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Chemotactic effect of mono- and disaccharides on the unicellular *Tetrahymena pyriformis*

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ABSTRACT

Chemotaxis is one of the most essential cell physiological responses, which was developed in parallel the molecular evolution of signal molecules. Previously good correlations were found between chemotactic moieties and physicochemical properties (SEA, solubility, pKa) of peptide type ligands in Tetrahymena model. However, references are rather weak in eukaryotic chemotaxis about significance of simple carbohydrates. In the present work our goal is (i) to investigate the chemotactic effect of 10 mono- and disaccharides in the eukaryotic *Tetrahymena pyriformis*; (ii) to describe effective ligands with physicochemical parameters; (iii) to test whether sugars are acting via induction of metabolic pathways. Our results are: (i) the tested sugars can trigger both significant attractant (D-glucose, D-mannose) and significant repellent (D-glucosamine, D-fructose, N-acetyl-D-galactosamine, D-arabinose) effects, while some of the sugars (maltose, lactose, sucrose, D-galactose) had no effect. (ii) Correlations were described between the chemotactic effectiveness of the ligands and their physicochemical characters (TPSA, XLogP), which are supposed to influence the internalization of the sugars. (iii) All ligands proved to have low selection potential, which refers to a 'short-term' receptor moiety or influencing specific metabolic pathways. (iv) Starvation elicited modified, strong chemoattractive responsiveness towards glucose; however, it was independent of concentration while 1 h insulin treatment resulted in an increased and concentration dependent chemotaxis induced by glucose.

1. Introduction

The chemotaxis (directed movement induced by chemical gradients) is one of the basic physiological activities of a cell. At unicellular level it takes part in feeding and avoiding toxic substances[1] while in multicellular organisms it has an essential role in fertilization, differentiation or in inflammatory responses.[1] Triggering ligands of chemotaxis are several, they are classified in two main groups (i) professional chemotactic ligands (their main biological function is to elicit chemotaxis), such as formyl-peptides,[2] chemokines or C5 and C3 derivatives of complement system; (ii) other molecules, which are able to evoke chemotaxis, but not as a main function, e.g. amino acids,[3] volatile oils,[4] lectins[5] and peptide hormones, such as insulin.[6] The two groups of ligands mentioned above could be associated also by Lenhoff-hypothesis.[7] This theory explains that the cells in the early phase of evolution had only primordial receptors, detecting food molecules and the ligand specificity of the receptor was limited. However, some of the ligands were exploited not solely as a source of energy, but they were able also to evoke a special kind of cell physiological responses, too. The parallel positive selection of ligands and their receptors have resulted in the group of signal molecules (e.g., hormones or chemokines), while the other molecules possessing no special signal character remained for nourishment. The process described above was theoretically valid for all classes of signal molecules including biogenic amines, peptides, lipids and a wide range of carbohydrates.

The eukaryotic ciliate *Tetrahymena pyriformis* is frequently used as a model for studies in molecular and cell biology. It is a suitable model as characteristics of its membrane receptors (e.g., down-regulation of insulin receptor),[8] activity and induction of second messenger systems (e.g., cAMP, IP3, Ca^{2+}- calmodulin)[9–11] or metabolic processes (e.g., effect of inducers or blockers of glucose metabolism),[12] show homology with mammalian models such as rodents, human etc. Over the well-investigated peptide/protein

Abbreviations: SEA, solvent exposed area; TPSA, topological polar surface area; XLogP, octanol/water partition coefficient.

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systems of Tetrahymena, a wide range of references is available describing significance of carbohydrates in this ciliate; nevertheless, the results from the published studies are rather weak in data about their chemotactic behaviour.[13] It was demonstrated that Tetrahymena is able to metabolize glycogen, glucose and fructose by glycolysis, although the utilization of glucose is 10-fold higher than fructose.[14–17] Special significance of glucose was proved by other works, too. In sugar supplemented medium the uptake of glucose was shown to be higher than for mannose, galactose and fructose. In contrast, mannose added to the medium stimulated the cell growth at a high rate, while glucose and galactose had reduced effects and fructose had no effect on growth.[18] Kaneshiro had shown very poor uptake of small organic compounds, including glucose, by Paramecium.[19] In protozoa no carriers for sugars are known, but uptake via phagocytosis is discussed in the literature and it was also demonstrated that Tetrahymena is able to internalize glucose even in the presence of phagocytosis inhibitors (e.g., cytochalasin),[20] while another work demonstrated the significance of a Na^+-independent glucose transporter system in the same model.[21] Other studies also reported facilitated diffusion of arabinose by stereospecific carriers and that glucose is a competitive inhibitor of the process.[22] Not only carbohydrates themselves but modulators of carbohydrate metabolism have also characteristic effects on *Ciliophora* level. It was observed that Tetrahymena can detect insulin as a ligand[23] and the receptor of insulin was also characterized as a close relative of the human one.[24] Endogenous production of insulin, and induction of this activity by exogenous insulins,[25,26] as well as enhancing effect of insulin on the glucose uptake[9] were described in this ciliate.

The number of publications about chemotaxis induced by sugars in eukaryotic organisms is few. In prokaryotes (e.g., *Escherichia coli*, *Catenuloplanes japonicus*) diverse sugars can elicit very strong attractant responses in a relatively wide range (10^{-6}–10^{-1} M); however, these studies were carried out only in inorganic medium.[27,28] Fan et al. have tested chemotaxis of mangrove zoospores by several sugars, but only at one, 10^{-2} M concentration.[29] In these experiments maltose had moderately attractant effect in the case of one strain, galactose and glucose proved to be weak attractants for all the five strains tested, while fructose, mannose, cellobiose, xylose and sucrose had a dual, weak attractant and repellent effect. We know only one experiment on vertebrate cells: among the five cell types tested only bovine corneal endothelial cells showed chemotactic and chemokinetic responses towards 10^{-2} M glucose and sucrose; however, this high dose is not considered as an adequate stimulus for vertebrate cells.[30]

In case of prokaryotes, Adler has identified nine sugar receptors displaying competitive inhibition in the presence of ligands.[27] As in eukaryotes, the references about the chemotactic responses elicited by carbohydrates are rather weak, experiments focused on receptor selection are more promising. The technique—chemotactic selection—deals with the chemoattractant effect of the ligand as a selector moiety at first, then the offspring generations derived from the responder cells are re-assayed as representatives of chemotactic responsiveness in cell physiological and genetical aspects, as well.[31] In the case of increased responsiveness towards the ligand, we can suppose a so-called '*long-term*' *receptor*, which is a genetically determined, permanent component of the membrane (e.g., chemokine receptors),[31] while failure of the phenomenon supports that '*short-term*' *receptors* are responsible for the chemotactic responses (e.g., vasoactive peptide receptors).[32] The second type of responsiveness is based on ad hoc, transient assembly of receptor components or induction of specific metabolic pathways.[33] The technique described above is essentially different from cross adaptation described before by Roberts and Orias:[34] in cross adaptation the time range of effectiveness is detectable only in the subsequent hours after the treatment while responsiveness of chemotactically selected subpopulations is retained in several (<70) offspring generations.[31]

Taking into account that simple sugars have appeared in the early phases of molecular phylogeny working of both signalling mechanisms—using genetically preformed or *ad hoc* receptors—are conceivable in eukaryotic level; however, the literature is still incomplete in relevant articles exploring this implication of the problem.

In the present study the objectives of our work are:

(i) to test chemotactic character of 10 mono- and disaccharides in Tetrahymena cells;
(ii) to analyze whether the chemical character of the examined mono- and disaccharides have any relationship with the chemotactic effect elicited;
(iii) to analyze whether chemotactic responses elicited by carbohydrates are developed via 'short-term' or 'long-term' signalling mechanisms in the unicellular ciliated model.

Depending on the responses received, our further aims were to understand the significance of underlying metabolic processes influenced by biolgically active chief regulator molecules like insulin and to construct a working model—could be valid even in human—of chemotaxis elicited by simple sugars.

2. Results

2.1. Concentration course of chemotaxis

Although sugars can serve as simple nutrients for protozoa organisms—which means a chemoattractant effect in general, the effects of the investigated sugars were rather diverse in Tetrahymena. In capillary assays, majority of the sugars investigated had chemoattractant or chemorepellent effects, whereas one of the tested sugars was neutral (Table 1). The repellent effect was significant at two concentrations of fructose (10^{-12}, 10^{-9} M), N-acetyl galactosamine (10^{-12}, 10^{-9} M) and arabinose (10^{-8}, 10^{-6} M) and it was significant at one concentration in the case of glucosamine (10^{-8} M). Four other sugars, including three disaccharides, had no significant effect; however, a weak repellent effect was still detectable with sucrose (10^{-11} M). A weak positive, attractant effect was elicited by galactose (10^{-17}, 10^{-11} M) and maltose (10^{-9} M). Two sugars elicited significant positive chemotactic responses: the maximal chemoattractant effect of glucose was elicited at 10^{-7}–10^{-8} M, while mannose was effective only at 10^{-6} M. Finally, lactose proved to be the only sugar possessing a wide range and neutral effect on chemotaxis of Tetrahymena.

2.2. Swimming behaviour

Changes of swimming behaviour in *Ciliophora* are considered as good indicators of even slight alterations of chemical composition of the environment. In the present work, chemotaxis assays were completed by computer assisted tracking analysis (mean velocity and tortuosity of swimming) in Tetrahymena cells. The selected test sugars were the four strong or weak chemoattractants: glucose, mannose, galactose, and maltose. As shown Fig. 1A 10^{-6} M mannose could elicit significantly higher swimming velocity than the control, while 10^{-7} M glucose and 10^{-11} M galactose could also increase the value of this parameter; nevertheless, it was not significant. Finally, the effect of the 10^{-9} M maltose was neutral; it was in the same range as the control. The other registered characteristic feature was the tortuosity of the path. This moiety describes the swimming path whether it is a linear one or it is interposed by short

Table 1
List of sugars investigated and their effects on chemotaxis in *Tetrahymena pyriformis* GL. The most effective (attractant or repellent) concentrations were determined in capillary chemotaxis assays in the given ranges. The overall chemotactic character of the individual sugars was determined on the basis of evaluation a database—520 data/sugar. (Data of significant repellent and significant attractant sugars are highlighted in gray.)

Mono- or disaccharide	Conc. range investigated (M)	Most effective concentration (M)	Ch_{idx}(% ±S.E.)	*p*	Effect
Glucosamine	10^{-18}–10^{-6}	10^{-8}	52±10	0.032	Strong repellent
Fructose	10^{-18}–10^{-6}	10^{-12}	63±5	0.001	Strong repellent
		10^{-9}	67±11	0.047	Strong repellent
N-acetyl galactosamine	10^{-18}–10^{-6}	10^{-12}	64±6	0.021	Strong repellent
		10^{-9}	72±10	0.036	Strong repellent
Arabinose	10^{-18}–10^{-6}	10^{-8}	74±9	0.019	Strong repellent
		10^{-6}	74±10	0.037	Strong repellent
Sucrose	10^{-18}–10^{-6}	10^{-11}	70±11	0.078	Repellent
Lactose	10^{-18}–10^{-6}	Not effective	101±5	—	Not effective
Galactose	10^{-18}–10^{-6}	10^{-17}	138±21	0.098	Weak attractant
		10^{-11}	134±21	0.099	Weak attractant
Maltose	10^{-18}–10^{-6}	10^{-9}	138±25	0.088	Weak attractant
Mannose	10^{-18}–10^{-1}	10^{-6}	160±25	0.028	Strong attractant
Glucose	10^{-18}–10^{-1}	10^{-7}	182 ±13	0.004	Strong attractant
		10^{-8}	160±21	0.047	Strong attractant

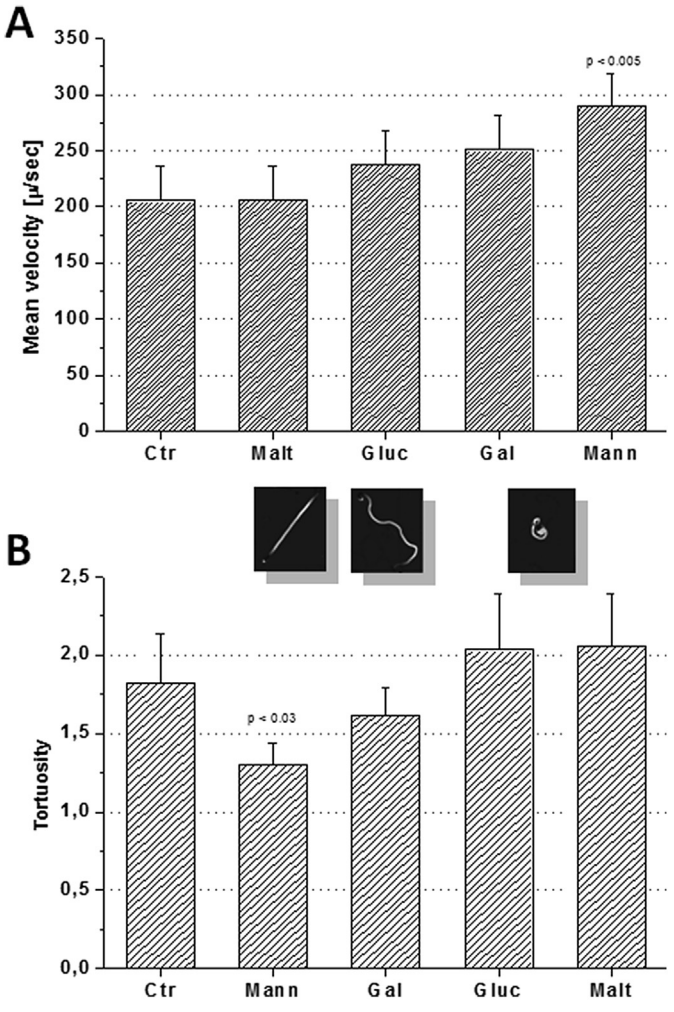

Fig. 1. Swimming behaviour evaluated by computer assisted tracking analysis in Tetrahymena cells treated with sugars. (A) Mean velocity of cells (μm/s); (B) Changes in tortuosity of swimming paths (ctr-control; Malt—maltose; Gluc—glucose; Gal—galactose; Mann—mannose; in upper panels the representative swimming paths are shown). Values of significance were represented with threshold limit $p<0.05$.

or longer loops. The maximal linear path value is 1, while the number of foldings in the path increase the value calculated. The sequence in activity of sugars shows a total overlapping and good correlation with the data of velocity: the most linear path of swimming was elicited by mannose, while galactose elicited more tortuous—slower—swimming. Glucose and maltose were even more effective and the increased value of tortuosity was due to the high number of creeping induced by these sugars in Tetrahymena (Fig. 1B).

2.3. Chemotactic range-fitting

Assay of the chemotactic responsiveness pointed to that the well-known difference of chemoattractant and chemorepellent ligands described by '**chemotactic range fitting**'[3]—a fitting of ranges (amplitudes; number of responder cells) and chemotactic activities where chemoattractant moiety was accompanied with wide ranges, while chemorepellent actions with narrow ones—were present in the case of sugars, too. It was shown that in case of the significantly repellent ligands (glucosamine, fructose, N-acetyl galactosamine, arabinose), the amplitude of chemotactic responsiveness varied in a narrow range, while with attractant

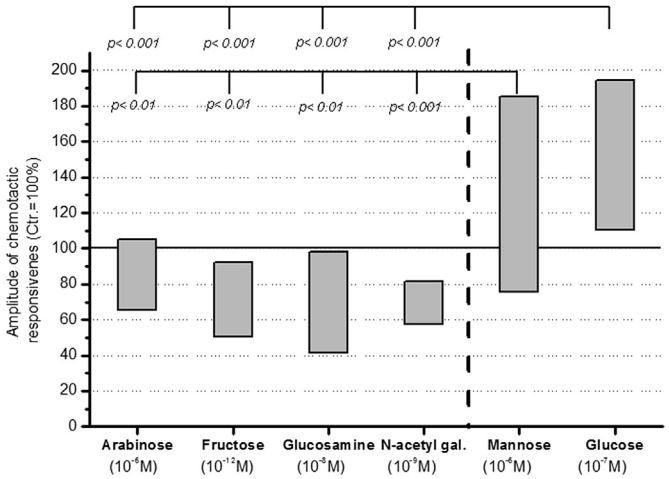

Fig. 2. Chemotactic responsiveness of Tetrahymena cells treated by sugars. The floating bars represent the differences of ranges in chemotactic activity in cultures treated with 6 different simple sugars. Values of significance calculated for corresponding pairs is shown above the columns.

ligands (glucose, mannose) the range of responsiveness was significantly wider (Fig. 2)

2.4. Chemotactic selection

Both in case of chemotactic selection and of reciprocal selection, the values of chemotactic activity were calculated close to or below 1.00 (formulas please see in 5.3 and 5.4) (Table 2). This means that at second encounter with the selector sugar, the cells of these subpopulations had similar or lower responsiveness than subpopulations selected by the control media. There were only two sugars—fructose and galactose—whose chemotactic selection index values (Ch_{sel}) were significantly different from 1.00. In the case of fructose, the selection index was as low as Ch_{sel}=0.61. As it was detected at a very low, 10^{-17} M concentration, it indicates that Tetrahymena has highly sensible receptor moiety to detect fructose and this system is working as short-term one for chemotaxis. Galactose embodies the other extremity, its high selection index (Ch_{sel}=1.82) shows that we can suppose preformed, long-term membrane recognition elements as responsible for the elevated chemotaxis responses detected in the first part of the experiment.

2.5. Chemical characteristics of sugars and their chemotactic effectiveness

Two chemical characteristics—the topological polar surface area (TPSA) and the octanol/water partition coefficient (XLogP) as important moieties of dissolved organic compounds—,were also investigated with respect of their influence on the biological activity of the sugars. In general, TPSA value is used to forecast whether a molecule has a high or low capacity to enter the cell. In this case, the limit value is 140 Å^2, smaller molecules usually enter the cell, but the bigger molecules cannot cross the membranes. In case of XLogP the smaller value than −3.0 indicates a hydrophilic ligand, while the bigger value refers to a lipophilic ligand, which can get through the surface membrane more easily.

Our present data show a close relationship between the two parameters mentioned above and the chemotactic character of the sugars investigated. Those sugars possessing either significant attractant (glucose, mannose) or repellent (glucosamine, fructose, N-acetyl galactosamine and arabinose) effects have got larger XLogP values than −3.0 and uniformly smaller TPSA values than 140 Å^2 (Fig. 3 and Fig. 4). XLogP values of other sugars, which had no significant effect (sucrose, lactose and maltose) were in the range −4.7 and −3.7 and theTPSA values were also higher than 140 Å^2.

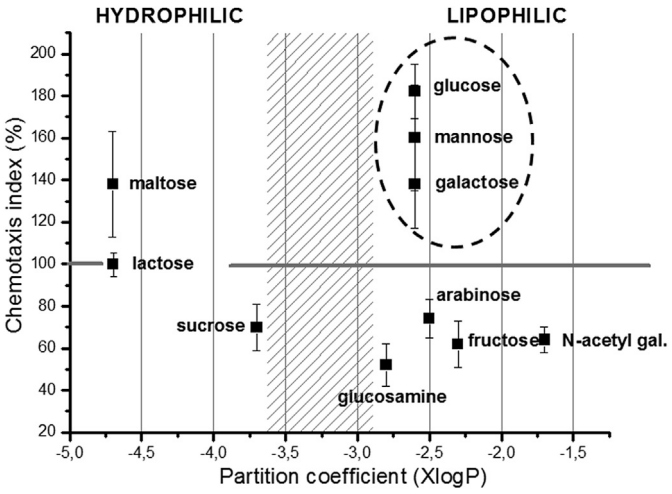

Fig. 3. Correlation between chemotactic responses induced by sugars and values of partition coefficients (XLogP) in Tetrahymena. Significant chemoattractant sugars are highlighted by a dotted circle.

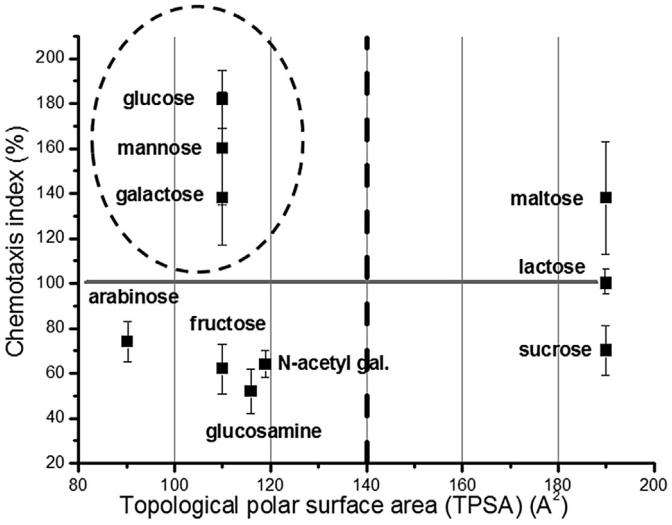

Fig. 4. Correlation between chemotactic responses elicited by sugars and the values of topological polar surface areas (TPSA) in Tetrahymena. Significant chemoattractant sugars are highlighted by a dotted circle.

Table 2
Effect of chemotactic selection and reciprocal selection implemented by sugars in *Tetrahymena pyriformis* GL cells. Ch_{sel}<1 refers to short-term while Ch_{sel}>1 refers to the long-term type of signalling. (Data of significant repellent and significant attractant sugars are highlighted in gray.)

Mono- or disaccharide	Conc. (M)	Ch_{sel}	*p*
Chemotactic selection			
Arabinose	10^{-13}	0.85	0.088
Fructose	10^{-17}	0.61	0.032
Glucose	10^{-7}	1.01	—
Mannose	10^{-6}	0.89	0.140
Maltose	10^{-9}	1.15	0.091
Maltose	10^{-16}	0.91	0.146
Galactose	10^{-10}	1.82	0.018
Reciprocal selection			
Mono or disaccharide	Conc. (M)	Ch_{rps}	*p*
Arabinose	10^{-8}	0.72	0.036
Fructose	10^{-12}	0.90	0.082
Glucosamine	10^{-8}	1.00	—
N acetyl- galactosamine	10^{-9}	0.94	—

2.6. Effects of glucose withdrawal

As study of chemotactic selection has raised the possibility that metabolic processes might also have direct or indirect influences on chemotactic responsiveness of our model cell, it was necessary to investigate the modulation of glucose metabolism as the most significant member of the carbohydrate metabolism even in Ciliophora.[35] For this purpose cultures were assayed in two ways: (i) effect of 10^{-6} M insulin was used as a strong reducing factor of intracellular glucose transport in Tetrahymena cells[12] and (ii) cells were cultured in starvation medium. Insulin treatment was also able to elicit an increased response to 10^{-10} M glucose, but only in the group, which had a 12 h lag between the treatment and the chemotaxis assay. Cultures tested immediately after the insulin treatment presented no change in the chemotactic responsiveness to glucose (Table 3). Starvation had a very strong effect on

Table 3
The effect of insulin treatment (immediately and 12 h after the treatment) and starvation on chemotaxis induced by glucose. In the case of starvation the assay was carried out in Losina-Losinsky inorganic medium. (Significant effects are highlighted in gray)

Insulin treatment (M)		Assay after insulin treatment		Assay 12 h after insulin treatment	
Outer	Inner chamber	Ch_{idx} (% ±S.E.)	*p*	Ch_{idx} (% ±S.E.)	*p*
Control	Contr.	100±12	—	100±11	—
	Glucose 10^{-10}	88±15	0.108	80±11	*0.081*
	Glucose 10^{-7}	79±10	0.087	83±14	*0.087*
	Insulin 10^{-6}	60±9	0.010	71±18	*0.025*
Insulin treated	Contr.	100±9	—	100±16	—
	Glucose 10^{-10}	82±10	0.071	144±9	0.030
	Glucose 10^{-7}	91±9	0.110	104±13	—
	Insulin 10^{-6}	86±10	0.093	107±20	—

Starvation			
Outer	Inner chamber	Ch_{idx} (% ±S.E.)	*p*
Starved cells	Contr.	100±11	—
	Glucose 10^{-10}	134±17	*0.065*
	Glucose 10^{-9}	157±13	0.001
	Glucose 10-8	143±16	0.030
	Glucose 10-7	155±14	0.003
	Glucose 10-6	136±7	0.008

chemotaxis, glucose was significantly an attractant in all tested concentrations (Table 3).

3. Discussion

3.1. Mechanisms induced by sugars in chemotaxis

The results of these experiments carried out on the ciliated eukaryotic model Tetrahymena support our previous observations on peptide and lipid type signal molecules,[36] about the high sensitivity and capability for discrimination of slight molecular differences between ligands.

On the basis of results of chemotaxis assays, we can distinguish three distinct groups of the mono- and disaccharide ligands.

(i) Wide range chemotactically effective monosaccharides are belonging to the first group. Glucose and mannose belong into this group, the sugars, which were able to elicit strong chemoattractant effects. However, their wide **chemotactic range-fitting** characteristics have ranged from strong attractant to mild repellent responses. The phenomenon shows close homology to the range fitting described for amino acids where the effective range of chemoattractant amino acids was significantly wider (Ch_{idx}=66.1±14.2%) than the chemorepellent one (Ch_{idx}=24.66±8.16%).[3]

A peculiar aspect of chemoattractant characteristics of glucose, mannose (and galactose mentioned below) is that these sugars are consumed as nutrients in our model cell Tetrahymena[18], which suggests that positive chemotactic responses have also a strong relation to the direct and instant utilization of the chemoattractant molecules. Activation of specific metabolic pathway is assumed in the backgrounds of the increased responsiveness. The mode of action supposed above is supported by the fast intake of these types of monosaccharides and that the two chemoattractants (glucose and mannose) are metabolized as energy sources of the cell, while the metabolism of the non-chemoattractants (e.g., fructose) is much slower. The ratio of fluxes measured glucose[16] (extracellular glucose → glucose 6-phosphate=460 nmol/10^6 cells/20 min) and fructose (extracellular fructose → fructose 6-phosphate=130 nmol/10^6 cells/20 min) have a good correlation with their strong chemoattractant and chemorepellent moieties found in the present work.

This theory is also supported by our current results gained in study of chemotactic selection and in experiments focused on insulin pre-treatment and starvation. The attractant monosaccharides did not elicit enhanced responsiveness in the experiments of chemotactic selection, which is dedicated to analyze receptor dynamics and the genetical determination of these receptors. Their neutral effect could be explained by action of 'short-term' receptors. In this case, the receptor components are thought to be assembled for a temporary basis and only in the presence of the ligand, while they fall apart in the absence of the inducer molecule. In this way chemotactic selection cannot result in increased responsiveness, which shows good agreement with the above mentioned 'energy source' role of these chemoattractant monosaccharides. Our results show that induced breakdown of intracellular carbohydrate pools by insulin or withdrawal of nutrients has a strong inducer effect on the chemotactic response elicited by glucose. The fact that concentration dependent chemotactic responses were provoked by insulin and that Tetrahymena has also mobilizable endogenous insulin pools[26] suggest that insulin might also work as a chief regulator of chemotactic responses of Tetrahymena via metabolic pathways.

The moderate chemoattractant, galactose, was grouped here as it has a relatively wide chemotactic range fitting. Sub-populations of Tetrahymena selected by this sugar have retained an increased chemotactic responsiveness (Ch_{sel} 1.82), which indicates the presence of constantly expressed, 'long-term' chemotaxis receptors in the surface membrane. It is known that galactose specific lectins—with identical in function to the vertebrate antibodies of several invertebrates,—are present in the surface membrane of our model cell.[37,38] Their galactose specificity could explain the enhanced functional affinity of the membrane. On the other hand galactose itself, which was detected by galactose specific lectins as a significant constituent of the membrane could explain the increased responsiveness to galactose—as an activity of the cell to get close to the source substance of a main constituent of the membrane.

Former Tetrahymena studies have referred chemotaxis as a receptor mediated response and in some cases the expression of these receptors, e.g. insulin receptor, was also identified.[39] In addition, glucose, galactose and mannose specific lectins were also identified in Tetrahymena membrane[37], which is considered to function as parts of short term receptors mentioned above. As feedback between surface membrane/its receptors and the cell physiological responses is rather fast, several signalling systems (e.g., activation of Ras, Akt/PkB, Rac, Scar/Wasp; polymerization and depolymerization of actin, myosin II activation), serve migratory and sensing apparatus of the cell to make its responsiveness fast and well tuned to the ligand.[40] Simple sugars can also work on these pathways, e.g. the prominin-1 dependent uptake of glucose is a model mechanism of the joint effect of glucose induced metabolism and cytoskeleton alteration. Based on these data, it is obvious that ligands influencing chemotaxis can act both via receptor mediated pathways and by direct ways following internalization into the cytoplasm and modulation of various metabolic processes or acting on dynamic transformation of cytoskeletal network.

(ii) Glucosamine, fructose, N-acetyl galactosamine and arabinose appear to belong to an individual group whose members' most representative characteristics were their chemorepellent moiety (in fructose and arabinose weak chemoattractant effects were also registered). The general, significantly repellent characteristic was confirmed by the narrow chemotactic range fitting values as well as a good matching with the theory described above concerning the nutrient moiety and the chemoattractant effects, as no information is available about the nutritional potency of the

sugars listed into this group in our model. The only member is the weak chemoattractant fructose, which can enter glycolysis in Tetrahymena, but its intensity is only 10% in comparison to glucose.[15] In cell proliferation studies—as good indicators of metabolic induction—the fructose containing medium could not result in a higher rate of growth, while media by containing glucose, mannose and galactose were more effective.[18] Characterization of the members of this group as chemorepellent and not metabolism inducer ones is further supported by their selection index values ($Ch_{rps} \approx 1$), which show that signalling by these sugars is assumed to be mediated by 'short-term' receptors.

The duality of nutritive (wide range effective) and signal moiety sugars (repellent ligands) seems to be very similar to the theory of initial phase of molecular phylogeny described by Lenhoff.[7] Previous studies had demonstrated that in the case of amino acids, there is a close correlation between chemotactic activity of amino acids and their consensus sequences in the primordial soup: those ones, which have appeared at first in phylogeny were chemoattractant (e.g., glycine, glutamate), while latter ones became repellent (e.g., phenylalanine, tyrosine).[3] According to our present results and their interpretations, this duality of attractant and repellent sugars could be also the result of an early, molecular level phylogenetical selection.

(iii) Non-effective, disaccharide sugars (sucrose, lactose and maltose) seem to belong to the third group tested. However, in two cases weak responses were also detected (sucrose—chemorepellent, maltose—chemoattractant) and we have to consider that Tetrahymena has also the ability to synthesize and release enzyme such as β-glucosidase (similar to maltase).[41] Therefore the concentration of these disaccharides is supposed to be very low in the close environment of Tetrahymena cells. Differences in chemoattractant effects of maltose [glucose-glucose]<glucose (138±25% <182±13%) and chemorepellent moieties of sucrose [glucose-fructose]<fructose (70±11%<63±5%) also support our theory concerning the significance of fast enzymatic cleavage of disaccharides. Another explanation of the low effectiveness of disaccharides in Tetrahymena is that the receptor mediated chemotactic response of sugars is specific as in prokaryotic e.g. *E. coli*, where monosaccharides and disaccharides possess distinct ligand carriers and membrane receptors.[27] Nevertheless, we cannot reject the possibility that Tetrahymena surface membrane is deficient in disaccharide receptors.

3.2. Motility induced by sugars

Motion analysis is a widely used method to evaluate responsiveness to signal molecules in protozoa. In Tetrahymena the varying types of swimming patterns (ethograms) and changes in swimming velocity provide the possibility to evaluate slight differences in the physical properties, e.g. temperature[42] and concentrations of signalling molecules, e.g. insulin or concanavalin A[43,44] of the environment. Sensibility in this kind of responsiveness is essential to be high. Even different taxa of Tetrahymena (e.g., *T. pyriformis* vs. *Tetrahymena malaccensis*) express identical behavioural patterns in trajectory, reorientation, sliding etc.[45] The above mentioned data explains also the fact that swimming behaviour has a close relationship to the dynamics of surface membrane receptors of Terahymena and the special molecular level 'memory' of these cells developed by pretreatments—imprinting—can also modify quality of ethograms for generations.[43]

The good matching between chemotaxis and the swimming velocities/tortuosity of the swimming paths underline the significance of our observation that even in sugars these migratory properties are closely related. Quantitative and qualitative gradual differentiation of the chemoattractant sugars by motility assays (mannose>galactose>glucose>maltose) confirm that these kinds of tests should be used as gold standards of chemotaxis research in the future.

3.3. Availability of sugars

Some results of our present study point to that availability of the sugars to be metabolized can really influence chemotaxis. The observed relation described between chemical characteristics determining internalization of the ligand (XLogP and TPSA) and the chemotaxis elicited support our theory that there is a close association between the readiness to join cytoplasmic metabolic processes and the chemoattractant behaviour of simple sugars.

If we suppose that sugars elicit or influence chemotactic responses via interactions with specific metabolic pathways, it is conceivable that the reason of their weak effects is that the cell metabolizes these molecules slowly, or these sugars cannot internalize into the cell within the time of incubation. The latter choice is supported by the good matching of chemical characters (XLogP, TPSA) and chemotaxis (sugars with chemotactic potency: TPSA<140 and XLogP>−3; chemotactically neutral sugar: TPSA>140 and XLogP<−3).

4. Conclusions

In conclusion, our chemotaxis studies supplemented by motion analysis call attention to that the chemotactic behaviour of Tetrahymena induced by mono- and disaccharides is also feasible to be

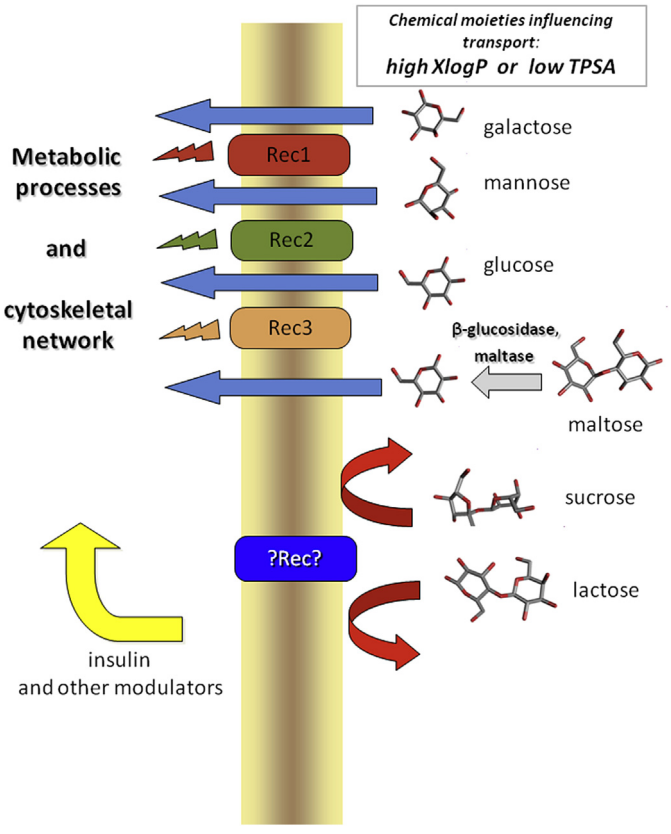

Fig. 5. Theory on the main pathways how chemotactically active sugars can elicit alone or in combination migratory responses in protozoa (Tetrahymena). Preferred values are: high XLogP and low TPSA. (Rec1, Rec2 …—supposed surface membrane receptors of sugars; lighting arrows—downstream signalling induced by sugars; linear arrows crossing surface membrane—transport of sugars; reflexed arrows—presumed as not membrane crossing sugars).

carried out by other means than receptor mediated mechanisms (e.g., internalization, extracellular enzymatic cleavage) (Fig. 5). As in chemotactic selection of the ciliate Tetrahymena, none of the examined sugars was a good selector except galactose, which refers to the weakness in 'long-term', permanent membrane receptors in general, but still permit the possible existence of functioning 'short-term' receptors. Nevertheless, examining the chemical characteristics of the sugars, we could conclude that physicochemical characteristics of the effective sugars are dominant, partition coefficient (XLogP) and topological polar surface area (TPSA) values shows that ligands fitting to the required ranges are able to pass the cell membrane easily, while chemotactically non-effective sugars require much longer time to complete this process. This correlation indicates a tight connection between chemotactic effects and internalization into the cytoplasm, which can also support the hypothesis describing positive relations between chemotaxis and induction of special metabolic processes. In wide range of effective sugars, we could find further evidence to the tight connection of chemotaxis and metabolism: the altered reactions in chemotactic range-fitting experiments and the starvation- and insulin-dependent chemotactic responses towards glucose also show that the physiological and metabolic states of the unicellular cell can largely affect chemotactical responsiveness induced by a carbohydrate.

Significance of chemotaxis elicited by simple sugars grows beyond signalling characteristics of our eukaryotic model Tetrahymena. References proved that sugars have basic cell physiological effects on higher levels up to human cells, e.g. they modulate responsiveness of human neutrophils in direct and indirect ways, too. Internalization of carbohydrates (glucose, fructose, sucrose) modulate phagocytic activity—a target reaction of chemotaxis—to engulf bacteria is a well known mechanism as well as that neutrophils synthesize and release series of sugarspecific lectins and in this way they indirectly regulate accessibility of extracellular sugars.[46] In spite of the well described and wide range of cellular activity of sugars, the molecular regulatory mechanisms are still unclear in several areas and chemotaxis belongs to this obscure group of cellular activities triggered by sugars. Therefore we call attention that XLogP and TPSA values—as determinants of intracellular aviability of sugars—could be significant regulators of chemotaxis in more levels in phylogeny.

5. Materials and methods

5.1. Cell culturing and chemicals

T. pyriformis GL cells were maintained in 0.1% yeast extract containing 1% Bacto tryptone (Difco, Michigan, USA). The cultures were grown at 28 °C; the investigated cultures were in the logarithmic phase (24 h) of growth. The sugars used in the chemotaxis assays were D-arabinose, D-glucose, D-mannose, D-galactose, D-glucosamine, N-acetyl D-galactosamine, sucrose, lactose (Reanal, Budapest, Hungary); maltose and D-fructose (Merck GmbH, Darmstadt, Germany).

5.2. Assay of chemotaxis

The chemotactic ability of Tetrahymena cells was evaluated using a two-chamber capillary chemotaxis assay.[4] Tips of an eight-channel-micropipette filled with tested sugars representing a concentration range have served as the inner chamber of the system. The outer chamber was a 96-well plate (Sarstedt 82.1581) filled with the model cells (10^4 cells/mL). The incubation time was 20 min. This relatively short time was advantageous for measuring pure, gradient directed chemotactic responses and prevented contamination from the randomly running chemokinetic responder cells.[33] The concentration dependence of chemotactic responses was determined in range 10^{-18}–10^{-6} M in general and in an extended range (10^{-18}–10^{-1} M) in case of glucose and mannose. In all experiments a concurrent run of pure medium served as controls. Each experiment was repeated 3 times in 5 parallels. After incubation the samples of the inner chamber containing the positive responder cells (cells responding to the ligand as chemoattractant) were fixed in 4% formaldehyde containing phosphate buffered saline (PBS, 0.05 M; pH=7.2). The number of the cells was determined using a Neubauer haemocytometer counting parallel samples from each run. Values were normalized to the control (fresh medium) and this value is given as 'Chemotaxis index' (Ch_{idx}) in percents.

5.3. Chemotactic selection

The technique is dedicated to evaluate the selector capacity of the ligands. The first step is a chemotaxis assay as described above. For this purpose the most effective chemoattractant concentration of the ligand was applied. In the control group, simple, fresh medium served as attractant. Following the assay, the positive responder cells were transferred to fresh culture medium and cultivated for 144 h with consecutive transfers at every 48 h. Prior to the re-assay the cells were transferred to fresh medium for 24 h. Then the two sub-populations (Tc/c—selected with control; Tc/s—selected with sugar) were re-assayed in the following combinations: the cells selected with control were tested with control medium (Tc/c→C) or the identical sugar (Tc/c→S), the cells selected with sugar were tested with control medium (Tc/s→C) or the identical sugar (Tc/s→S). The experiments were made in 8–8 parallels. To quantify the degree of selection potency of the sugars the values of '*Chemotactic selection index* (Ch_{sel})' were also calculated on the basis of the following formula.[33]

$$\mathrm{Ch_{sel}} = \frac{(\mathrm{Tc/s \rightarrow S})/(\mathrm{Tc/s \rightarrow C})}{(\mathrm{Tc/c \rightarrow S})/(\mathrm{Tc/c \rightarrow C})}$$

When value of Ch_{sel} is above 1.15 action of a 'long-term' receptor is supposed while under that we assume that receptors are working as 'short-term' type or some specific metabolic pathway is triggered independently of receptors.

5.4. Reciprocal selection

The classic type of chemotactic selection was used to evaluate chemoattractant ligand specific chemotactic receptors. The reciprocal type of selection was developed to allow us to evaluate selection for chemorepellent ligands. In this case the following four combinations were set in the first step of the assay: Tc/c—cells are maintained in their own medium and the applied inducer is also fresh medium; Tc/s—cells are maintained in their own medium while the applied inducer is the optimal concentration of the identical sugar solved in medium; Ts/c—cell culture is supplemented with the effective concentration of the identical sugar and the inducer is fresh medium; Ts/s—both the cell culture and the inducer substance are supplemented with the similar sugar in the same concentration solved in medium. The positive responder cells were transferred to fresh culture medium and were maintained for 144 h with consecutive transfers as it was done in the case of simple chemotaxis selection. In the re-assay the following 8 combinations were tested (all subpopulations were tested with plain medium—C or sugar solution containing fresh medium—S): Tc/c→C; Tc/c→S; Tc/s→C; Tc/s→S; Ts/c→C; Ts/c→S; Ts/s→C; Ts/s→S. In each combination 8 parallel samples were prepared. 'Reciprocal

chemotaxis index' (Ch_{rps}) was also calculated to evaluate results obtained numerically on the basis of the following formula:

$$Ch_{rps} = \frac{(Ts/c \rightarrow C)/(Ts/c \rightarrow S)}{(Ts/s \rightarrow C)/(Ts/s \rightarrow S)}$$

Interpretation of Ch_{rps} values was similar to the Ch_{sel} mentioned above.

5.5. Motion analysis

Samples of Tetrahymena cultures ($5x10^2$ cell/ml) were treated with the four chemotactically effective, attractant sugars in their most effective concentrations (galactose -10^{-11} M, glucose -10^{-7} M, maltose -10^{-9} M, mannose -10^{-7} M). Prior to the analysis, the cultures were treated with the sugars for 5 min. Tracks of the cells were recorded in Zeiss Observer A1 microscope. For evaluation of tracks, the Tracking module of AxioVision V4.7.1.0 was used. Parameters of recording were: Multidimensional Acquisition/Time lapse mode (interval: 'maximal speed'; duration: 5 s). In evaluation the 'auto detect' mode was used to select the cells for tracking, the sampling frequency was 25 frame/sample. Each data point represents evaluation of swimming behaviour of 100 cells/group. The mean velocity of cells and the tortuosity of the swimming tracks (the ratio of the distance of starting and end point of the path and the real length of the swimming path) were used to characterize the swimming behaviour.

5.6. Calculation of XLogP and TPSA values

XLogP is the partition coefficient that is a measure of differential solubility of a compound in two solvents. Values of octanol (hydrophobic)/water (hydrophilic) XLogP coefficient were calculated on the basis of Cheng publication[47] and was used to characterize our ligands. TPSA (topological polar surface area) is an estimate of the area (in $Å^2$), which is polar. Formula used a simple method to determine value of TPSA, only N and O are considered, 3D coordinates are not used, and there are various precomputed factors for different hybridizations, charges and participation in aromatic systems.[48] The used database of XLogP and TPSA was http://pubchem.ncbi.nlm.nih.gov.

5.7. Effect of starvation

Cells were isolated by a low-speed centrifugation (1 min on 100x g). Then the cells were transferred to Losina-Losinsky (LL)[49] starving solution composed of inorganic salts (0.01 g KCl, 0.01 g $MgCl_2$, 0.01 g $CaCl_2$, 0.1 g NaCl, and 0.2 g $NaHCO_3$ in 1000 mL solution) for 180 min (the generation time of *T. pyriformis* is 150 min). Then a regular chemotaxis assay was done, in this case, LL medium was used as a minimal medium of cells and sugars were also dissolved in LL medium. The experiment was done in 5 parallels.

5.8. Insulin treatment

Cell culture in logarithmic phase of growth was treated with 10^{-6} M insulin (Actrapid, Novo, Denmark) for 60 min, while an identical group of non-treated cells was also incubated as a control. After incubation cells were isolated by a low-speed centrifugation (1 min on 100×g) and the cells were transferred to fresh culture medium. Effect of insulin treatment was evaluated in two ways (i) a chemotaxis assay was carried out immediately after the treatment and isolation of the cells; (ii) insulin treated and the control cells were cultured for 12 h to get logarithmic phase cultures and then the chemotaxis assays were applied. In both cases 8–8 combinations of assays were done: control and insulin treated cultures were tested by medium as a control and by the effective concentrations of insulin (10^{-6} M) and glucose (10^{-7} and 10^{-10} M). The experiment was done in 5 parallels.

5.9. Statistical evaluation of data

The statistical analysis of data was done by inbuilt statistical routines of Origin Pro8.0 (OriginLab Corp, USA). Significance of chemotactic responses was analyzed by one-way ANOVA. Values of significance were represented in figures with $p<0.05$ threshold limit, while in tables this limit is $p<0.15$. On figures the error bars represent standard errors (±S.E.).

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References

1. Eisenbach M, Lengeler JW. *Chemotaxis*. UK: Imperial College Press; 2004.
2. Kőhidai L, Török K, Illyés E, Tamási J, Sebestyén F, Láng O, et al. *Cell Biol Int* 2003;**27**:695–700.
3. Kőhidai L, Láng O, Csaba G. *Cell Mol Biol* 2003;**49**:OL487–495.
4. Kőhidai L, Lemberkovits É, Csaba G. *Acta Protozool* 1995;**34**:181–5.
5. Kőhidai L, Csaba G. *Acta Microbiol Immunol Hung* 1996;**43**:83–91.
6. Csaba G, Kovács P. *Microbios* 1993;**76**:35–9.
7. Lenhoff HM. On the mechanism of action and evolution of receptors associated with feeding and digestion. In: Muscatine L, Lenhoff HM, editors. *Coelenterate Biology*. USA: Acedemic Press; 1974. p. 238–9.
8. Csaba G, Kőhidai L. *Acta Protozool* 1986;**25**:331–8.
9. Csaba G, Nagy SU. *Acta Biol Med Ger* 1976;**35**:1399–401.
10. Kovács P, Csaba G. *Acta Physiol Hung* 1987;**69**:167–79.
11. Kovács P, Csaba G. *Microbios* 1994;**81**:231–9.
12. Kőhidai L, Csaba G. *Acta Biol Hung* 1985;**36**:281–5.
13. Hellung-Larsen P, Leick V, Tommerup N. *Biol Bull* 1986;**170**:357–67.
14. Borowitz MJ, Stein RB, Blum JJ. *J Biol Chem* 1977;**252**:1589–605.
15. Stein RB, Blum JJ. *J. Biol Chem* 1979;**254**:10385–95.
16. Stein RB, Blum JJ. *J. Biol Chem* 1980;**256**:2752–60.
17. Stein RB, Blum JJ. *J. Biol Chem* 1980;**255**:4198–205.
18. Seaman GR. *J. Biol Chem* 1951;**191**:439–46.
19. Kaneshiro ES, Merkel SJ, Reuter SF. *Eur J Cell Biol* 1989;**49**:55–60.
20. Hoffmann EK, Rasmussen L, Zeuthen E. *J Cell Sci* 1974;**15**:403–6.
21. Rasmussen L, Hoffmann EK, Jørgensen B. *J Comp Physiol B* 1978;**125**:97–9.
22. Cirillo VP. *J. Bacteriol* 1962;**84**:754–8.
23. Csaba G, Kovács P, Kőhidai L. *Microbios* 1994;**80**:215–21.
24. Christensen ST, Guerra CF, Awan A, Wheatley DN, Satir P. *Curr Biol* 2003;**13**:R50–2.
25. Csaba G, Kovács P. *Cell Biol Int* 1995;**19**:1011–4.
26. Csaba G, Gaál A, Kovács P, Simon G, Kőhidai L. *Cell Biochem Funct* 1999;**17**:165–73.
27. Adler J, Hazelbauer GL, Dahl MM. *J. Bacteriol* 1973;**115**:824–47.
28. Hayakawa M, Ariizumi M, Yamazaki T, Nonomura H. *Actinomycetologica* 1995;**9**:152–63.
29. Fan KW, Vrijmoed LLP, Jones EB. *Mycologia* 2002;**94**:569–78.
30. Vogel T, Blake DA, Whikehart DR, Guo NH, Zabrenetzky VS, Roberts DD. *J Cell Physiol* 1993;**157**:359–66.
31. Kőhidai L, Csaba G. *Cytokine* 1998;**10**:481–6.
32. Kőhidai L, Tóth K, Ruskoaho H, Csaba G. *Cell Biol Internat* 2001;**25**:1173–7.
33. Kőhidai L, Schiess N, Csaba G. *Comp Biochem Physiol* 2000;**126C**:1–9.
34. Roberts CT, Orias E. *J Cell Biol* 1974;**62**:707–16.
35. Shrago E, Brech W, Templeton K. *J Biol Chem* 1961;**242**:4060–6.
36. Kőhidai L. *Acta Biol Hung* 1999;**50**:375–94.
37. Csaba G, Kovács P. *Acta Histochem* 1983;**73**:53–61.
38. Kovács P, Csaba G. *Acta Histochem* 1983;**73**:181–92.
39. Christopher GK, Sundermann CA. *Exp Cell Res* 1992;**201**:477–784.
40. Affolter M, Weijer CJ. *Dev Cell* 2005;**9**:19–34.
41. Müller MJ. *Cell Biol* 1972;**52**:478–87.
42. Goto M, Ohki K, Nozawa Y. *Biochim Biophys Acta–Biomembranes* 1982;**693**:335–40.
43. Mugnaini D, Ricci N, Banchetti R, Kovàcs P. *Cytobios* 1985;**81**:87–95.
44. Leick V, Iversen CG, Olesen JD, Bøg-Hansen TC. *Eur J Protistol* 2003;**39**:390–3.
45. Ricci N, Russo A, Banchetti R, Kovács P. *Cytobios* 1995;**83**:139–58.
46. Sanchez A, Reeser JL, Lau HS, Yahiku PY, Willard RE, McMillan PJ, et al. *Am J Clin Nutr* 1973;**26**:1180–4.
47. Cheng T, Zhao Y, Li X, Lin F, Xu Y, Zhang X, et al. *J Chem Inf Model* 2007;**47**:2140–8.
48. Ertl P, Rohde B, Selzer P. *J Med Chem* 2000;**43**:3714–7.
49. Losina-Losinsky LK. *Arch Protistenk* 1931;**74**:18–20.