To analyze polyribosome sedimentation pattern, aliquots of polyribosome fractions containing approx. 0.05 mg of RNA were layered on 14.5 ml of 15–35% (w/w) convex sucrose density gradient[11]. After centrifugation in a 6×16.5 ml MSE swingout rotor for 4 h at 4 °C and $110{,}000 \times g$ (r_{av}. 10.97 cm) the gradients were displaced upwards through a Perkin-Elmer 124 spectrophotometer and extinction at 260 nm was continuously recorded. Protein synthesis in a cell-free system was measured as described previously[10], except that cytosol was used (1.5 mg of protein per 0.5 ml of incubation mixture) instead of pH 5 fraction.

Results and discussion. Sedimentation pattern of the polyribosome fraction derived from the livers of mice killed 1 h after i.p. injection of 20 μmoles/kg cadmium chloride shows a high increase of the monomeric ribosome peak and a partial disaggregation of heavy polyribosome aggregates (figure 1).

The cell-free system composed of polyribosomes and cytosol isolated from the livers of cadmium-treated mice has a reduced ability to incorporate ^{14}C-leucine into proteins (table). The cytosol derived from control mice added to the cell-free system containing polyribosomes prepared from cadmium-treated mice did not alter the activity of the system. Also the cytosol of cadmium-treated mice did not affect the activity of the polyribosomes derived from control animals. The results indicate that cadmium affects the polyribosome fraction rather than the cytosol. Cadmium chloride added to the cell-free system composed from polyribosomes and the cytosol derived from control mice in concentrations between 30 and 100 μM decrease the ability of the system to incorporate ^{14}C-leucine into proteins (figure 2). The results presented indicate that cadmium inhibits protein synthesis, apparently by producing a defect in the polyribosome fraction, possibly damaging either the ribosomes or some ribosome-bound factor or factors engaged in protein synthesis. In addition to the references cited[5–8], the described effect of cadmium on polyribosome structure and function indicates a complex effect of this toxic element on nucleic acid and protein biosynthesis. More detailed studies of the effects of cadmium on polyribosome structure and function and RNA biosynthesis could lead to a better understanding of the mechanism of cadmium-produced cell damage. This is the subject of our further investigations.

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Mechanism of potassium deficiency-induced retardation of chlorophyll biosynthesis in Zea mays

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Summary. Potassium deficiency decreased the formation of protochlorophyll and retarded the rate of transformation of protochlorophyll to chlorophyll in maize seedlings.

The retardation of chlorophyll formation during potassium deficiency is well-documented[1,2], but the mechanism of this effect is not fully understood[2,3]. Such an effect may be due, amongst other things, to a decreased formation of protochlorophyll, or to a decreased rate of conversion of protochlorophyll to chlorophyll. In this report, investigations are described which were carried out to test these hypotheses.

Materials and methods. Seedlings of Zea mays Linn. cv NS1 were raised under potassium deficiency and full nutrient regimes as previously described[4]. On the 7th day, and at intervals of 3 or 4 days thereafter, chlorophylls were extracted from shoots harvested at random from

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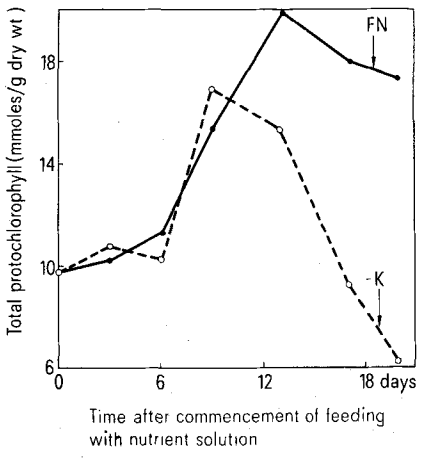

Fig. 1. Time-course of protochlorophyll formation in seedlings of Zea mays maintained under potassium deficiency (–K) and full nutrient (FN) conditions. SD varied between 0.1 and 0.5.

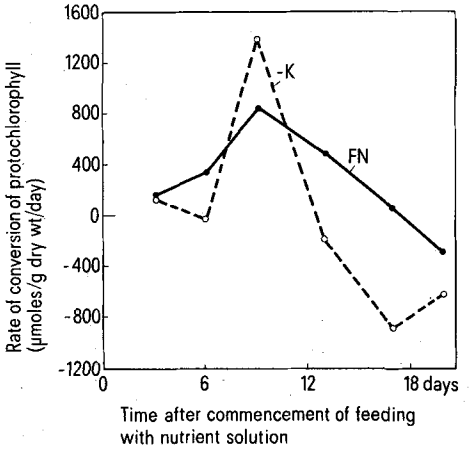

Fig. 2. Rate of conversion of protochlorophyll to chlorophyll in seedlings of Zea mays maintained under potassium deficiency (–K) and full nutrient (FN) conditions.

each nutrient regime. Details of the extraction procedure have been given in a previous communication[5]. Total chlorophyll, chlorophyll a and chlorophyll b were determined by the method of Vernon[6]. Total protochlorophyll (assuming no conversion to chlorophyll), and the amount of protochlorophyll transformed to chlorophyll were estimated by the method of Smith and Benitez[7]. From the values thus obtained, the rates of transformation of protochlorophyll to chlorophyll were calculated.

Results and discussion. Figure 1 shows the time-course of formation of protochlorophyll in seedlings grown under potassium deficiency and full nutrient conditions. During the first 10 days of starvation, potassium deficiency did not affect the concentration of protochlorophyll in the maize seedlings. Beyond this point the deficiency resulted in a drastic decrease in the concentration of total protochlorophyll. A similar pattern of effect was observed for chlorophylls a and b.

Seedlings maintained under potassium deficiency showed initial rates of conversion of protochlorophyll to chlorophyll that were approximately equal to the rates recorded for control seedlings (figure 2). The rates increased under both nutrient regimes during the first 10 days of feeding with nutrient solutions and thereafter decreased. This decrease was more pronounced in the potassium-deficient seedlings than in the control seedlings.

It can thus be concluded that the decrease in chlorophyll formation during potassium deficiency is due, at least in part, to a decreased formation of protochlorophyll and a decreased rate of transformation of protochlorophyll to chlorophyll. Bogorad[8] suggested that in plants chlorosis is generally due to a block in porphyrin formation at a very early point in the biosynthetic chain. Potassium deficiency-induced chlorosis would seem to be due to an additional block later in the chain.

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Increased RNA synthesis during preconjugation and its effect on pair formation in Tetrahymena[1]

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Summary. Increased RNA synthesis, mainly mRNA, occurs in Tetrahymena very shortly before the pairing which takes place after conjugation has been induced. Specific inhibition of mRNA synthesis by cordycepin delays pairing.

The sexual phase in the ciliated protozoan Tetrahymena is an inducible developmental process. Formation of a cell pair between 2 mating-types requires several steps: 1. Deprivation of nutrients. 2. A specific cell-cell interaction. 3. Fusion of cell surfaces of both mating-types. The actual pairing is preceded by a process which makes possible the cellular recognition and attachment. This process involves at least 2 major stages: initiation and costimulation[2]. Initiation is induced by starvation in buffers such as 10 mM Tris or 50 mM Tricine for 2 or more h (depending on the mating types used at 28–30 °C) and is independent of the presence of the complementary mating-types[2,3]. After mixing mating types which were previously initiated, there is a lag period of 45–60 min until the first pairs of conjugates are formed. Obviously, therefore, during this lag period some form of communication is needed between cells of complementary mating types[3,4]. Since the aforementioned processes of initiation, costimulation and fusion occur sequentially[2], it is possible to examine each step separately. In order to understand more about the macromolecular events during costimulation, we have investigated the pattern of RNA synthesis in relation to this period of conjugation in Tetrahymena. In addition, the effect of cordycepin, an RNA synthesis inhibitor, was examined on RNA synthesis as well as on pair formation.

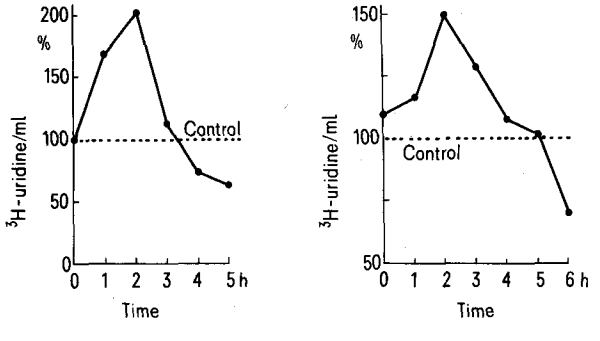

Fig. 1. RNA synthesis during costimulation and pair formation during the process of conjugation. After mixing starved cultures of mating types, 10 μC/ml ^{3}H-uridine was added at 1-h-intervals for 10 min. The radioactivity was determined by precipitation with cold 10% TCA. Solid line: RNA synthesis during costimulation and pair formation of conjugating Tetrahymena. The curves were calculated and drawn relative to the straight control line. Dotted straight line: RNA synthesis in starved controls. Each starved mating type alone served as a control. The dotted line represents the calculated mean of both mating types. Left curve: ^{3}H-uridine incorporation into phenol-chloroform extracted RNA. Right curve: ^{3}H-uridine incorporation into entire Tetrahymena cells.

Materials and methods. Cell cultures. Tetrahymena pyriformis mating types WH_6 and WH_{52} of syngen I (obtained from the American Type Culture Collection) were maintained separately in 2% proteose peptone (Difco) at 28 °C. Cells used in the experiments were obtained by inoculating 100 ml of a medium containing 0.5% proteose peptone, 0.5% Bacto tryptone, 0.01% yeast extract, 0.1%

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