Transfilter associations of nonleguminous plants with rhizobia: channelling of N_2 fixed by rhizobia into the nitrogen-metabolism of *Portulaca grandiflora* callus tissues

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Summary. In transfilter associations of *Portulaca grandiflora* callus tissues with *Rhizobium* sp. 'cowpea' 32H1 the nitrogenium fixed by the bacteria was channelled into the normal pathway of nitrogen metabolism of higher plants. In associations kept in an $^{15}N_2$ containing atmosphere 10% of the ^{15}N taken up into the plant cells was incorporated into *Portulaca* proteins. The results demonstrate that cells of nonleguminous plants are profiting from the quasi-symbiotic situation.

Nonleguminous tissues and plants are able to induce nitrogenase activity in rhizobia[1]. Among others, callus tissues of *Portulaca grandiflora* induce nitrogenase activity in *Rhizobium* cowpea 32H1[2]. Induction was possible even if rhizobia and plant cells were separated by a membrane impermeable to the bacteria[3]. For possible practical use, the main question that arose was whether the plant cells were profiting from the nitrogenium fixed by the bacteria. Using ^{15}N analysis in transfilter cultures we were able to demonstrate that following fixation of $^{15}N_2$ by the rhizobia ^{15}N-containing substances passed the membrane and were enriched in the *Portulaca* cells[4]. The present paper deals with the question whether the N transferred from rhizobia to plant cells was used for the normal N metabolism of higher plants, and especially, whether it was incorporated into proteins.

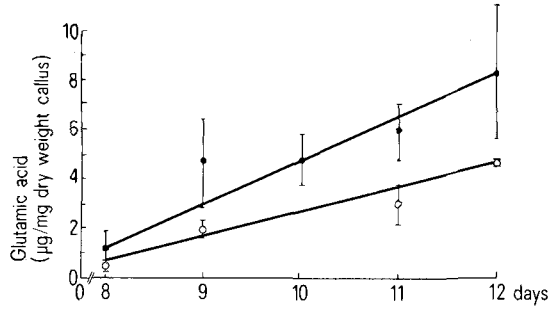

Fig. 1. Time course of glutamic acid accumulation in transfilter associations of *Portulaca* callus tissues with *Rhizobium* cowpea 32H1 (●—●), and in control calli not associated with rhizobia (○—○). Abscissa: days after initiation of the association. Rhizobia in the association showed nitrogenase activity, control bacteria not associated with plant cells showed no nitrogenase activity.

Materials and most methods, especially the transfilter culture, have already been described[3,4]. Lyophilized callus was extracted to obtain amino acids using 70% ethanol (3.5 ml/50 mg callus). Following centrifugation (10,000 × g for 15 min) the supernatant was used for TLC. (Cellulose plates Merck Nr. 5577, solvent system n-butanol/aceton/acetic acid/water 35:35:7:23[5].) The amino acids were stained by ninhydrin and then eluted for colorimetry. Extraction, purification, and photometry of *Portulaca* betacyanins have been described elsewhere[6]. Proteins were extracted from lyophilized callus using McIlvain buffer pH 8.0, precipitated with ice-cold ethanol, centrifuged down (40,000 × g for 15 min), and dried. Quantitative protein determinations were performed using the biuret reaction[7] with γ-globulins as standard.

The agar medium of associations *Portulaca* callus-*Rhizobium* showed a strong reaction with Nessler reagent. On the other hand, Nessler coloration of control agar medium (agar medium with bacteria alone) was very weak. The *Rhizobium* used by us was found to excrete, in suspension cultures, up to 80% of the fixed ^{15}N in the form of NH_4^+[8]. Therefore, the assumption seems to be justified that in our

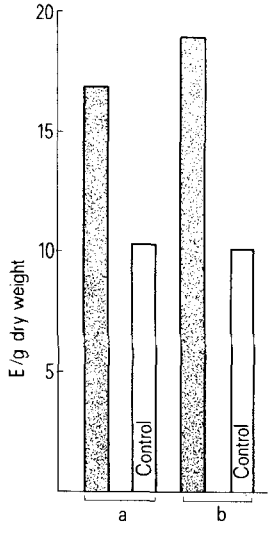

Fig. 2. Betacyanin accumulation in associations of *Portulaca* callus tissues with *Rhizobium* cowpea 32H1 (black), and in control calli not associated with rhizobia (white). E, betayanin concentration in relative extinction units 42 days after initiation of the associations. a, plant cells and rhizobia not separated; b, plant cells and rhizobia in transfilter culture. Rhizobia in the associations showed nitrogenase activity, control bacteria not associated with plant cells showed no nitrogenase activity.

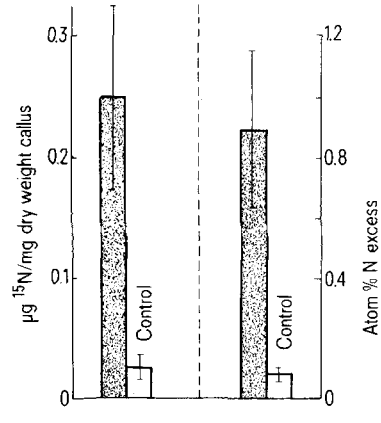

Fig. 3. Incorporation of ^{15}N into the proteins of *Portulaca* callus. The transfilter associations (black) as well as the controls (white, callus without bacteria) were kept in a $^{15}N_2$ containing atmosphere. ^{15}N analysis 14 days after initiation of the association and 4 days after addition of $^{15}N_2$. Rhizobia in the association showed nitrogenase activity, control bacteria not associated with plant cells showed no nitrogenase activity.

transfilter cultures the nitrogenium fixed by the bacteria migrates in the form of NH_4^+ to the plant cells. This assumption, of course, needs further confirmation.

Portulaca cells associated with rhizobia showed an increased accumulation of glutamic acid compared with control callus (figure 1). The stronger ninhydrin coloration of glutamate was even visible to the naked eye. None of the other free amino acids, including glutamine, showed a comparable increase. The glutamine synthetase - glutamine oxo-acid aminotransferase pathway represents the predominant route for ammonia assimilation in higher plants[9], glutamate being both the acceptor of ammonia and the product of its assimilation[10]. Apparently the ammonia transferred into the *Portulaca* cells follows this pathway, causing an increase in glutamate. That no comparable increase in glutamine could be detected, might be due to a low stationary concentration.

As for the further fate of the transferred N, it seemed sufficient to check just 2 classes of substances: the betacyanins, representing N-containing secondary plant products, and the proteins, representing N-containing substances of primary importance.

Rhizobia are unable to synthesize betacyanins. Therefore it was not absolutely necessary to separate plant cells and bacteria. In any case, *Portulaca* cells associated with rhizobia showed an increased betacyanin accumulation, whether we used the transfilter technique or not (figure 2).

Total protein from callus associated with rhizobia was somewhat, but statistically insignificantly higher than from control callus. That no marked difference could be detected, could perhaps be due to the comparatively rough colorimetric procedure. ^{15}N analysis, however, revealed differences between experiment and control. *Portulaca* callus associated with bacteria and control callus not associated with rhizobia were kept in an atmosphere containing $^{15}N_2$. The ^{15}N content of proteins extracted from control callus was within the limits of the method. Proteins from callus associated with rhizobia, however, showed a more than 10-fold ^{15}N-enrichment (figure 3). About 10% of the ^{15}N incorporated into the callus material were found within the proteins.

This ^{15}N analysis confirmed the interpretations of the results based on accumulation studies; in associations of rhizobia with cells of the nonlegume *Portulaca*, nitrogenium fixed by the bacteria was transferred to the plant cells and channelled into the normal pathway of ammonia utilization. Cells of nonleguminous plants are able to induce nitrogenase activity in rhizobia, and vice versa are able to profit from the nitrogenium fixed by the bacteria.

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Nonrandom association of acrocentric chromosomes in human epithelial cells[1]

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Summary. In diploid, aneuploid and polyploid cells of a human epithelial finite cell line, a statistically significant higher frequency in the involvement of D's than G's in acrocentric chromosome associations was found.

Studies on acrocentric chromosome associations have been very contradictory, revealing either randomness[2-4] or specific variations in different individuals[5-9], regarding the frequency of participation of different acrocentric chromosomes as well as the percentage of metaphases containing associations. Moreover, the almost exclusive utilization of PHA-stimulated lymphocytes rather limits the significance of chromosome associations, so that there is no information on a possible relationship with a) gene functional activation or suppression in other types of normal or neoplastic cells, and b) the obvious rearrangements of the genetic material in the chromosomes of neoplastic cells. In this work, we examined the frequency of associations and the involvement of D's and G's in acrocentric chromosome associations in diploid, aneuploid and polyploid cells of a human epithelial finite cell line.

Methods. The finite cell line KOS-ROV was derived by trypsin dissociation from a sterile tissue specimen removed from the right ovary of a 43-year-old female on October 27, 1978. Both ovaries of the patient were removed prophylactically, when the patient exhibited extensive osteolysis and pains in the pelvis; 18 months before this operation, the patient had been subjected to a breast tumor removal and radiotherapy. McCoy's 5a medium supplemented with 15% fetal bovine serum, penicillin 100 IO/ml, streptomycin 100 µg/ml and kanamycin 100 µg/ml and buffered with Hank's salts at pH 7.4 was used. Cells were proved to be free of mycoplasma and other contaminations, as shown by ^3H-thymidine labelling and autoradiography[10].

Cells in the logarithmic phase of growth were treated with colcemid 0.1 µg/ml for 4 h, harvested by trypsinization, subjected to hypotonic treatment with 0.95% sodium citrate pH 7.5 for 30 min and fixed in methanol-glacial acetic acid (3:1). Metaphase preparations were stained with Giemsa.

Results. A KOS-ROV monolayer showed a pavement-like arrangement with anaplastic and pleomorphic features, and cell overlapping during confluency (figure). Each cell was polygonal epithelial-like with abundant, well expanded cytoplasm. The ultrastructure of KOS-ROV cells revealed considerable similarities with benign Brenner tumour cells, regarding the plasma membrane foldings, the morphology of nuclei, mitochondria and secretory granules and the