

GENETIC EVIDENCE FOR TWO POTASSIUM TRANSPORT SYSTEMS IN THE GREEN ALGA, *CHLAMYDOMONAS REINHARDTII*

David Polley
Biology Department
Wabash College
Crawfordsville, Indiana 47933

ABSTRACT: The genetic evidence for two independent potassium transport systems in *Chlamydomonas reinhardtii* is presented. The first system was previously described and is encoded by the *TRK* genes. The second, described here, is encoded by the HKR gene.

KEYWORDS: *Chlamydomonas reinhardtii*, potassium transport, transport mutants.

INTRODUCTION

Potassium is the major monovalent cation in plant and algal cells, where it plays an important role in several cellular processes, such as osmoregulation, protein synthesis, and charge balance (Leigh and Wyn-Jones, 1984). Because of the essential role potassium plays in these processes, its concentration within the cytoplasm and various other cell compartments is highly regulated. This control is achieved through the regulation of potassium transport across the membrane barriers of various compartments. For example, the cytoplasmic potassium concentration is maintained at a fairly constant level of approximately 150 mM even though plants grow successfully in media with potassium concentrations ranging from 10 μ M to 10 mM. A higher plant's ability to respond to a range of external potassium concentrations is achieved by two kinetically distinct transport systems. The first, a high-affinity system, exhibits typical Michaelis-Menten kinetics and reaches saturation in the micromolar range; the second, a low-affinity system, operates in the millimolar range and is often said to be non-saturable (Kochian and Lucas, 1982). In a recent study of potassium transport in the green alga *Chlamydomonas reinhardtii*, Malhotra and Glass (1995) reported kinetic data that support the existence of two potassium transport systems, a saturable, high-affinity system (HATS) and a non-saturable, low-affinity system (LATS).

In recent years, much progress in our understanding of the nature of potassium transporters has been made through a variety of kinetic and electrophysiological analyses (*e.g.*, Kochian and Lucas, 1982; Maathius and Sanders, 1994). Insight into the structure of transporters has been enhanced greatly with the cloning of plant potassium channel genes (Anderson, *et al.*, 1992; Sentenac, *et al.*, 1992). This progress notwithstanding, gaps remain in our understanding.

One approach to the study of potassium transport that has not been explored fully is the isolation and characterization of potassium transport defective clones. This approach, employing *Chlamydomonas* as a model system, was first outlined by Polley and Doctor (1985), who isolated and conducted a preliminary characterization of three potassium transport defective clones. Subsequently, additional mutant clones have been isolated and characterized, and the presence of at least three unlinked genes (*trk*) that encode the high affinity transport system has been demonstrated (Polley, in review). In work reported here, genetic evidence for a second potassium transport system is presented. Isolation of mutant transport alleles should make possible the identification of additional components of the potassium transport system and the future cloning of genes involved in transport and its regulation.

MATERIALS AND METHODS

Strains. The mutant clone DP2 (*trk2-1 act1 mt-*) was derived as a recombinant from a cross between the potassium transport defective clone KDP5 (*trk2-1 mt+*) and CC1680 (*act1 ac80 mt-*). KDP5 was isolated after UV-mutagenesis of wild-type strain 137c (Polley, in review). Both the wild-type and mutant strain CC1680 were obtained from the *Chlamydomonas* Genetics Center. Mutant clone 10KDP1 was isolated after UV-mutagenesis of clone DP2.

Media and Growth Conditions. Cells were grown axenically at 25° C under continuous illumination either in aerated liquid cultures or on medium solidified with agar. Medium 0K0N is a modification of tris-acetate-phosphate medium (TAP medium) that possesses only trace amounts of potassium or sodium (Polley and Doctor, 1985). The potassium requirement of mutant clones was determined by measuring the rate of growth in 0K0N supplemented with different concentrations of KCl. Growth was monitored by following absorbance (light scattering) at 560 nm. Absorbance is directly proportional to cell number, and the same correlation between cell number and absorbance exists for cells grown in media of different potassium concentrations.

Mutagenesis and Genetic Analysis. Four milliliters of DP2 cells grown to a density of approximately 5×10^6 cells/ml were placed in a 4.5 cm Falcon petri dish and exposed to UV-irradiation for 90 sec in a Strategene UV Crosslinker. After exposure, cells were grown overnight in the dark to fix the mutation. The cells were then plated on 10K0N (0K0N medium supplemented with 10 mM KCl). Approximately 5-10% of the cells survive UV irradiation. Survivors were screened by replica-plating to 1K0N (0K0N supplemented with 1 mM KCl). Mating and tetrad analysis were done as described by Harris (1989).

RESULTS AND DISCUSSION

Approximately 1,000 DP2 clones that survived UV-mutagenesis were screened for their ability to grow on 1K0N. Of these, 5 failed to grow, and they were designated as putative high K^+ requiring (*hkr*) mutants. One of these, clone 10KDP1, was selected for further study. The concentration of potassium required

Table 1. Growth rate of wild-type and mutant clones (growth rate constants, k , where $A_t = A_0 e^{kt}$, are the averages of three experiments $\pm$ standard error).

Strain	Alleles	Growth Rate Constant ($k \times 10^{-2}$)			
		KCl (mM)			
		10	5	1	0.1
137c	<i>TRK2-1 HKR</i>	ND*	7.5 $\pm$ 0.5	7.7 $\pm$ 0.9	7.9 $\pm$ 1.0
DP2	<i>trk2-1 HKR</i>	8.0 $\pm$ 0.3	ND	7.0 $\pm$ 0.1	0.6 $\pm$ 0.9
10KDP1	<i>trk2-1 hkr</i>	7.0 $\pm$ 0.3	5.2 $\pm$ 0.6	0.0 $\pm$ 0.0	ND

* ND = not done.

for growth and the specificity of the requirement were determined by measuring the rate of growth in liquid media supplemented with different concentrations of KCl (Table 1). Growth was monitored by measuring absorbance (see methods); readings were taken every 3-5 hours during exponential growth of the culture. The growth rate constant, k , is based on 6 time points. The reported values in Table 1 are the averages of three experiments. As the data clearly show, *trk2-1* cells require KCl concentrations greater than 0.1 mM to achieve a wild-type growth rate; *trk2-1 hkr* cells require concentrations greater than 1 mM. In both cases, NaCl cannot substitute for KCl (Polley and Doctor, 1985, and data not shown).

The *trk2-1* allele maps to linkage group II and is linked to the *act1* allele (PD > NPD, Table 2, Tetrad Analysis; Polley, in review). The mutant clone 10KDP1 was crossed to wild type (*trk2-1 hkr act1 mt- x TRK2-1 HKR ACT1 mt+*), and tetrad analysis was performed in order to map the genetic lesion, *hkr*, relative to the *trk2-1* and *act1* alleles. The possible genotypes resulting from this cross and their respective phenotypes and tetrads are shown in Table 2 (Genotypes and Phenotypes). The phenotypes are defined as low KCl when cells achieve wild-type growth rate on 0.1 mM KCl, as intermediate KCl when cells require 1 mM KCl, and as high KCl when cells require 10 mM KCl in order to grow at wild-type rates. Of the four genotypes listed, only the phenotype of the recombinant *TRK2-1 hkr* could not be predicted *a priori*. The wild-type *TRK2-1* allele was assumed to be epistatic to *hkr*, and, therefore, the phenotype of *TRK2-1 hkr* would be low KCl. That this assumption is correct is supported by the observation that only three classes of tetrads, as defined by the phenotypic ratios of tetrad products, were obtained (Table 2, Tetrads and Phenotypes). Based on the criteria PD = NPD and NPD/T > 0.25 (Perkins, 1953), the tetrad data (Table 2, Tetrad Analysis) show that the *hkr* gene is unlinked to *trk2-1*.

While the *hkr* lesion in 10KDP1 might actually be a mutation in one of the other two, unlinked *TRK* genes, this possibility seems unlikely for two reasons. First, genetic recombinants harboring two mutant *TRK* alleles (*trk1 trk2-1*, *trk1 trk3*, or *trk2-1 trk3*) do not exhibit growth rates different from cells with just one mutant *TRK* allele (Polley, in review). This finding suggests that the *TRK* gene

Table 2. Genetic analysis of clone 10KDP1.

Genotypes and Phenotypes

Genotype	Phenotype
<i>TRK2-1 HKR</i>	Low KCl
<i>trk2-1 hkr</i>	High KCl
<i>trk2-1 HKR</i>	Intermediate KCl
<i>TRK2-1 hkr</i>	Low KCl

Tetrads and Phenotypes

Tetrads	Phenotypic Ratios
Parental Ditype	2 High : 2 Low
Nonparental Ditype	2 Inter. : 2 Low
Tetratype	2 Inter. : 1 Low : 1 High

Tetrad Analysis

Cross	<i>trk2-1 act1</i>	<i>trk2-1 hkr</i>
KDP5 x CC1680	16:0:12*	
10KDP1 x CC125	16:0: 5	8:5:8

* PD:NPD:T.

products interact functionally; *i.e.*, if one mutant polypeptide disrupts function of a multimeric protein complex, a second mutant polypeptide would not have a noticeable effect because the complex is already disabled. The *hkr* allele in a *trk2-1* background, however, does increase the potassium requirement for growth. The *hkr* allele must, therefore, affect the function or regulation of some other potassium transport system.

Second, and consistent with the above interpretation, is the observation that *TRK2-1* is epistatic to *hkr*. If the *TRK* genes encode for a potassium transport system that is able to transport potassium from media with low concentrations (0.1 mM) of potas-

sium, and if the *HKR* gene encodes for a transport system that is effective only in media with intermediate levels (1.0 mM) of potassium, then one would expect *trk hkr* cells to require high concentrations (10 mM) of potassium to grow. By similar reasoning, one would also expect cells with a functional *TRK* system not to need an *HKR* system; *i.e.*, *TRK2-1 hkr* cells will grow on low levels of potassium.

In summary, the above genetic data support the conclusion that two distinct transport systems exist: the *TRK* system which operates at low levels of potassium and the *HKR* system which operates at higher levels of potassium. Future work will involve a kinetic analysis of potassium transport by 10KDP1, mapping the *hkr* mutant allele, and determining if *hkr* in a *trk1* or *trk3* background also require, as would be expected, high concentrations of potassium.

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