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ION AND WATER TRANSPORT IN LIMONIUM

VI. THE INDUCTION OF CHLORIDE PUMPING

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SUMMARY

During the development of chloride pumping in the glands of Limonium in response to a salt load, low-temperature treatments and inhibitors of both RNA and protein synthesis indicate that a synthesis of components of the chloride transport mechanism occurs during the response. Separate stages of the process are analysed and discussed.

INTRODUCTION

It may well be that a major feature of euryhalinity in mature higher organisms is an inducible ion-transport system, *i.e.* that a net synthesis of ion pumps occurs in response to an increased salt load in the special cells that function as desalinators. In a previous paper[1] it was shown that the response of the glandular extrusion in low-salt-adapted Limonium leaves to an increase in NaCl concentration is much slower than could be explained by any diffusive lag in the tissue, and is not due to the existence of a concentration threshold below which pumping activity is negligible. In this paper the effects of low temperature and of certain inhibitors of RNA and protein synthesis on the rise of pump activity during a salt load, have been studied. Measurements were made of the current drawn from short-circuited leaf discs as this current is a measure of an electrogenic component of the active chloride transfer; the short-circuit technique for this preparation has been described elsewhere[2]. The typical response to NaCl is shown diagrammatically in Fig. 1, where the curve has been conveniently trisected into stages. The behaviour of each stage to the various treatments is described below.

METHODS

All experiments were done in triplicate with 100 mM NaCl as the salt load, on leaf discs of *Limonium vulgare*, cultured in the laboratory without salt water.

Temperature studies

To study the temperature dependence of the stages, discs were incubated in pretreatment media on a cooling bath or in a refrigerator. The lighting was not con-

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trolled as the induction shows no apparent sensitivity to the level of illumination. After treatment, discs could be transferred to the short-circuit apparatus within 2 min.

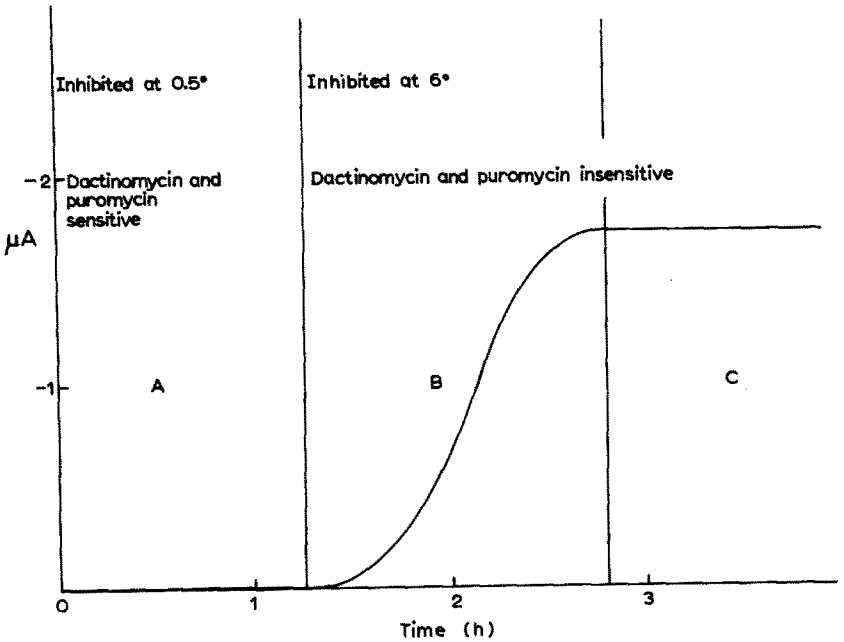

Fig. 1. A diagram of the development of short-circuit current in low-salt-adapted Limonium discs in response to a salt load of 100 mM at 25°. A. Lag period. B. Sigmoid rise. C. Steady state. The sensitivities of the three stage to inhibition are indicated.

The pretreatment temperatures were 0.5°, 2°, 3°, 4° and 6°, after which the time-course of the current was studied at 25°. Control discs taken from the same leaf were run at 25° for all treatments, and the final current levels in all experiments were always close to these (±10%).

Inhibitor studies

For a general review of these inhibitors in plant biochemistry see ref. 3. Isopropyl-*N*-(3-chlorophenyl) carbamate (CIPC) is described elsewhere[4]. The specific inhibitors of RNA synthesis used were dactinomycin (actinomycin D) 25 mg/l; CIPC, 20 mg/l; and 5-fluorouracil, 1 mg/l. Those of protein synthesis were puromycin, 250 mg/l; 5-fluorophenylalanine, 50 mg/l; and cyclohexamide (actidione), 10 mg/l. Discs were either pretreated with salt-free inhibitor or experiments were run from time zero with inhibitor and 100 mM NaCl in the short-circuit chambers.

RESULTS

The effects of low temperature are summarised in Fig. 1, where it can be seen that the lag and the sigmoid rise stages have different temperature coefficients. At 0.5° the lag period was effectively inhibited, although controls run at this low temperature showed that occasionally physiological damage resulted; such cases were rejected. By 3° the lag period could proceed at its normal rate and discs transferred to

25° immediately entered the sigmoid phase. The sigmoid rise was very temperature dependent for even at 6° it was strongly inhibited, and no initial current was observed immediately after a return to 25°, nor was the sigmoid phase shortened.

Inhibitors of RNA synthesis all blocked the time-dependent current rise. If dactinomycin was applied at the same time as the salt load no inhibition was registered, presumably due to slow penetration; if a pretreatment with any inhibitor was given, however, then the salt had no effect. Removal of the inhibitors had the effect of allowing a slow lift of the inhibition. This is unusual in view of the fact that dactinomycin inhibition is generally considered to be irreversible. In one experiment a combination of low temperature and inhibitor was used. At 3° the lag period is completed, and the tissue was filled with dactinomycin at this temperature without releasing the sigmoid rise; the effect of the inhibitor on the rise could then be seen by raising the temperature to 25°. The sigmoid rise occurred with similar time-course to the control, showing that this phase is not blocked by dactinomycin.

The effects on the process of the protein synthesis inhibitors was similar. Pretreatment with puromycin was also required to give any effect, as with dactinomycin, but pretreatment with any inhibitor completely blocked the current rise. Loading with puromycin at 3° was also used to test the sensitivity of the sigmoid rise, and with the same result. Taken as a whole it can be said that the effects of the two classes of inhibitors was virtually identical. When any inhibitor was applied in the steady state,

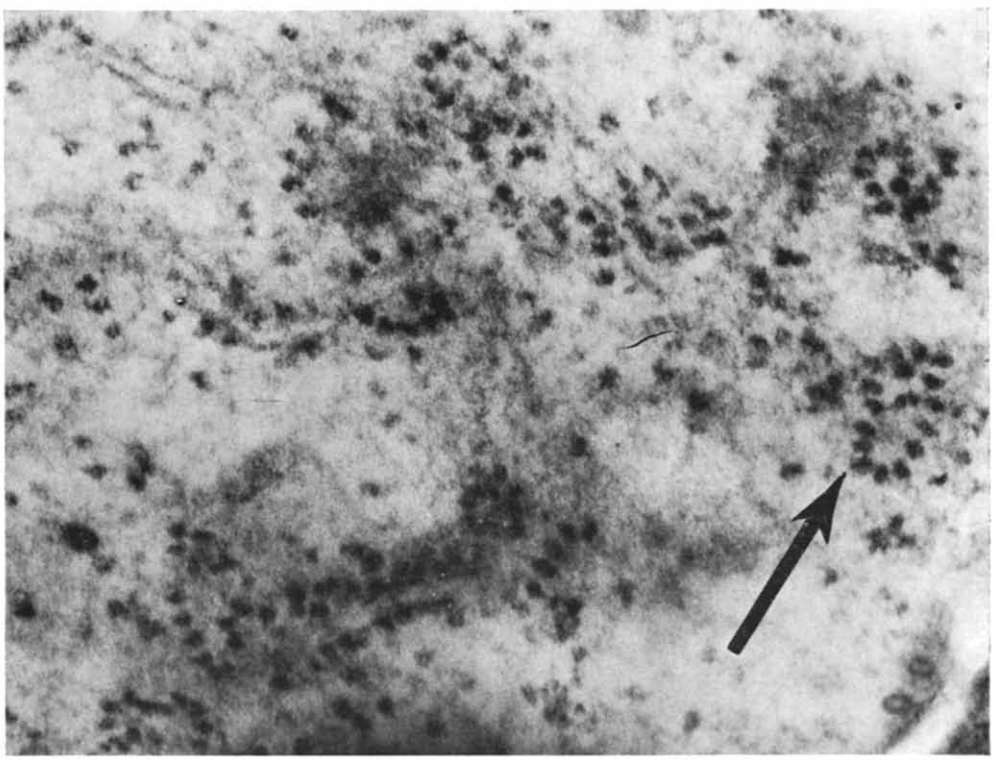

Fig. 2. Ground cytoplasm of a Limonium gland cell, magnification × 150 000. Many polyribosomes with RNA threads can be seen (arrow). Fixation with OsO_4 and lead citrate stained.

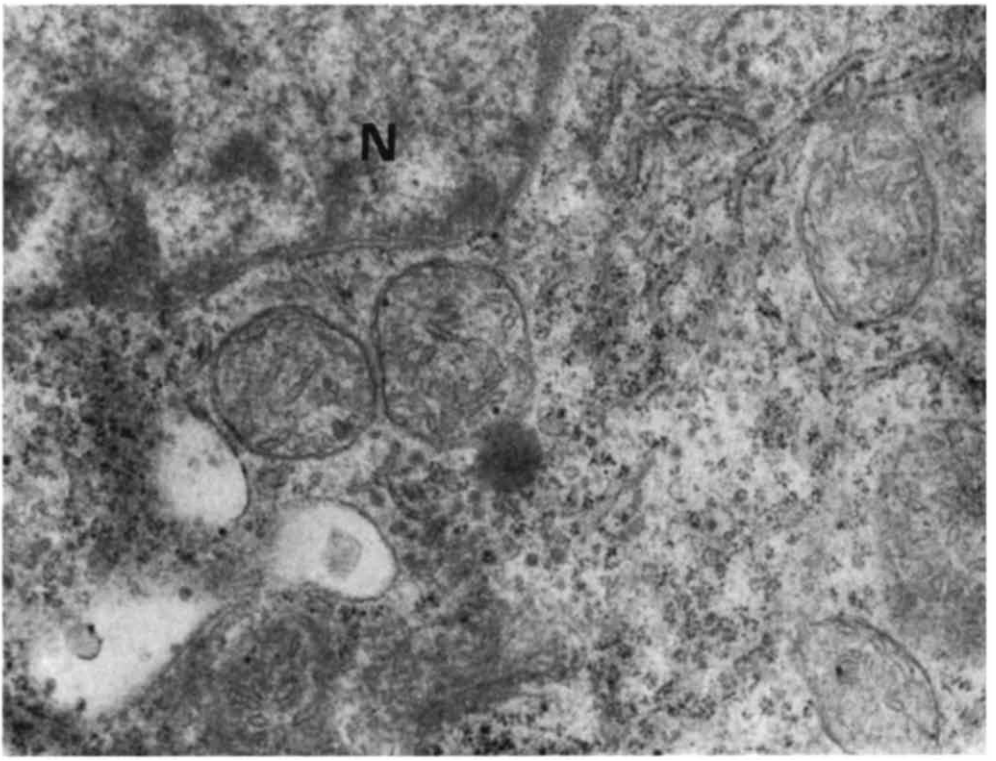

Fig. 3. The cytoplasm of a pumping Limonium gland cell, magnification × 30 000. Nucleus (N), ribosomes, and rough endoplasmic reticulum can be seen. Fixation in OsO_4 and lead citrate stained.

it had no effect whatsoever, with the exception of cyclohexamide. This was immediately inhibitory to pumping discs in the steady state, reducing the current to very low values. In view of recent findings it seems that this compound may have side effects in plant tissues, apparently even on the chloride pump itself[5]. This seems to be confirmed here and must invalidate its use as a specific inhibitor of protein synthesis in this tissue.

DISCUSSION

The high temperature sensitivity of the sigmoid rise relative to that of the lag period merely serves to emphasise that these are in fact two distinct physiological processes, and the specific inhibitors of RNA or protein synthesis block only the latter; it thus appears that the response as a whole is a true inductive process. The whole sensitive period, from the reception of a derepression signal to the appearance of the primary products of translation, must therefore lie within the lag period, and it is probable that transcription and translation are overlapping one another in time. As to the sigmoid rise, there are two relatively straightforward interpretations. (1) The proteins synthesized are enzymes controlling metabolism of the energy supply for the pump; the sigmoid rise therefore reflects the accumulation of that energy supply. (2) The pump machinery, or part of it, is being synthesized and transferred to the

active membranes. The first alternative is incorrect for two reasons. Firstly, the energy supply to the pump would not be expected to be completely zero before induction; it might be low, but not zero, and the response studied here is an on–off phenomenon, not a graded one. Secondly, a pumping disc in the steady state will function, albeit at a reduced level, for a very long period at low temperature; the energy supply is not inhibited at 6°. Alternative (2) is also favoured more by the data, as the temperature sensitivity of the sigmoid rise and its slow course (approx. 1 h) suggest a complex process with a concomitantly low rate coefficient. If we assume that the pump is entirely constructed of protein, or is a complex of more than one protein molecule, then this stage can only represent the transfer of material to the membrane and its subsequent binding there.

Electron microscopy of the gland cells does not indicate any immediately obvious difference between actively pumping or nonpumping states[6], but the cytoplasm is very rich in ribosome clusters, and polyribosomes can be clearly discerned at high magnification (Fig. 2). The exact signal for the start of induction remains to be elucidated, but preliminary experiments with different ion ratios show that it is not a simple one.

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REFERENCES

1. A. E. HILL, *Biochim. Biophys. Acta*, 196 (1970) 73.
2. A. E. HILL, *Biochim. Biophys. Acta*, 135 (1967) 461.
3. K. T. GLASZIOU, *Ann. Rev. Plant Physiol.*, 20 (1969) 63.
4 J. D. MANN, E. COTA-ROBLES, K. YUNG, M. PU AND H. HAID, *Biochim. Biophys. Acta*, 138 (1967) 133.
5 I. R. MACDONALD AND R. J. ELLIS, *Nature*, 222 (1969) 791.
6 B. SHACHAR-HILL, to be published.