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The hexose carrier from *Chlorella*

cDNA cloning of a eucaryotic H^+-cotransporter

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The cDNA coding for the inducible H^+/hexose cotransporter of *Chlorella kessleri* has been cloned and sequenced. It was isolated by differential screening of a cDNA library prepared from glucose-induced cells. The increase in expression of the gene correlates quantitatively with the increase in uptake activity due to induction; it is not expressed in a hexose transport mutant. An open reading frame allows for a membrane protein of 533 amino acids with a relative molecular mass of 57 kDa. This protein is highly homologous to the human and rat glucose transporters catalyzing facilitated diffusion and to the bacterial H^+/pentose cotransporters. It is not related to the H^+/lactose cotransporter of *E.colli* and to the mammalian Na^+/glucose cotransporter.

Cotransporter, H^+/hexose; Differential screening; (*Chlorella kessleri*)

1. INTRODUCTION

Inducible uptake of hexoses has been intensively studied with the unicellular alga *Chlorella* [1,2]. These algae accumulate glucose analogues by proton cotransport [3,4], a general feature of active transport in plants [5]. Whereas photosynthetically grown cells of *C. kessleri* take up hexoses at a very low rate, this rate increases up to 400-fold when cells are pretreated with glucose under non-growing conditions [6]. Synthesis of a plasma membrane protein with an apparent molecular mass of 47 kDa as determined by SDS-polyacrylamide gel electrophoresis correlates with this induction [7]. Here we describe the molecular cloning of a cDNA clone coding for the *Chlorella* H^+/hexose cotransporter. It was obtained by differential screening of a cDNA library prepared from glucose induced cells.

2. MATERIALS AND METHODS

2.1. *Materials*

The *Chlorella* strain used in our work and so far named *Chlorella vulgaris* has recently been determined as *Chlorella kessleri* by Dr Erich Kessler, Erlangen. The cells are grown in a mineral medium in light as previously described [8].

2.2. *Construction and screening of the cDNA library*

A cDNA library of poly (A^+) RNA from *C. kessleri* induced for glucose transport [1] was constructed in λgt10 as described by Gubler and Hoffmann [9] and screened differentially [10] with radiolabelled cDNA from induced versus non-induced cells. The insert of λ clone 14, which reacted only with cDNA from induced cells, was subcloned (pTF14) and sequenced [11]. The clone was not full length but exhibited homology to other glucose carriers. It was therefore used as a probe to screen another cDNA library in λgt10, which had been made from size-selected cDNA. From this library five full length cDNA clones were obtained (pTF201-pTF205). Hybridizations for differential screening were performed in 50% formamide, 5×SSC, 1× Denhardt's, 0.1% SDS, 5 mM EDTA, 100 μg/ml carrier-DNA, and 20 μg/ml poly-U at 37°C overnight. Filters were washed at 37°C twice with 1× SSC, 0.1% SDS and once with 0.2×SSC, 0.1% SDS. Hybridizations with the pTF14 insert were similar, but with 2× SSC and at 42°C, the poly-U was omitted. Washes were done at 42°C with 0.1× SSC, 0.1% SDS.

2.3. *Southern and Northern blot analysis*

2.3.1. Southern blot DNA was isolated [12], digested with several restriction enzymes (see fig.3), separated on a 0.8% agarose gel, and blotted onto a nitrocellulose filter. The filter was probed with the cDNA clone pTF14, which starts at base 1042 of the sequence shown in fig.1 and ends with the poly A-tail. This fragment does not contain sites for *Eco*RI, *Bam*HI, or *Hin*dIII, and only one site for *Kpn*I. Hybridization and washing conditions were as described below. *Cla*I digested λWT DNA was used as molecular weight marker.

2.3.1. Northern blot Total cellular RNA was isolated as described in [13]; mRNA was further purified by oligo-dT-cellulose chromatography [14]. 5 μg of poly(A^+) RNA were loaded per lane, separated, and blotted onto a nitrocellulose filter. The conditions for hybridization were 50% formamide, 2×SSC, 1× Denhardt's, 100 μg/ml salmon sperm DNA, 42°C. The filter was washed at 42°C with 0.1×SSC, 0.1% SDS.

3. RESULTS

Since autotrophically grown cells of *Chlorella kessleri* take up hexoses and glucose analogues at a very

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession no. Y07520

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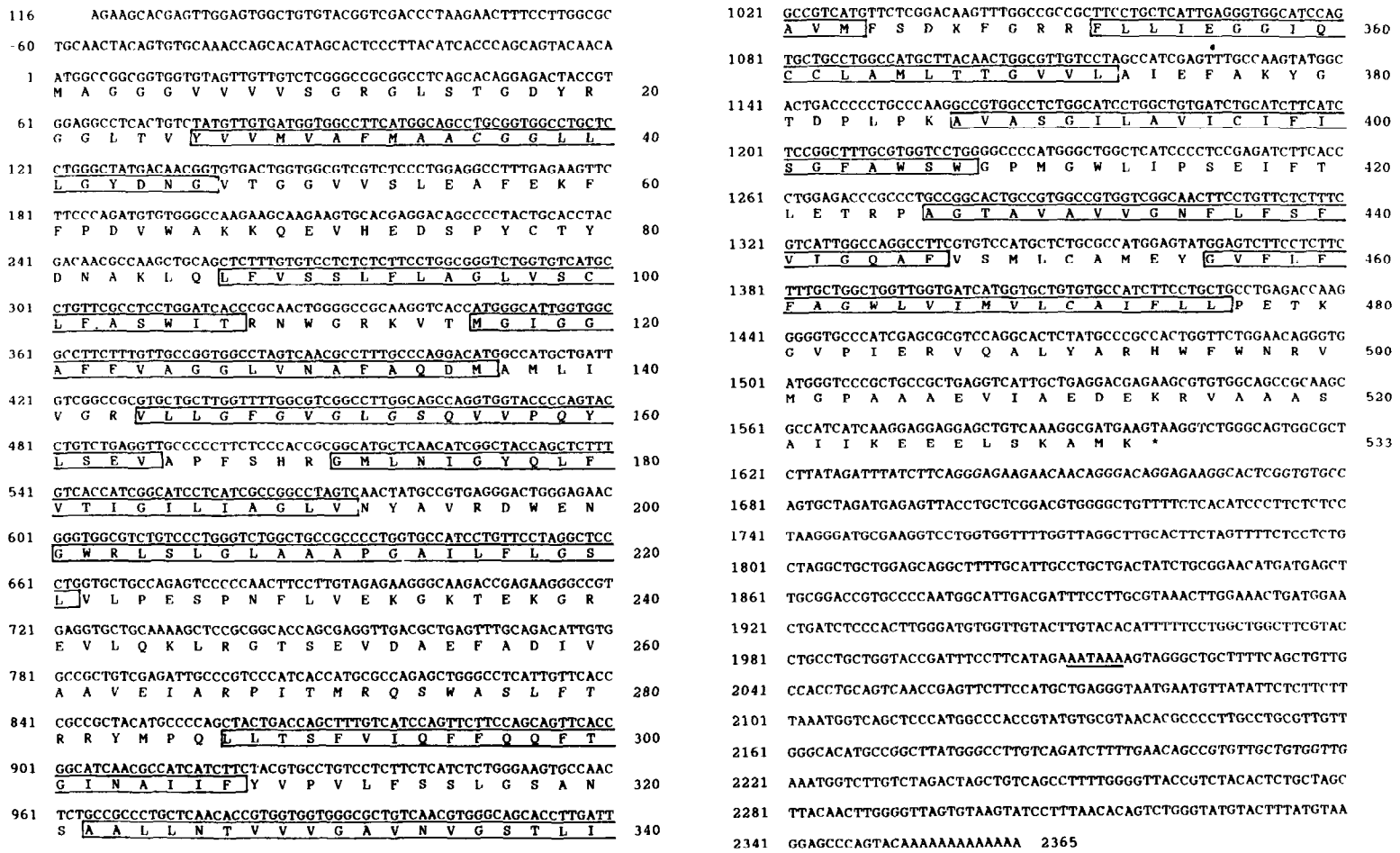

Fig.1. Nucleotide and predicted amino acid sequence of the insert of cDNA clone pTF205. The 12 putative membrane spanning domains of the protein are boxed, the presumed polyadenylation signal sequence is underlined.

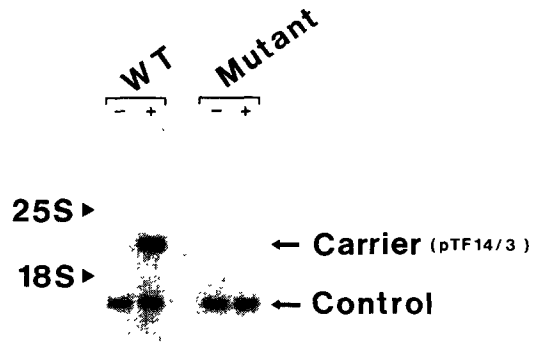

Fig.2 Northern blot analysis of poly(A$^+$) RNA of wild type or mutant *Chlorella* cells, induced (+) or not induced (−) for glucose transport. The filter was probed simultaneously with a 3′-fragment of the cDNA-clone pTF14 starting at the *Kpn*I-site at position 1990 (fig.1) and with the insert of cDNA clone pTF30C, which is used as a control clone, since its mRNA level is not influenced by induction. The result obtained with the 3′-specific probe was identical to the result with the full clone (data not shown); both recognize an mRNA with an apparent length of about 2500 bp.

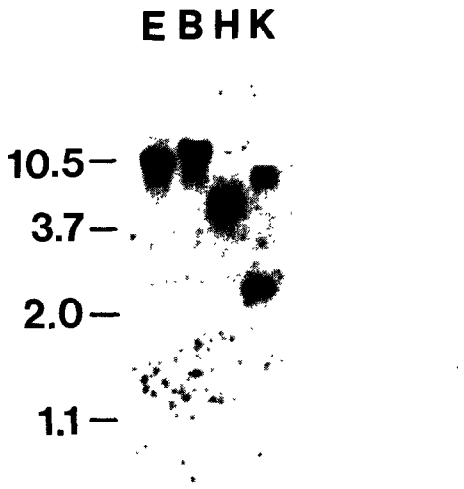

Fig.3. Southern blot analysis of *Chlorella* genomic DNA. Genomic DNA was isolated from light grown *Chlorella* cells and digested with *Bam*HI(B), *Eco*RI(E), *Hin*dIII(H), and *Kpn*I(K). For details see section 2.

low rate, but induce an H$^+$/hexose cotransporting system several hundred fold in the presence of transport substrates [1,6] a cDNA library from mRNA of induced cells was constructed in λgt10. This library was screened with radiolabelled cDNA from induced and from non-induced cells. Clones were picked that lit up only or considerably stronger with cDNA prepared from induced cells. Of about 20 clones 8 turned out to be unrelated to each other; they were sequenced. Five of them most likely code for membrane proteins according to their hydropathic profile. The sequence of the clone pTF14 with a 1400 bp insert showed a high degree of homology to the carboxy terminal sequence of published glucose transporters from mammalian cells [15–17] and to the SNF3 gene product of yeast cells [18]. The sequence of a full length *Chlorella* clone (pTF205) obtained subsequently is given in fig.1. An open reading frame of 1599 bp allows for a protein of 533 amino acids with a relative molecular mass of 57 445 Da. This protein when aligned to the insulin-regulatable glucose transporter [17,19] possesses 29.7% and to the SNF3 gene product of yeast [18] 29.0% identity.

When mRNA from induced and non-induced *Chlorella* cells was probed with a radiolabelled segment of pTF14 the signals of fig. 2 were obtained. An mRNA of about 2.5 kb is synthesized due to glucose induction. The increase in signal strength correlates with the degree of transport induction (> 100-fold in this experiment, data not shown). In addition mRNA of a *Chlorella* glucose transport mutant [7] does not give rise to the signal (fig.2). Glucose and glucose analogues also induce two amino acid transport systems in *C. kessleri* [20]. Mutants defective in these amino acid uptake systems [21] showed the identical mRNA signal with pTF205 as hybridization probe as the wild type (data not shown).

Genomic Southern blots of *Chlorella* genomic DNA digested with four different restriction enzymes are shown in fig.3. Hybridization at high stringency yielded only one or two autoradiographic bands (the probe pTF14 has one internal *KpnI* site). This makes it very likely that only one copy of this gene is present in the *Chlorella* genome.

4. DISCUSSION

To our knowledge no eucaryotic H$^+$-cotransporter gene has been cloned so far. The hexose transporter gene of *Chlorella*, except for ATPases, is also the first plant gene of a plasma membrane transporter. Although the evidence is not absolutely tight yet, the high degree of sequence identity with mammalian, yeast, and procaryotic sugar transporters [15,18,22], the qualitative and quantitative induction behaviour of expression, and the lack of the corresponding mRNA in a specific glucose uptake mutant, strongly suggest that the DNA in question codes for the *Chlorella* hexose transporter.

It has been shown previously that a protein present only in the plasma membrane of induced *Chlorella* cells correlates well with glucose uptake [23]. The difference in relative molecular mass of this protein of 47 kDa (as determined on SDS polyacrylamide gels [7]) to 57 kDa as deduced from the DNA sequence, is a phenomenon typical for hydrophobic membrane proteins [8,22,24].

So far three classes of sugar transporter genes have been sequenced and characterized: (i) the H^+-Lac permease of *E. coli* [25]; (ii) the Na^+-glucose cotransporter of rabbit intestine [26]; and (iii) a large group consisting of the mammalian glucose transporters [15-17], the procaryotic pentose H^+-cotransporters [22] and fungal glucose and even lactose transporters [18,27]. The *Chlorella* H^+/hexose cotransporter shares all characteristics of the class 3 sugar transporters: the 12 hydrophobic segments are divided 6 by 6 by a relatively long (63 amino acids) hydrophilic sequence (fig.1); the typical RXGRR (R can be replaced by K) motive [22] after the second and eighth potential transmembrane segment reads RXXGRR (amino acids 108-113) and KXGRR (347-351) in *Chlorella*; typical is also a PESP (224-227) after the sixth, and a PETKG (477-481) sequence after the 12th potential transmembrane segment. A striking homology is in addition existing within the seventh potential membrane spanning segment of class 3 transporters with QXXQQXS/TGXNXXXF/YY (amino acids 294-308 in *Chlorella*). The typical glycosylation site of mammalian and yeast sugar translocators at Asn^{45} [15], Asn^{57} [17,19], and Asn^{383} [18] is missing in *Chlorella*, which explains the previous observation that tunicamycin did not affect at all the induction of glucose uptake [8]. As in all the other transporters, also the *Chlorella* one does not possess an N-terminal signal peptide.

Among the class 3 sugar transporters are those catalyzing active transport (H^+-cotranslocators of bacteria and of *Chlorella*) as well as those catalyzing facilitated diffusion (in mammalian and yeast cells). No obvious sequence difference can be detected between the two groups. An aspartic acid residue present in the first potential transmembrane segment of all H^+-translocaters is also present in the corresponding SNF 3 segment of yeast.

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