Estimation of Diffusible Auxin Under Saline Growth Conditions

Of all the chemical substances that plants may encounter in their external environments, none impairs or inhibit their growth on so large a scale as salt[1]. Studies from various aspects have been reported in the literature, but the influence of salinity on auxin physiology has mostly remained unexplored. Though the effect of sodium salinity on auxin transport has recently been reported[2], we still do not know its effect on the diffusible auxin. The present studies were, therefore, undertaken to estimate the amount of diffusible auxin recovered from *Zea mays* L., coleoptile tips, raised under saline conditions.

Seeds of *Zea mays* L. (Orla-266) were thoroughly washed and soaked for 5 h in tap water and NaCl solution (0.4% w/v in tap water) and planted on 0.75% agar, prepared in the respective solutions, in medium-sized beakers, which were then covered. The seedlings were raised in complete darkness for 94 h, except for 4 h beginning at 48 h, when they were exposed to red light to suppress mesocotyl growth.

Tips, 5.0 mm in length, were removed from 94 h coleoptiles. Four tips were grouped and placed on a 1.5% agar block ($11 \times 8 \times 1$ mm) in replicate assemblies for a diffusion time of 2.0 h. After diffusion the tips were discarded and the blocks were kept at 4 °C in a water-saturated atmosphere for the next 24 h, and then assayed by the standard Avena curvature test (*Avena sativa* L. cv. Victory). These experiments were repeated 3 times on different days with essentially similar results. The temperature throughout was maintained at 25 ± 1 °C and only green safelight[3] was used for manipulations. Statistical evaluation of the data was made by Student's *t*-test.

The seedlings raised under saline growth conditions were smaller, as compared to the control, confirming the earlier finding[2]. Also from the results of the *Avena* curvature bio-assay (Table) it can be observed that salinity treatment significantly reduced the curvature response, thus indicating a reduction in the amount of diffusible auxin recovered from the treated seedlings. It has also been reported[2] that salinity did not affect either the polarity or the amount of auxin transported through *Zea* coleoptile segments. Therefore, it can be concluded from the present studies that salinity reduced the amount of diffusible auxin rather than its transport, which in turn inhibited the growth. This reduction may have been a direct effect of salinity or an indirect one, i.e. through reduction in the supply of cytokinins[4,5] which in turn influences the amount of diffusible auxin recovered[6]. Further studies to delineate the role of salinity and cytokinins are in progress.

Zusammenfassung. Der Auxingehalt von Koleoptilenspitzen von *Zea mays* L.-Keimlingen wird durch saline Wachstumsbedingungen herabgesetzt.

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Effect of salinity on the 'diffusible' auxin of the *Zea mays* L. coleoptile tips

Treatments	Mean curvature degrees ± S.E.
100 µg/l IAA	21.88 ± 0.52
Control	15.38 ± 0.32
Salinity	11.13 ± 0.40

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Electron Microscope Studies on the Dissociation of Actomyosin by Pyrophosphate

Glycerol-extracted muscle fibres in the rigor state relax if Mg-pyrophosphate (Mg-PP) is added to the bathing solution[1,2]. Biochemical studies demonstrated a dissociation of the actomyosin complex by Mg-PP at high ionic strength[3,4] and suggested that the contractile proteins of the Mg-PP relaxed fibres are likewise dissociated into actin and myosin. However, the interpretation of these findings is complicated in the light of mechanical experiments: the stiffness of Mg-PP relaxed fibres is comparable to that of rigor muscle if the fibres are stretched by applying small amplitude sinusoidal length changes at frequencies above 1 Hz[5]. In correspondence with these apparently contradictary results, electron microscope, optical[6] and X-ray[7] diffraction studies of Mg-PP relaxed fibres seem to combine characteristics of both the ATP-relaxed and the rigor state.

HUXLEY[8] demonstrated that shearing forces disintegrate myofibrils into separate actin and myosin filaments if relaxing conditions (presence of ATP, absence of Ca^{++}) are maintained during homogenization. Likewise it should be possible to isolate single filaments if the ATP of the homogenization solution is replaced by PP or nonsplittable ATP-analogs, e.g. β, γ-imino-ATP (AMPPNP)[9], which are thought to imitate only the plasticizing action of ATP produced by dissociation of actomyosin. Conversely the presence or absence of single filaments after the homogenization of myofibrils in such

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seen only occasionally and are then separated by a small modified relaxing conditions should allow conclusions to be made on the dissociation effect of these plasticizers.

For this reason purified myofibrils[8] of freshly prepared flight muscles of *Locusta migratoria* and of frog sartorius muscle have been homogenized with a MSE homogenizer in solutions containing 50 m*M* KCl, 10 m*M* NaN_3, 4 m*M* EGTA, 20 m*M* histidine pH 6.5 and various concentrations of $MgCl_2$, ATP, $Na_4P_2O_7$[10] or AMPPNP[11]. After centrifugation (10 min at 2100 *g*), the supernatant was used for negative staining with uranyl acetate on carbon coated grids[8].

The grids were covered with single filaments as described by HUXLEY[8] using 5 m*M* Mg-ATP as a plasticizer in the homogenization solution. The result was similar if Mg-AMPPNP (in concentrations of 5 m*M* or 30 m*M*) was added instead of Mg-ATP (Figure 1). However, if the Mg-ATP of the homogenization medium was replaced by Mg-PP (in concentrations of 1 m*M* Mg + 10 m*M* PP or 2 m*M* Mg + 5 m*M* PP or 5 m*M* Mg + 5 m*M* PP) single filaments were rare. Their number seemed to be little larger than in control experiments where myofibrils had been homogenized in pure rigor solution. The appearance of Mg-PP isolated filaments differed in yet another respect from those isolated in ATP relaxing solution. In the latter case thick filaments lying alongside thin filaments are gap bridged by cross linkages[8]. In the case of Mg-PP isolated filaments, this observation can be stated as a rule: myosin filaments always seem to be surrounded by parallel arranged actin filaments (Figure 2) and are often grouped in bundles. These bundles can be observed especially in the neighbourhood of fragments of myofibrils damaged by the blades of the homogenizer (Figure 3). It seems that Mg-PP relaxed myofibrils first disintegrate into bundles of myofilaments which may become smaller by further homogenization, but are not broken up completely to single actin and myosin filaments as are myofibrils relaxed by ATP or AMPPNP.

It is tempting to look for reasons to relate this observation to the high dynamic stiffness[5] of Mg-PP relaxed fibres mentioned above. If we suppose that all cross bridges are detached from the actin filaments during ATP relaxation, thereby enabling the isolation of single filaments, we should conclude that this is not the case in Mg-PP solutions and that here some cross bridges at least remain attached. The difficulty is to reconcile this hypothesis with the biochemical results[3,4] and the reversible disappearance of the lines of attached cross bridges (388 Å lines) in single actin filament layers[6]. These findings demonstrate that dissociation of the actomyosin complex and detachment of cross bridges by Mg-PP are indeed possible. The contradiction could be resolved by postulating an equilibrium state between detached and attached myosin heads. LYMN and HUXLEY[7] have suggested this to explain the resemblance of X-ray patterns of PP-relaxed fibres to those of rigor muscle. It is possible that the equilibrium is shifted slightly in the direction of its dissociated components by high ionic strength and increasing pressure[12], which may accelerate the separation of actin and myosin and facilitate the formation of distinct fractions in the ultracentrifuge. On the other hand, the high dynamic stiffness of Mg-PP relaxed fibres at sinusoidal length changes[5] could be explained as a result of the passive stretch of the elastic elements of attached cross bridges.

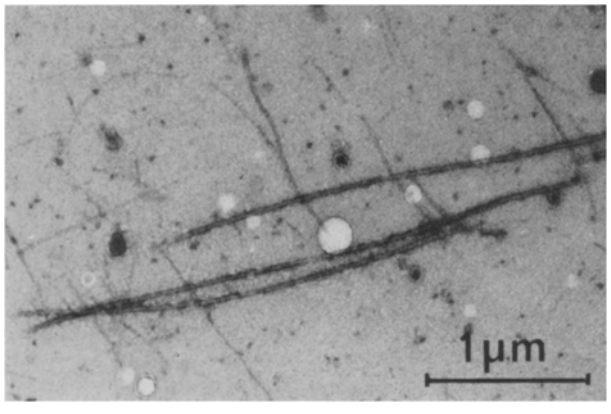

Fig. 1. *Locusta migratoria*. Preparation of separated actin and myosin filaments isolated in relaxing solution containing 5 m*M* Mg-AMPPNP.

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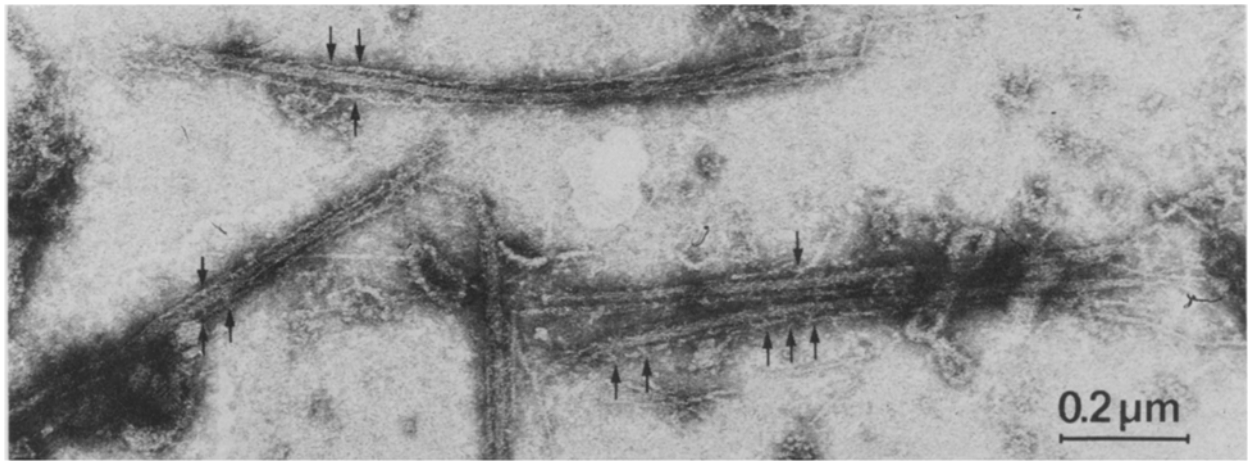

Fig. 2. *Locusta migratoria*. Preparation of actin and myosin filaments from myofibrils homogenized in relaxing solution containing 2 m*M* Mg and 5 m*M* PP. Fragments of myosin filaments (natural length is about 3 µm in Figure 1) lying alongside actin filaments are connected with them by cross bridges (arrows).

It is clear that the results described in this paper do not permit conclusions regarding the size of the shearing forces acting during homogenization, or the number of attached cross bridges necessary to prevent the complete disintegration of myofibrils into single filaments. However, mechanical experiments with 5 m*M* Mg-AMPPNP[13] suggest that a reduction of the dynamic stiffness to about 80% is enough to allow the formation of the single filaments demonstrated in Figure 1[14].

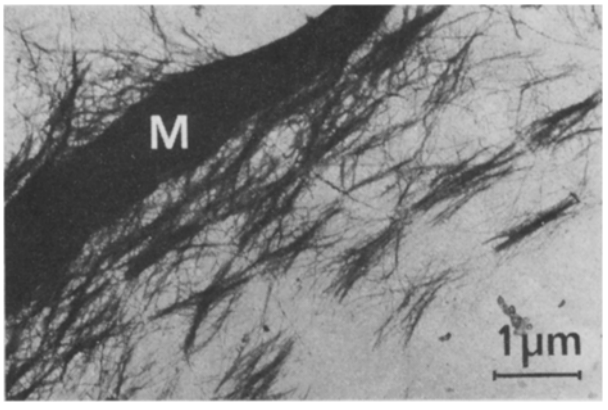

Fig. 3. Frog sartorius muscle. Preparation of myofilaments. Myofibrils have been homogenized in relaxing solution containing 5 m*M* Mg-PP. The myofibril (M) crossing the micrograph seems to disintegrate into bundles of myofilaments.

Zusammenfassung. Myofibrillen, die in einer Erschlaffungslösung mit Mg-ATP oder Mg-β, γ-imino-ATP homogenisiert werden, zerfallen in einzelne Myofilamente. Im Gegensatz dazu scheinen Myofibrillen bei einer Verwendung von Mg-Pyrophosphat (Mg-PP) als Weichmacher allenfalls zu Bündeln von Myofilamenten zerschlagen zu werden, wobei die Filamente noch durch Querbrücken verbunden bleiben. Hieraus wird auf eine unvollständige Dissoziation des Aktomyosin-Komplexes (bzw. die Bildung eines Gleichgewichtes zwischen losgelösten und angehefteten Querbrücken) in Gegenwart von Mg-PP geschlossen.

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Chromosomes of *Molgula manhattensis* de Kay (Ascidiacea)[1]

Since the family Molgulidae, suborder Stolidobranchiata, is one of the least known at a karyological level in the whole class Ascidiacea, the only cytogenetic studies being those regarding the chromosome number of *Molgula manhattensis*[2,3], the present study is intended to give a preliminary description of both mitotic and meiotic chromosomes of *Molgula manhattensis* in order to reduce the gap in our knowlegde of the chromosomes of ascidians.

Material and methods. The present study deals with the chromosomes in male gonads, unfertilized and cleaving eggs of *Molgula manhattensis* from the Lagoon of Venice. Fixation, squash preparations and observations were made according to the methods described elsewhere[4,5].

Meiotic chromosomes. At metaphase-I oocyte bivalents (Figure 1) appear as 2 deeply stained, more or less closely connected roundish bodies; heterotypic elements are not present and only 1 bivalent can be recognized by its greater size. Although these bivalents show the tendency to join randomly together, a count of 15 metaphase-I plates has confirmed the haploid number of 16.

At early pachitene (Figure 2) each spermatocyte bivalents consists of a rod-shape homogeneously stained body in which some segments appear to be thicker and more condensed than others; although heterotypic chromosomes are not present, the small chromosomes are often slightly more condensed than the long ones. The homologous are not distinguishable from each other and neither kinetochore nor terminal zones are differentiated. One chromosome can be distinguished from the others by its distinctly greater length.

At late pachitene (Figure 3) spermatocyte bivalents are thick rod-shaped and homogeneously stained bodies in which neither the homologues nor differentiated zones are distinguishable. Measurement of pachitene chromosomes from 4 plates indicates that the relative chromosome lengths vary from one plate to another. Although the presence of some random connections between chromosomes, the haploid number 16 was consistently determined on pachitene chromosomes.

At diakinesis, the bivalents (Figure 4) are very short and deeply stained. The analysis of 30 diakinetic plates indicates that there are only 2 types of bivalents: rod shape and cross shape. The rod-shaped bivalents often show thicker roundish ends and from their morphology it is not possible to determine whether they bear chiasmata. The cross-shaped bivalents probably possess unterminalized chiasmata but it might be, at least in some cases, that the cross-shape outline be due to the precocious separation of daughter kinetochores. The diakinetic bivalents are randomly dispersed throughout the nucleus and a random association often occurs between these. The haploid number 16 was determined in most of the 30 plates examined.

Mitotic chromosomes. Although the number of observations was rather limited, it was established that a precocious separation of daughter kinetochores occurs from prometaphase till metaphase in cleaving eggs of *M. manhattensis* (Figure 5). At prometaphase the chromosomes appear as homogeneously stained rods in which no differentiated zones are present. The position of kine-

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