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Utilization of phosphate compounds for growth of *Tetrahymena*

L. Rasmussen, H. Toftlund, M. Florin-Christensen* and J. Florin-Christensen*

Institute of Anatomy and Cytology, and Department of Chemistry, Odense University, DK-5230 Odense (Denmark), 15 April 1985

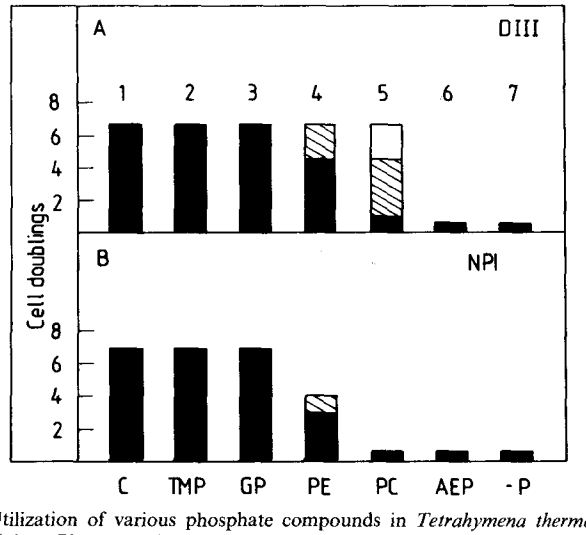
Summary. Taking advantage of a synthetic nutrient medium, we have studied which compounds phosphate-starved *Tetrahymena thermophila* can use as phosphate sources for growth and cell multiplication. Ortho-, trimeta- and α-glycero-phosphate are good sources for both the wild type and a food-vacuoleless mutant; phosphorylcholine is used only by the wild type, and 2-aminoethyl phosphonic acid fails to serve as a phosphate source. Since at least two phosphatases are found in the extracellular fluid these results indicate that *Tetrahymena* can make use of extracellular digestion of nutrients.
Key words. Tetrahymena; growth studies; utilization of organic phosphate; role of phagocytosis.

The ciliate *Tetrahymena thermophila* can be grown in a synthetic medium in which we can control the concentrations and the chemical forms of added compounds[1]. This gives us a possibility to see which compounds – in this case, which phosphate compounds – can support cell growth under certain circumscribed conditions of cultivation.

We have used a synthetic medium[1] supplemented with 0.2 mg tyrosine per ml, prepared without the usual orthophosphates and then supplemented with one of the six phosphate compounds to be tested. When cells from the complete medium are transferred to a phosphate-free medium (at least a 100-fold dilution) they complete 5–6 generations on internal reserves and on phosphates carried over upon subcultivation. Such cells go through at most one cell doubling upon transfer to new phosphate-free medium. The growth and multiplication tests have been carried out on two cell types: a nonphagocytic mutant of *T. thermophila,* NP1, which does not form food vacuoles when grown at restrictive temperature, and the wild type from which it is derived, DIII[2]. The cells were inoculated into test medium at a population density of 5000 cells per ml in small test tubes (10 mm wide, 100 mm high) holding 2 ml sterile-filtered synthetic medium. The cultures were incubated at 38 °C, the restrictive temperature for NP1 and near optimum for the wildtype. Population densities were monitored in an electronic counter on samples removed 18, 42, and 66 h after inoculation. The number of cell doublings was calculated and plotted (fig.).

Tetrahymena can utilize a number of phosphate compounds for growth and multiplication. Ortho-phosphate, trimetaphosphate, and α-glycerophosphate (Nos. 1–3, respectively, fig.) support growth at the rate of more than six cell generations in 18 h in both wild type (frame A) and nonphagocytic cells (frame B). Phosphorylethanolamine (No. 4) supports cell growth better in the wild type than in the mutant, and phosphorylcholine (No. 5) supports growth only in wild-type cells, and the growth rates are low. 2-Aminoethyl phosphonic acid (No. 6) is not utilized by *Tetrahymena,* not even in 10 mM concentrations (Not shown). The cells do not grow in phosphate-free medium (No. 7).

Taking in account that *Tetrahymena* secrets large amounts of phosphatases into the extracellular medium[3], and assuming that only ortho-phosphate can pass the plasma membranes, we may conclude the following from these results: 1) trimetaphosphate, α-glycerophosphate and phosphorylethanolamine are broken



Utilization of various phosphate compounds in *Tetrahymena thermophila. A* Phagocytozing wild-type cells; *B* a non phagocytozing temperature-sensitive mutant derived from the wild type of frame A and grown under restrictive conditions[9]. Phosphate-starved cells were transferred to media containing 1) orthophosphate, 2) trimetaphosphate, 3) α-glycerophosphate, 4) phosphorylethanolamine, 5) phosphorylcholine, 6) 2-aminoethyl phosphonic acid (a structural analogue of phosphorylethanolamine, but containing a P–C bond instead of a P–O–C bond), and 7) no phosphate. The concentrations of the phosphate sources were in all cases 3 mM. Cell multiplication during the first 18 h: ■; during the next 24 h: ▨; and during the next 24 h: □.

down by phosphatase secreted into the medium; 2) utilization of phosphorylcholine requires the mediation of food vacuoles; it is possible that the enzyme concentrations required to break down these compounds are high enough only in food vacuoles, in view of findings that wild type DIII and food-vacuole-less NP1 secrete comparable amounts of enzyme activities (Silberstein[4] and confirmed by own unpublished results); and 3) 2-aminoethyl phosphonic acid cannot be hydrolyzed and therefore it is not utilized as a phosphate source by our cells, although they produce large amounts of this compound[5] and it has been reported that *Tetrahymena* homogenates cleave it[6,7].

We want to point out that the results of the figure correlate well with our unpublished results on extracellular phosphatase activities. We found values of 100, 4, and 2% against α-glycerophosphate, phosphorylethanolamine and phosphorylcholine, respectively, whereas no activity was observed against 2-aminoethyl phosphonic acid.

The synthetic growth medium for *T. thermophila* is easy to prepare and supports good growth (doubling times down to 1.6 h) under optimal conditions. It can be used to get insight into aspects of nutrient utilization and interactions between components of the medium[8,9] and, when combined with suitable mutants, the roles of various uptake systems, like cell surface and food vacuoles[10], can be separately analyzed.

Here we have shown that *T. thermophila* cannot use 2-aminoethyl phosphonic acid as a phosphate source for growth and cell multiplication; that it needs food-vacuolar functions to utilize phosphorylcholine; and that these functions are not required for the utilization of trimetaphosphate, α-glycerophosphate or phosphorylethanolamine. Our results also suggest that the activities of the external phosphatases suffice to satisfy the cells' need for phosphate for fast growth. The ability of *Tetrahymena* to use phosphate esters in the absence of food vacuole formation is the first reported evidence for the exploitation of extracellular digestion of nutrients by this type of cell.

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* Present address: Laboratorio de Invertebrados I, Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, Pab. II 4°P, 1428 Buenos Aires, Argentina.

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Tetrasomy and quadruple trisomy in pea

H. Kumar[1], V. C. Mercykutty[2] and R. M. Singh

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005 (India),
28 February 1984

Summary. Two off-type plants, morphologically distinguishable from each other and from their respective sister euploid, were isolated in the M_3 generation of pea interchange heterozygotes. Pollen sterility was very high, ranging from 63.0 to 90.0%. Cytologically one of them was tetrasomic (2n+2 = 16) and the other one was quadruple trisomic (2n+1+1+1+1 = 18). In the tetrasomic plant 1IV+6II was the most frequent (46.7%) chromosome configuration, while cells with 4III+3II were predominant (40.0% cells) in the quadruple trisomic plant.

Key words. Pisum sativum; pea interchange heterozygotes; tetrasomics; quadruple trisomy; cytomorphology; off-type plants.

Tetrasomics have been reported in guava[3], datura[4] and other species. However, neither tetrasomy nor quadruple trisomy seem to have been reported in pea. In view of the importance of tetrasomics and multiple trisomics as a source of primary trisomics, and also in determining the homologous series[5-7], the present communication on the cytomorphological behavior of such mutants in pea is of considerable significance.

Two off-type plants were isolated in the M_3 generation of the selfed progeny of interchange heterozygotes induced through gamma-irradiation (10 krad) of the F_1 seeds from the diverse cultivars of pea (*Pisum sativum* L.); namely, T 163 (a local cultivar), 5806-S (a normal plant selection from the progeny of chlorophyll mutant, L-5806 of Dr S. Blix, Sweden), 68 C (Dr W. Gottschalk, West Germany) and PI210613 (Dr A. E. Slinkard, Canada).

The tetrasomic plant was (L613-2, T163 × 5806-S-1-11-13-2) dwarf (52.3 cm; ⅓ of the sister euploid) and characterized by the presence of a slender stem, profused branching with small and yellow green foliage, reduced leaf-length (fig. 1) and late flowering (by 10 days) as compared to its sister diploids. Chromosome configurations 1IV+6II (46.67% cells, fig. 3) and 2IV+4II (33.33% cells, fig. 2) were most frequent, which obviously indicated that the tetrasomy was in interchange heterozygote background. Moreover, presence of cells with III or V or both were not observed. At anaphase I (AI), normal disjunction was observed in 31.8% cells; 22.7% cells had laggards (fig. 5). Pollen grains were of variable sizes and were highly sterile (about 90%). This plant did not set any seed.

The quadruple trisomic plant (L421-5, 68 C × PI210613-11-5-11-5) was very dwarf (15.5 cm, compared to 55.5 cm of its sister euploid), and weak with a slender stem. Stipule and leaflets were yellow-green and very small. Leaf-length was highly reduced (fig. 6) and it was early in flowering by 18 days. At metaphase I, 40% cells showed 4III+3II configuration (fig. 7) indicating the presence of trisomy for four different chromosomes. The next most frequent configuration, 3III+4II+1I (fig. 8) was observed in 30% cells. Univalent frequency was high, ranging from 1 to 12 per cell. At AI, 10-8 separation was most frequent (50.0% cell, fig. 9). Laggards ranging from 1 to 4 per cell were observed. Pollen sterility was high (63.0%) and the plant did not set seed. Morphologically, the tetrasomic plant differed the quadruple trisomic with respect to shape and size of stipules and leaflets. Tetrasomics in guava were also shorter than diploids and male sterile[3]. Reduced vigor and viability of these mutants indicate