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Membrane redox as an essential component of how cells increase in size following cell division

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ABSTRACT Under investigation is the hypothesis that cell enlargement in both plants and animals is not a passive process but the result of an ECTO-NOX-driven physical membrane displacement. Cell enlargement correlates with ECTO-NOX activity and is stimulated when ECTO-NOX activities are stimulated and inhibited when ECTO-NOX activities are inhibited. Both are blocked by thiol reagents. Additionally, cell enlargement emerges as having an energy requirement. An energy requirement is universal among membrane displacement models and is met at the cell surface through coupling with a plasma membrane-associated AAA-ATPase.
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KEY WORDS

2,4-D
cell enlargement
ECTO- NOX
growth
membrane-displacement

Cell enlargement is a necessary requisite for sustained growth of both plant (Taiz 1984) and animal (Baserga 1985) cells. However, a prevailing view for plants established early (Lockhart 1965), is that cell enlargement is the result of a passive yielding of cell walls in response to turgor pressure. In this report we combine information from various sources and cell free systems (Morré 1998b) together with more recent findings to suggest that cell enlargement in both plants and animals is the result of an active, energy-driven process mediated by ECTO-NOX (cell surface NADH or hydroquinone oxidases with protein disulfide-thiol interchange activity) proteins as a major physiological function.

Materials and Methods

NADH oxidase activity was determined as the disappearance of NADH measured at 340 nm in a reaction mixture containing 25 mM Tris-Mes buffer (pH 7.2), 1 mM KCN to inhibit any low levels of mitochondrial oxidase activity, and 150 μM NADH at 37°C with stirring. Activity was measured using a Hitachi U 3210 spectrophotometer as recorded over two intervals of 5 min each in the presence or absence of hormone or growth factor. A millimolar extinction coefficient of 6.22 was used to determine specific activity.

Other methods have been published in detail elsewhere and will not be repeated here (Eisinger and Morré 1968; Morré 1994, 1995, 1998a,b).

Results

Turgor pressure is not the driving force of cell enlargement in plants.

In early experiments, plant stem sections were treated with the plant growth hormones such as 2,4-dichlorophenoxyacetic acid (2,4-D) or indole-3-acetic acid (IAA) to accelerate growth and to loosen cell walls. If the sections were then treated with N-ethylmaleimide (NEM) or other thiol reagents, wall extensibility remained high, at the control level, whereas cell enlargement ceased (Fig. 1).

Solute leakage measured by conductivity changes did occur in the presence of NEM but the change after 1.5 h when growth inhibition was complete accounted for only a 5 to 6% reduction in internal osmotic pressure (Eisinger and Morré, 1968). Moreover, when sections first incubated in NEM were then transferred to a sulfhydyl protectant dithiothreitol (DTT), growth was restored with no effect on the rate of solute leakage. These experiments demonstrate that plant cell enlargement, at least, is not the result of turgor-drive expansion of auxin-loosened cell walls but occurs by a sulfhydryl reagent-blocked active mechanism.

There is no obligatory requirement for delivery of new membrane materials to the plasma membrane for cell enlargement to occur

Previous studies demonstrate that delivery of new membrane materials to the plasma membrane is not required for cell enlargement to occur (Morré 1994). One of these is that in response to temperature, elongating segments of soybean show extensive accumulations of membranes at the trans Golgi network at temperatures of 18°C or less. These accumulations are reminiscent of temperature blocks seen in other plant and animal cells (Tartakoff 1986). On the other hand, auxin-induced growth showed no sharp transition in response to temperature over the entire range of 4 to 25°C (Morré 1994). This would argue that elongation growth in plants induced by auxin occurs in a manner independent of the vesicular transport pathway.

Similar conclusions were reached earlier based on results

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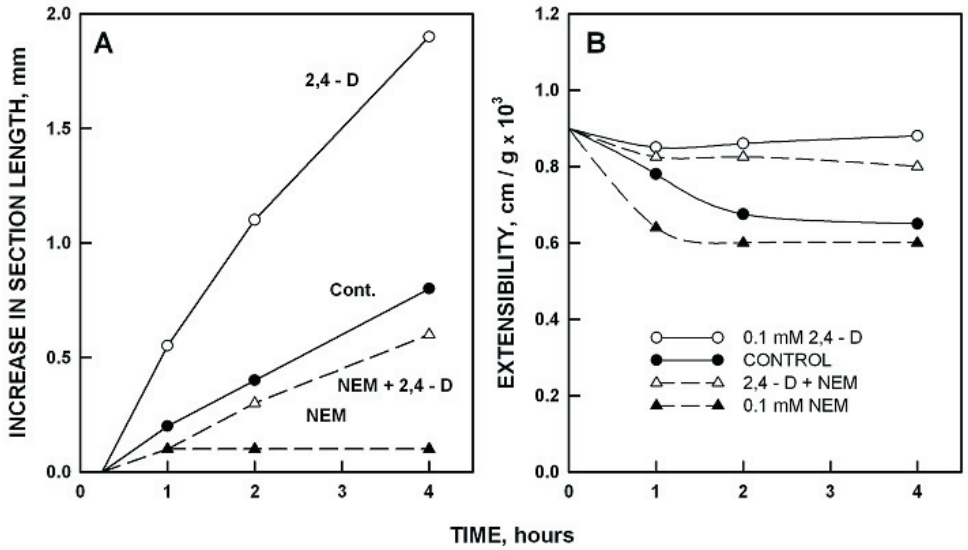

Figure 1. Time course of cell elongation (A) and extensibility (B) of 1 cm etiolated pea 3rd internode sections in the presence and absence of 0.1 mM 2,4-D with and without 0.1 mM N-ethylmaleimide (NEM). Redrawn from Eisinger and Morré (1968).

with monensin done with the narrow setae of the moss Pellia (Morré et al. 1986a). The setae elongate very rapidly and respond to auxin. Yet they are thin enough that the monensin is able to penetrate all of the cells in the section. That the monensin is able to penetrate is evidenced by electron microscope observations of swollen trans elements following fixation with glutaraldehyde (Morré et al. 1986b). Despite the nearly complete inhibition by monensin of normal trans Golgi apparatus functioning, treated setae responded in a relatively normal fashion to auxin for times of 4 h or more after the onset of monensin inhibition. Beyond 4 h, auxin-induced elongation ceased rather abruptly, possibly due membrane rupture as a result of the depletion of essential plasma membrane precursors required for sustained cell expansion.

ECTO-NOX proteins as drivers of cell enlargement

Growth-related ECTO-NOX proteins were first observed with isolated plasma membrane vesicles of soybean where activity was stimulated in response to the synthetic auxin 2,4 dichlorophenoxyacetic acid (2,4-D; Morré et al. 1986b) and inhibited by thiol reagents (Morré et al. 1995a). The acceptor was oxygen (Morré and Brightman 1991) or, in some cases, protein disulfides (Chueh et al. 1997).

Also extensively investigated, was a corresponding NADH oxidase activity of the mammalian plasma membrane (Brightman et al. 1992). The mammalian activity was both simulated by growth factors and hormones (Bruno et al. 1992) and inhibited by thiol reagents (Morré and Morré 1995). A strong correlation between cell enlargement and ECTO-NOX activity has since been demonstrated using both inhibitors and activators for plant as well as animal cells (Morré 1998a; Morré and Morré 2003).

Discussion

The marked susceptibility to inhibition by thiol reagents of both 2,4-D-induced growth and the 2,4-D-induced NADH oxidase of soybean plasma membrane suggested initially an involvement of essential active site thiols of the oxidase in the auxin growth mechanism. A similar thiol dependency was seen with the constitutively-activated NADH oxidase activity (CNOX) of hepatoma and HeLa cell plasma membranes.

If, indeed, the hormone- and growth-factor-stimulated NADH oxidase is in reality a thiol oxidoreductase or a thiol interchange protein, then the activity should exhibit, as well, a protein disulfide isomerase-like activity. Plasma membrane vesicles do exhibit protein-disulfide isomerase activity (Morré et al. 1995b). This activity with plant plasma membrane vesicles is stimulated approximately two-fold by auxin. While it is clear that long-term cell elongation extending over periods of several hours may be dependent upon the vesicular pathway of membrane addition for a source of membranes and membrane precursors, short term auxin-induced growth, that which occurs over an initial period of several hours, seems to occur more or less independently of a vesicular mechanism and independently of turgor as a driving force.

Based on the above findings, we have developed a model whereby cell enlargement is an active process involving both

ECTO-NOX proteins and an ATP-requiring step amenable to evaluation in a completely cell-free system with recombinant proteins and completely synthetic membrane vesicles. Findings support a central role of plasma membrane redox as an essential component of the cell enlargement process.

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