rather

frequently, the dictyosome locates in a wide layer of cytosol with its *cis*-side oriented to chloroplast, but *trans*-side oriented to plasma membrane [Figs. 1C(D9) and 3A]. A tight touch of the middle part of the *cis*-cisterna with a chloroplast and budding of small vesicles from the periplastidal space in this zone was observed. It suggests that periplastidal space is functionally similar to ER. Another end of the same dictyosome was bowed near to the plasma membrane. The annulate profile that dictyosome points to conducted reversible coiling and opening of dictyosomes export face is probably made by the help of cytoskeleton regulating the activity and polarity of processes [Figs. 1C(D2) and 3B]. Mostly the

1C(D1) and 3C]. Frequently, the periphery of dictyosome is close to a chloroplast. Exchange of vesicles between the rims of cisternae and the damaged envelope of chloroplast, as well as creation of a great autophagosome from *trans*-side of dictyosome towards the plasma membrane is evident (Fig. 3D). Greatly widespread is also an opposite situation where the chloroplast is associated directly or by mediation of a mitochondrion with a dictyosome that lies on its back—it is turned with *cis*-side to the plasma membrane [Figs. 1C(D8) and 3E]. A sickle shaped dictyosome, transverse both to chloroplast and to plasma membrane, exactly reflects dictyosome rotation, evidently caused by the change of polarity/orientation of cytoskeleton [Figs. 1C(D6) and 3F].

Simultaneously in the same cells a great transversal bridge between the chloroplast and plasma membrane was observed. The bridge is developed by approach of rims of two neighbouring dictyosomes. It is difficult to ascertain whether they are interconnected only by transport vesicles or also by some tubular structures. The X-shaped profiles of both dictyosomes and expanded rims of lateral cisternae point to increased activities of their *cis*- and *trans*-pathways. In addition, a lengthwise return transport through the horizontal axis of medial cisternae of the two conjoined dictyosomes could be one of the reasons for such a coupling [Figs. 1C(D4,5) and 3G].

The chloroplast-dictyosome complexes near vacuole are also different. The location of dictyosome in a triangle of cytosol among the chloroplasts, plasma membrane, and tonoplast is typical [Fig. 1C(D10), however, the position of dictyosome and address of vesicular dispatches can be different (Fig. 4A,B). A plain sight of a dictyosome-mediated traffic of substances from a chloroplast into vacuole is general in highly vacuolised cells: the middle region of *cis*-cisterna is close near the chloroplast; small exosomes, separating from the periplastidal space, move towards *cis*-cisterna, but vesicles, budding both from the rims of medial cisternae and from *trans*-network, transport probably various secretory products into the vacuole [Figs. 1C(D3) and 4C]. In the same cells, a distinct chloroplast-dictyosome-vacuole association is also frequent, in which the *cis*-cisterna is near to the twisted tonoplast showing that in this case the vacuole must be the donor compartment, but the target of transport vesicles are two chloroplasts located near each other and ER, squeezed between them [Figs. 1C(D7) and 4D].

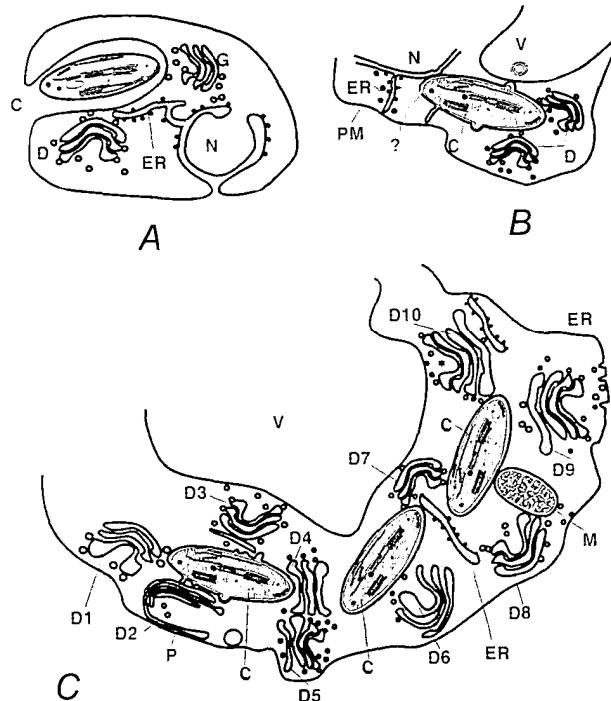


Fig. 1. The chloroplast relation with other cell compartments: A - interrelated origin; B - chloroplast communication with other cell compartments during the effect of ethylene; C - total view of diversity of chloroplast-dictyosome interaction in mesophyll cells of winter rye during the effect of ethylene. C - chloroplast, D - dictyosome, ER - endoplasmic reticulum, M - mitochondrion, N - nucleus, P - plasma membrane, V - vacuole.

chloroplast and dictyosome are located side by side and small vesicles migrate between the periplastidal space and *cis*-cisterna, whereas the overloaded *trans*-cisternae threw their products near the plasma membrane [Figs.

Discussion

The mechanism of fast spread of ethephon and ethylene in all tissues after a local treatment of plants is unknown. The different structural junctions of variously interconnected ethylene producing/binding sites with chloroplasts in rye mesophyll cells affirm our previous results on several centres of primary activity of ethephon (Selga M. *et al.* 1985). They also reveal possible successive co-action of several ethylene forming systems

described in the literature: ethylene production by decomposition of ethephon having penetrated into leaf tissues (Levinsh *et al.* 1990), autocatalytic effect of this exogenous ethylene causing multistage process of synthesis: methionine-SAM (S-adenosylmethionine)-ACC (1-aminocyclopropane-1-carboxylic acid)-ethylene, or formation from peroxydised linolenate upon breakdown of membranes by peroxidases (Lieberman 1979,

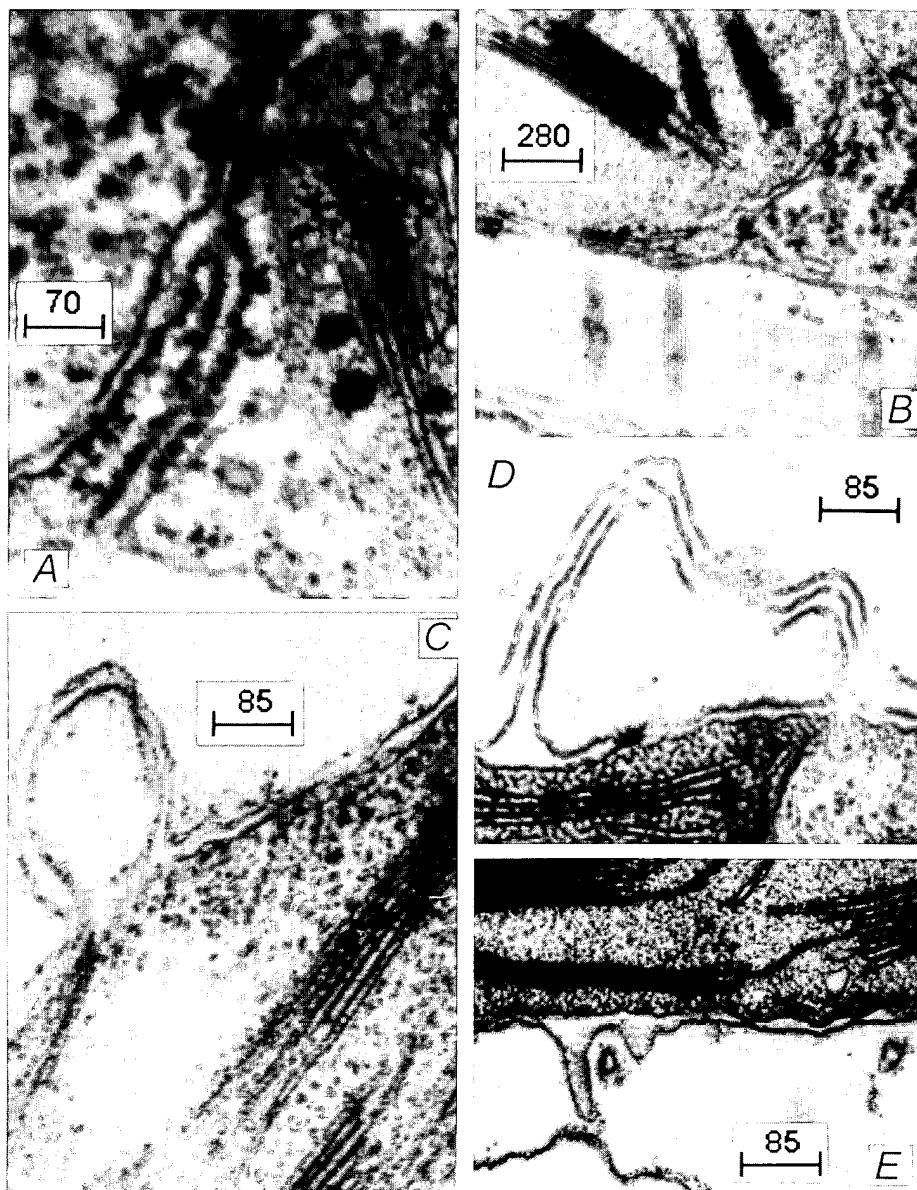


Fig. 2. Several communication activities of chloroplasts related with plasma membrane: *A* - direct contiguity of the chloroplast and nuclear envelopes near the initiation of ER tubes, which nearly touch plasma membrane; *B* - the chain of direct contacts: thylakoid-chloroplast envelope-plasma membrane-plasmodesmata; *C* - discharge of chloroplast exosome; *D* - inclusion of chloroplast envelope protuberance with tonoplast; *E* - approach of chloroplast envelope to plasma membrane. Bars show dimension in nm.

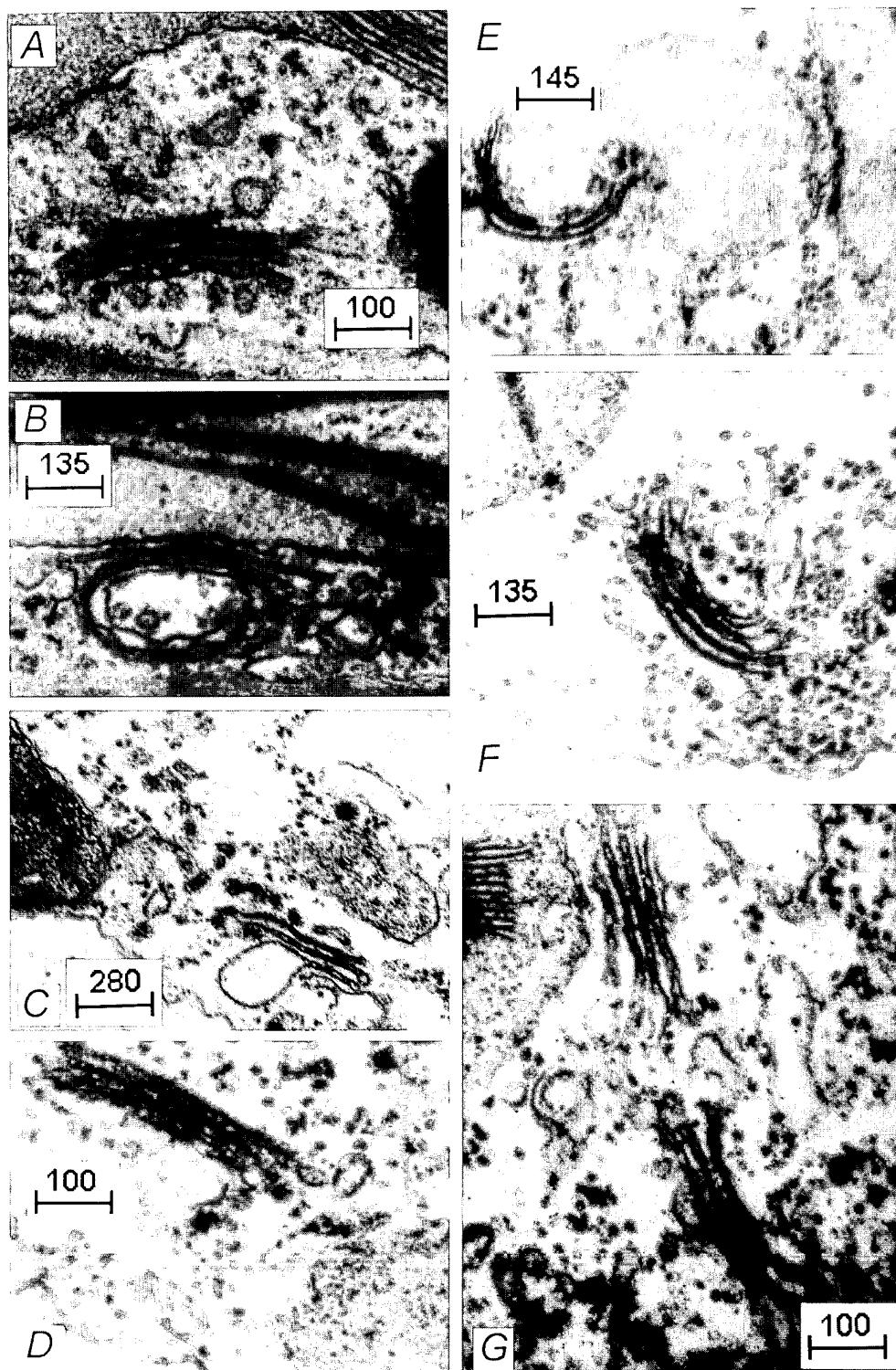


Fig. 3. The chloroplast-dictyosome associations related with plasma membrane: A - flat position of dictyosome between chloroplast and plasma membrane; B - the cis-side of shut dictyosome touches the chloroplast envelope; C, D - association of chloroplasts with the rimes of dictyosomes that separate great vesicles towards plasma membrane; E - a chloroplast-mitochondrion-rim of dictyosome turned with cis-side to plasma membrane; F - the rotation of dictyosome; G - a chain of two transversal dictyosomes between chloroplast and plasma membrane. Bars show dimension in nm.

Yang 1980). Thus methionine, which is both an initial substance for ethylene biosynthesis (Leninger 1972, Lieberman 1979) and the initial amino acid of protein chains (Kozak 1986), is probably an object of competition. Even more, methionine molecule may break apart, releasing ethylene and CO_2 (Lieberman 1979). Ethylene may reflexively bind with proteins in ER and dictyosomes (Goodwin and Mercer 1983). Competitive

protein/ethylene production and action evidently adjusts by flexible action of dictyosomes among several compartments of rye mesophyll cells (Fig. 1C). It causes a gradual activation of the lysosomal system, splitting and emission of superpolymer photosynthate storage, destruction of chloroplasts (Fig. 3D). The mode of contacts between chloroplast and nucleus (Fig. 2A) and between chloroplast and mitochondrion (Fig. 3E)

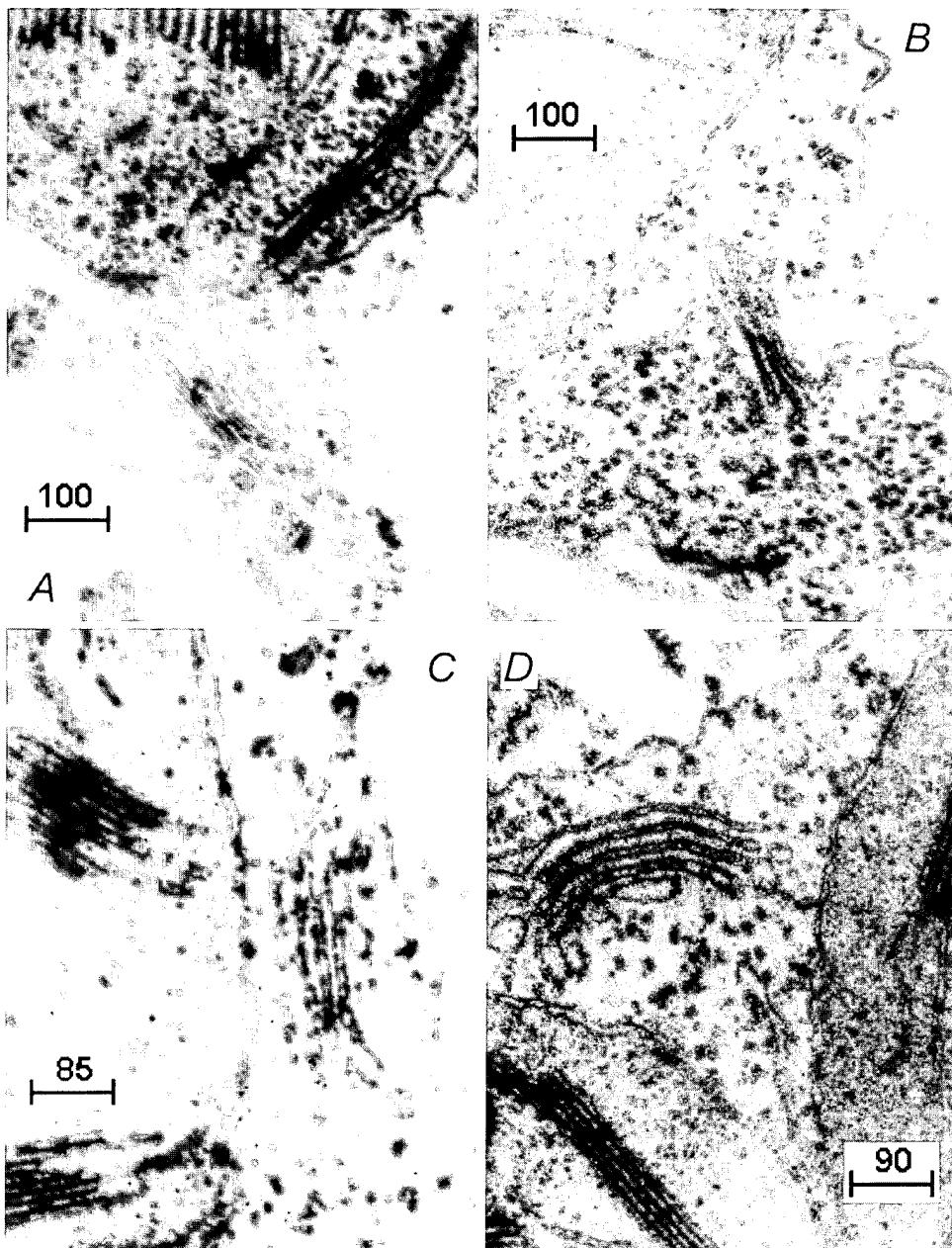


Fig. 4. The chloroplast-dictyosome complexes near the vacuole: A, B - side close association of chloroplasts with the rimes of dictyosomes performing vesicular traffic on several sides; C - a flatwise close association of a chloroplast with cis-side of dictyosome showing secretory vesicles directly into vacuole; D - the cis-cisterna receive transport vesicles from the vacuole and from the rimes of medial and trans cisternae vesicles migrate to two close located chloroplasts. Bars show dimension in nm.

reminds the gap junction described for electric synapses (Purves and Lichtman 1980, Edelman *et al.* 1987, Warner 1988).

Other cases show a two-directional exo/endocytosis where chloroplasts and dictyosomes can be mutually a source of target, that is, both a presynaptic and postsynaptic organelle in the local translation of substances (probably precursors and/or conjugates of ethylene, *etc.*) (Figs. 3B,D,E,G and 4A-D). Those associations are rather similar to synaptic clefts related to chemical synapses (Purves and Lichtman 1980, Edelman *et al.* 1987). Hence ethylene, soluble in water and diffusible, a derivative of an amino acid methionine (Goodwin and Mercer 1983), evidently functions both as a local chemical mediator in intra/intercellular signalling and as phytohormone, which can migrate into a DNA-containing organelle thus regulating gene expression. Increased content of nucleoles shows the production and transport of ribosome precursors from the nucleus into cytosol near the contact zone of dictyosome. Nucleus or organelle chains during the change of morphogenesis affirm this opinion (Selga M. *et al.* 1985, Selga M. and Selga T. 1994, 1995, Selga T. and Selga M. 1995, 1998).

Every displayed organelle complex could realise ethylene signalling together with several responses. As an example serves the association of a chloroplast with dictyosome which is turned with trans-side towards plasma membrane (Fig. 3A,C,D). It could take part both in ethylene production in this locality and in exporting its precursors or conjugates together with avalanche-like assimilate from chloroplasts into apoplast for the usage of new active acceptors. Exported assimilates are used in secondary growth of the cell walls, causing bulge of mesophyll tissues among wavelike veins in the leaves and increase of the dry mass per leaf area unit (Selga M. *et al.* 1985, Selga T. and Selga M. 1995).

The opposite examples, in which dictyosomes are turned with cis-side or perpendicular to plasma membrane (Fig. 3E,F,G) put a notion that dictyosomes, the derivatives of nuclear envelope, function as flexible axons, which can change their position and in that way also the direction and activity of signalling process. The revolution of dictyosomes probably is a sequel of ethylene interference in Ca^{2+} -conducted action of cytoskeleton. Change of orientation of dictyosomes could also be induced by increase of Ca^{2+} concentration due to partial hydrolysis of cell wall pectins.

In the other case, the association of a chloroplast with the cis-side of dictyosome which transports vesicles into vacuole from several cisternae (Fig. 4C), could carry out temporary accumulation into vacuole of several substances. These are ACC (Mayne and Kende 1989), the conjugated form of ACC - the MACC to reduce the ethylene production rate (Bouzen *et al.* 1989), or

photosynthates, chloroplast destruction products, hydrolytic enzymes, and ions (Kursanov 1976, Matile 1978). Other situation, when dictyosome takes vesicles from the vacuole by its cis-side and sends secretory vesicles into chloroplasts, is probably a gradual activation in metabolism of some vacuole store products.

Membranes and organelles, involved in the observed organelle associations, are well-documented sites of flexible ion-pumping systems, Ca^{2+} , H^+ , and energy reservoirs. Therefore the observed direct structural contacts of membranes and contacts mediated by dictyosomes may provide transport of Ca^{2+} and H^+ among symplastic compartments and apoplast, without decrease of stromal and cytosolic pH. For instance, the chloroplast contains a H^+ -pumping system, which drives ATP synthesis by pumping H^+ out of the stroma into thylakoid lumen, and a P-type H-ATPase in the inner membrane of chloroplast envelope (Peters and Berkowitz 1998). In the tonoplast, plasma membrane, dictyosomes, lysosomes, coated and secretory vesicles, and V-type H^+ pumping ATPase were found (Anraku 1995). Obviously, the above displayed organelle complexes participate also in transport, storage, and release of Ca^{2+} , which is a highly dynamic second messenger that controls many cell mechanisms. Ca^{2+} stimulates last step of ethylene biosynthesis—the conversion of ACC to ethylene (Ferguson 1983), but ethylene in its turn causes elevation of cytosolic free calcium levels in plants (Knight *et al.* 1995). The peripheral reticulum and intermembrane space of chloroplast envelope, which are considerably extended during the production and action of ethylene (Figs. 2A and 3G), participate in Ca^{2+} sequestration (Mosejev and Romanovskaja 1988). The Ca^{2+} -ATPase and $\text{Ca}^{2+}/\text{H}^+$ -antiporters pump Ca^{2+} ions into the lumen of ER, into vacuole, into cell exterior (Stein 1988), and into Golgi apparatus (Okorokov 1995).

Though the *in vitro* studies have many advantages in elucidation of the separate functions of chloroplasts and dictyosomes, they give a delusive notion about all embracing nature of the described processes. The results of present ultrastructural study show extremely high variability in structure and location of chloroplast-dictyosome complexes *in vivo* at the same or neighbouring mesophyll cells of rye leaves (Fig. 1C). These cells are involved both in promotion and inhibition in ethylene induced stress response, in order to prolong living of these cells and export the energoplastic substances to other tissues.

We propose that the above displayed organelle complexes reveal an adaptive, inducible complicated synapse-like process of several signalling systems regulating catabolism, anabolism, and morphogenesis of plants treated with the ethylene producer ethephon.

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