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MICROBIOROBOTICS

Biologically Inspired Microscale Robotic Systems

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CHAPTER

Tetrahymena Pyriformis in Motion

3

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3.1 Introduction

Microbiorobotics embodies one of the best examples of interdisciplinarity developed in the last five years. Nanoscale to mesoscale engineering and chemistry provide the most significant elements of background sciences required to use biological entities as test objects representing the basic cellular level of organization. Artificial model particles are also available in experimental systems of microbiorobotics, such as liposomes; however, feasibility of prokaryotic and eukaryotic organisms is still very important. There is still a need for living unicellular objects as biological models providing a wide range of characteristics which are not endogenous moieties of purely chemical experimental models. The most important moieties of unicellular models of microbiorobotics are as follows: (i) open systems – they provide the possibility of monitoring internalization, transport processes, and release of native or artificial test substances; (ii) the surface membrane is furnished with receptors, the most significant signal converters of living organisms – in this way ligand dependent signaling processes are inducible, down-regulation of receptors can regulate responsiveness of

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Table 3.1 Representation of Protozoa as Model Cells in Research

Taxon	Number of References Using the Taxon as a Model Cell (Source: PubMed 2005–2010)
Dictyostelium	1117
Tetrahymena	569
Paramecium	292
Dunaliella	189
Euglena	149
Euplotes	77
Oxytricha	43
Vorticella	29
Stylonychium	24
Stentor	19
Dileptus	16
Blepharisma	13
Colpidium	9

the cell; (iii) intracellular compartmentalization – provides the possibility to target test particles and to induce only isolated parts/pathways in the cell; (iv) individual genetical matter – provides the possibility of selection experiments and tracking sub-populations as well as comparison consecutive generations following treatments.

As unicellular, eukaryotic protozoa complies in all respects to/with the above-mentioned requirements they are very frequently used model cells of experiments in genetics, biochemistry, cell biology, and cell physiology as well (Table 3.1).

As we can learn from the data of two model cells, the ameba *Dictyostelium* and the ciliate *Tetrahymena* are considered as the most explored protozoan objects with scientific values. The objective of the present chapter is to describe the ciliate member *Tetrahymena* and to give a coverage of its experimental value as a living test object among biologically inspired microscale robotic systems.

3.2 *Tetrahymena* as a model cell

Tetrahymena is one of the most frequently used unicellular, eukaryotic models in genetics, and molecular and cell biology. The cellular structure (e.g., membrane compartments, cytoskeletal network) and functional complexity (e.g., signaling pathways, metabolic processes) of these cells represents a good homology to higher ranked vertebrates. Two members of the taxon *Tetrahymena* proved to be the most appropriate in research: the amicronucleate *T. pyriformis*, which division shows a

non-conjugating, asexual form due to the lack of micronucleus, and *T. thermophila*, which shows a good example of sexual reproduction and provides a wide range of experiments interfering with micronucleus exchange between the partner cells.

The suitability of the model cell is supported by several molecular level homologies to higher ranked vertebrates like (i) identical receptor pools of the surface membrane (e.g., insulin receptor [1]) and cytoplasm (e.g., steroids [2]); (ii) similar elements of signaling pathways (e.g., cyclic nucleotide phosphates [3, 4], Ca^{2+} -calmodulin system [5], phosphatidylinositol metabolism [6]); (iii) homologous cell physiological responsiveness (e.g., chemotaxis [7], phagocytosis [8], proliferation [9], and metabolic processes [10]) induced by natural ligands (e.g., peptide hormones, chemokines, artificial signals – drugs); (iv) high-level sensitivity and molecular level distinctiveness are shown by diverse activity or structurally closely related molecules (e.g., bradykinins [11], crystalline and amorphous insulins [12]).

Investigations carried out on these model cells (*T. pyriformis* and *T. thermophila*) presented arguably the most scientific evidence among protozoa rewarded by the Nobel Prize. These cells served as models to *Christian de Duve* in first description of lysosomes and peroxisomes (Nobel Prize in Physiology or Medicine, shared with Albert Claude and George E. Palade, 1974) and to *Thomas R. Cech*, who has described the catalytic character of RNA (ribozyme – self-splicing), which was a fundamental to propose the new RNA world theory on the origin of life (Nobel Prize in Chemistry, shared with Sydney Altman, 1989). The third Nobel laureate working on *Tetrahymena* is *Elizabeth H. Blackburn*, who has described how chromosomes are protected by telomeres and characterized the enzyme telomerase (Nobel Prize in Physiology or Medicine, shared with Carol W. Greider and Jack W. Szostak, 2009).

One of today's hottest research area is epigenetics, the study of heritable changes in phenotype or investigations on different forms of gene expression caused by mechanisms other than changes in the underlying DNA sequence. However, few people realize that this research has started also in *Tetrahymena* cells in the late 1970s. György Csaba pioneered this research and his "hormonal imprinting" theory has suggested a ligand-dependent amplification of responsiveness detectable in several molecular and cell physiological levels [13, 14]. The well-documented (more than 60 papers in peer-reviewed journals) theory can be considered as the basis for epigenetics, as the effect of pretreatments was proven not only in *Tetrahymena* but also later on in more vertebrate models as well as the clinical significance of the phenomenon is also derived from the theory of hormonal imprinting.

Aside from the aforementioned scientific discoveries, there are many other significant biological processes or cell organelles in which more detailed description and characterization were carried out using the *Tetrahymena* model. The first cytoskeletal motor protein dynein was identified and their directional activity in ciliary beating was described in these cells [15]. *Tetrahymena* was the first cell which division was synchronized, and in this way, the cell cycle control mechanisms were studied in detail [16]. More nuclear mechanisms were also described at first in this

ciliate. Among others, molecular level characterization of somatic genome rearrangement [17], the role of RNA interference-like pathway in heterochromatin formation [18], and the function of histone acetylation [19] were all discovered in this cell. In addition, some protein synthesis related processes like physiological roles of the post-translational modification (acetylation and glycylation) of tubulins [20] and description of the crystalline structure of the 40S ribosome subunit in complex with the initiation factor eIF1 [21] were also described at first in *Tetrahymena*.

The ability of cells to regulate themselves or the adjacent neighboring cells by their products (auto- and paracrine activity) appears to belong to the most essential cellular processes. It was described also in some bacteria; however, *Tetrahymena* shows a surprising richness in this kind of endogenous materials. In the last decade, production of substances typical of regulatory molecules of mammals has been reported in ciliates. In *Tetrahymena*, a wide range of biomodulators are present and released, among others biogenic amines: histamine [22], serotonin [23]; peptide hormones: insulin [24], ACTH [25], relaxin [26], endothelin-1 [27]; cytokine: IL-6 [28], and other bioregulators like melatonin [29]. The role of these well known substances is only hypothetic. Some researchers see the early appearance of these substances in phylogeny as evidences of their fundamental genetic and evolutionary significance, while others consider these signal molecules as early "attempts" in phylogenesis or simply errors attributed to the early appearance of these molecules. (A more detailed summary of characterization of *Tetrahymena* genus is available as an appendix of a concept paper was submitted to the Trans-NIH NonMammalian Models Committee in 2001.)

Some practical properties are also advantageous to researchers. The size of the cells ($20 \times 50\,\mu m$) provides the possibility close to macroscopic interventions (e.g., electrode insertions, manipulations on the nucleus). The short generation time (about 150 min) is very advantageous as several generations could be tested within a relatively short period of time (in *Tetrahymena* 1 day = 10 cell cycles, which means that 1 week = 70 generations; the 70 generation period is identical to 3500 years in the human lifespan scale, considering 50 year/generations). Growth conditions of *Tetrahymena* cultures possess decisive importance for the design of experiments. There are several types of growth media (e.g., inorganic "starving" medium or microbiologically determined medium); however, the chemically defined media provide the best choice, a well determined environment of culturing and experiments. Fast and easy handling, as well as the potential of accurate application of test substances, supports using *Tetrahymena* cells not only as an advantageous model of biology and medicine but also as a good candidate of microbiorobotics.

The characteristics described above present the opportunity to consider the *Tetrahymena* model more than a good target of biological assays. The well-compartmentalized internal structure and highly organized biochemical pathways provide the possibility to design and understand information transfer between the environment and *Tetrahymena* by the basic models of information transfer used also in biorobotics (Fig. 3.1).

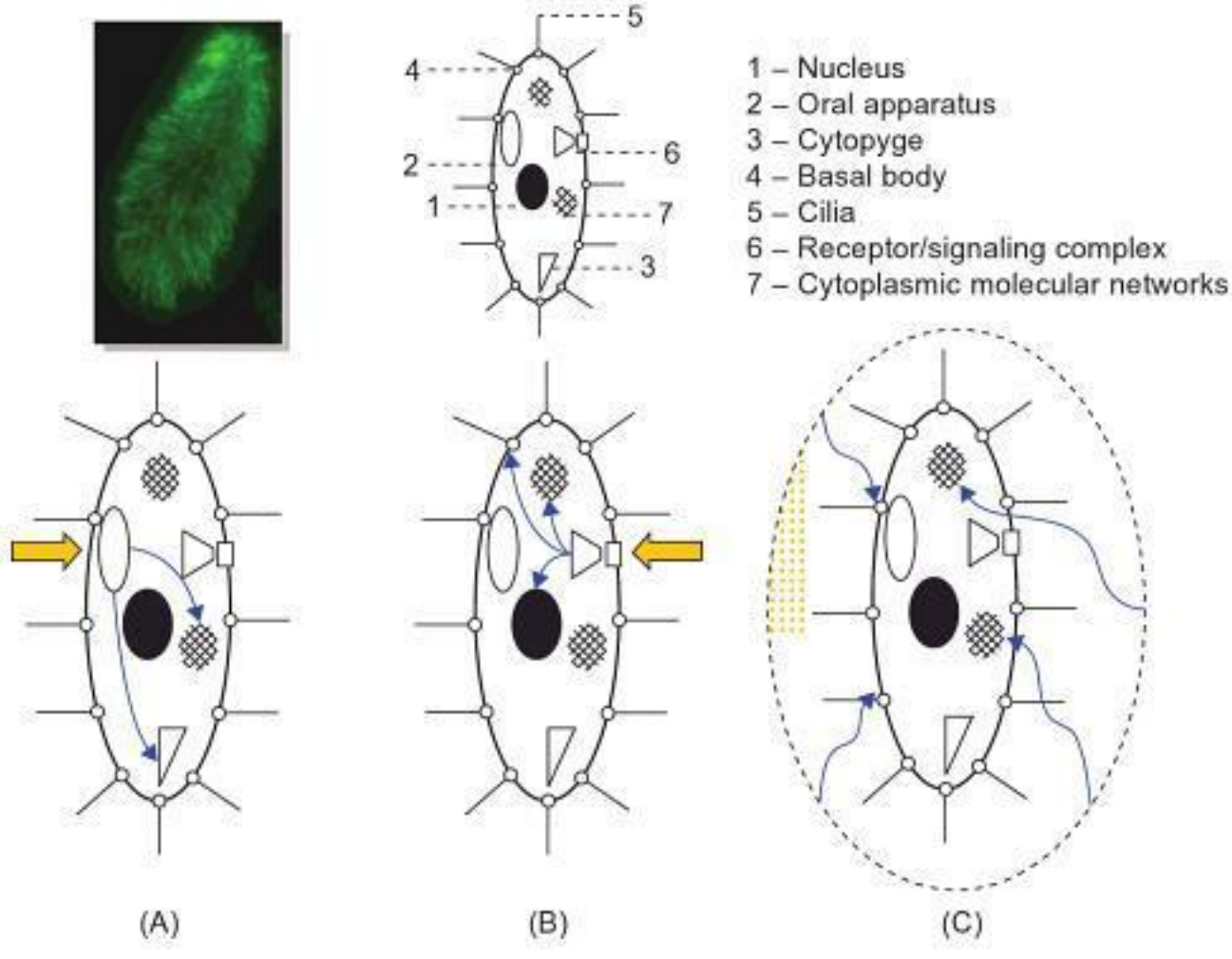


FIGURE 3.1

Environmental stimuli acting via main intervening pathways of microbiorobotics in *Tetrahymena*. Top panel: structure of *Tetrahymena* surface detected by FITC-insulin (left) and main structural targets of the model cell (right). Bottom panel: main pathways to modulate *Tetrahymena*. (A) Nutritional tract linked; (B) Surface receptor linked; (C) Physical filed linked.

As we can see on the top panel of Fig. 3.1, there are several structural targets of the ciliate (e.g., oral apparatus, receptors, cilia) which form also a functional intracellular network. There are two chief classical ways to modulate *Tetrahymena*. Via oral apparatus, natural and artificial substances are internalized and can modulate intracellular pathways by the interaction of cytoplasmic molecular networks (e.g., metabolic processes, cytoskeleton) (Fig. 3.1A). Following an action characteristic lag, the excess or remnants of these substances are released on one of the most physiological ways, the cytopyge. The second possibility to interact with these cells represents a more molecular level (Fig. 3.1B). Surface membrane-receptors are the most significant signal receivers and transformers which can modulate a wide range of intracellular processes. In migratory responses, phosphatidylinositol 3-kinase (PI3K) is one of the key enzymes triggered by several receptors [30]. This enzyme can modulate gene level activities via ERK/MAPK pathways, while other intracellular targets (e.g., cytoskeleton or basal bodies of cilia) are also controlled by PI3K dependent processes

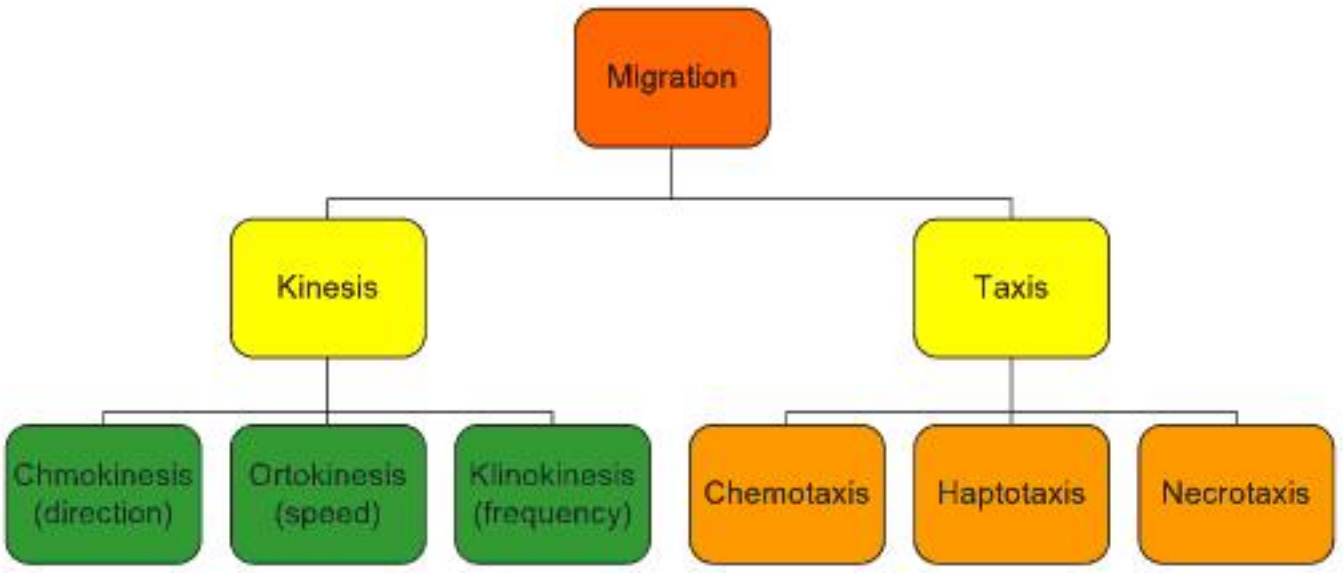


FIGURE 3.2

Most fundamental forms of migratory responses described in prokaryotic and eukaryotic organisms.

(e.g., Ras-MEK). In addition to these two main signaling mechanisms, we have to distinguish a third, which is performed by pure physical effects (e.g., magnetic fields or lighting) or by interactions between these physical effects and the biochemical networks/pathways (Fig. 3.1C). This latter group of exposures is probably the most widely used in applications of microbiorobotics in *Tetrahymena*.

3.3 Migratory responses in biology

Migratory responses belong to the most ancestral cell physiological reactions that have developed as prerequisites of survival of basic cellular organization during phylogeny. The migratory ability of these early cells has not constituted selective advantage itself; however, the primitive forms of cell surface receptors have already made possible to take advantage of the two most essential cell physiological functions. They were able to detect food molecules which were present in the environment and generated concentration gradients and whether there were toxic or other dangerous substances in the environment, they were also detected by sensitive relays which have prevented these cells by induction of movement away from the dangerous concentration zones. The two activities described above were conserved, and they are present in the entire cross section of phylogenetic tree as chemoattractants, chemorepellents (advantageous and disadvantageous), ligands, and reactions.

Quality of the ligand is very crucial considering the migratory responses elicited; however, more other aspects are also available to characterize the motion of prokaryotic and eukaryotic cells (Fig. 3.2).

Kinesis and *taxis* are the most fundamental forms of active motion. The most significant difference between the two forms is that while in kinesis, some elements of

cell motility (direction, speed, and frequency) are changed in random, non-vectorial ways, the taxis is composed by vectorial changes of the motion. While some types of migration are characteristic to prokaryotes (e.g., orto- and klinokinesis), there are more general types which were described in pro- and eukaryotes as well. The most frequently studied forms of these general forms are *chemotaxis* and *haptotaxis*. Both forms are induced by chemical stimuli. The significant difference between the two forms is that in chemotaxis, the concentration gradient of the chemoattractant or chemorepellent ligand develops in the fluid phase, while in haptotaxis, biological structures (e.g., surface membrane of endothel or extracellular matrix) provide the surface to develop the gradient of surface-bound ligands. Term chemotaxis is still used more frequently to classify swimming behavior of unicellular organisms or cells migrating in multicellular levels; however, it is getting more and more accepted that majority of cells in tissue level organization prefer movement by haptotaxis in contrast chemotaxis. A special type of migration *necrotaxis* requires mentioning due to its wide range effect at unicellular and multicellular levels. In this case, the quality of ligands is rather complex as they are released from necrotic or apoptotic cells. In ciliates, e.g., *Terahymena, Paramecium*, necrotaxis was also elicited by introduction of laser beam which could disintegrate the surface membrane. As a result of disturbing the membrane, cytoplasmic substances could work as chemoattractants or chemorepellents on cells belonging to the same taxon due to induction of specific or nonspecific ion channels of chemoreceptors [31]. As the type and life cycle of the cells are varying on a wide-scale quality and quantity of substances released are also very diverse; however, the main biological significance of necrotaxis is to promote clearing and restoration of microenvironment of cells.

Besides the aforementioned forms of migration, cells may be also triggered by a list of other external influences. As Fig. 3.3 summarizes, gravity (geotaxis/gravitaxis), mechanical (tigmotaxis), light (phototaxis), magnetic (magnetotaxis), electric (electro- and galvanotaxis), heat (thermotaxis), and the above discussed chemical (chemotaxis) signals are considered as the most significant inducers of migratory behavior of cells. Nevertheless, there are some other effects, like moisture (hydrotaxis), oxygen (aerotaxis), pressure (barotaxis), flow of fluid (rheotaxis), sound (phonotaxis) etc., which can also induce or complement migratory responses.

Tetrahymena is considered as one of the most accepted models in experiments on migratory behavior of cells. Theoretically, this positive rating is supported by the fact that chemotaxis and chemokinesis are belonging to the most essential, physiological responses of these ciliates; however, it is a good model of other migration-based assays like phototaxis, galvanotaxis, or gravitaxis. The well-developed signaling system of *Tetrahymena* provides specific responses to administration of different types of stimuli and molecules, too. Its swimming apparatus is also highly developed. The ordered beating of about 600 cilia (regulated by a collated work of basal body complexes) makes these cells able to develop rapid and tinctured swimming responses. Experiments have proven that there is a long list of chemotactic ligands and the sensibility of *Tetrahymena* toward these groups of molecules is exceptional. Also, there are several chemoattractant and chemorepellent ligands which molecules are

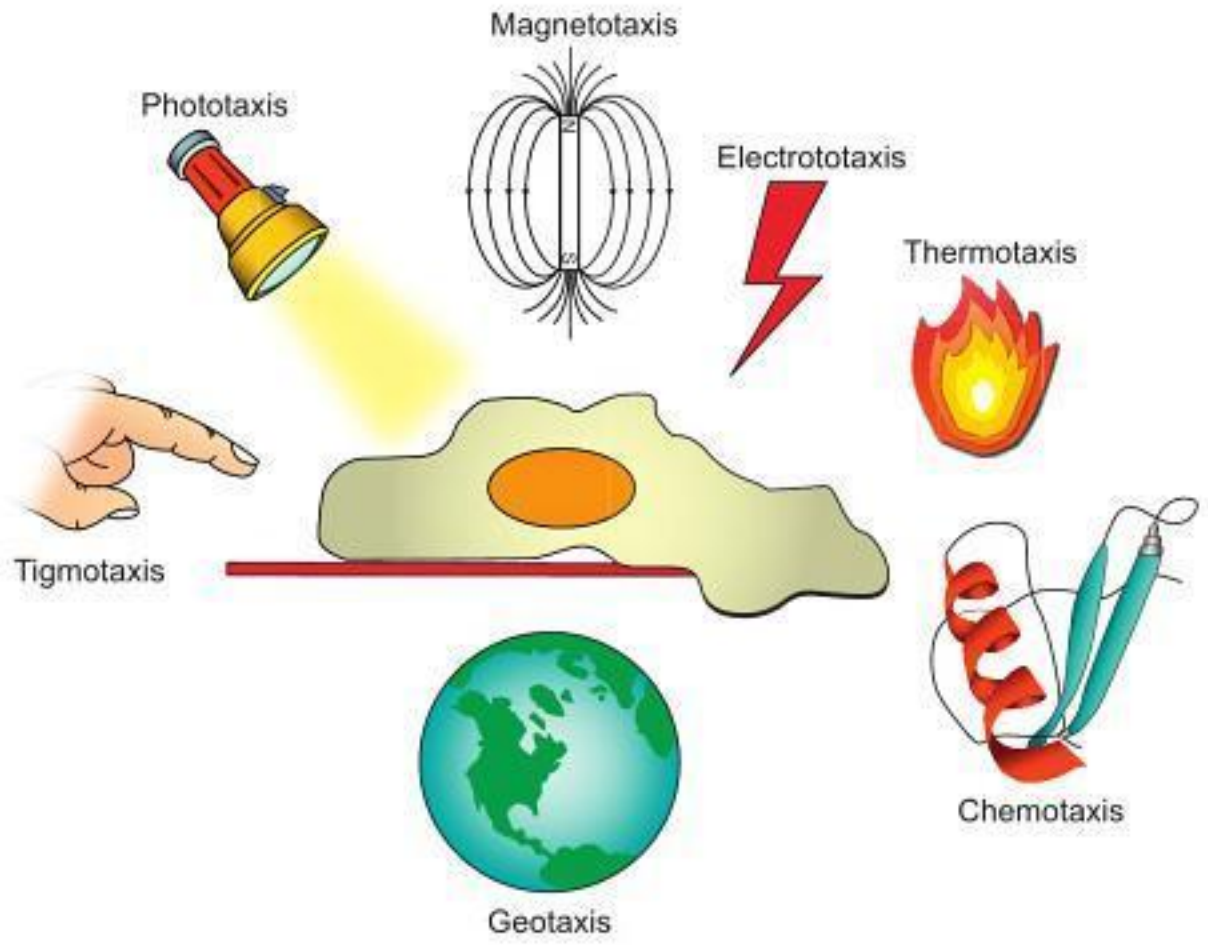


FIGURE 3.3
Main types of migratory responses of cells elicited by external stimuli.

acting similarly in vertebrates and *Tetrahymena*. Amino acids [32], oligopeptides [7], chemokines [33], lectins [34], or volatile oils [35] were detected with a high chemotactic sensitivity by these unicellular organisms. A high-level distinctiveness between molecular structures was also recorded as slight molecular differences of proline containing dipeptides [36] or chemical conditions of crystalline and amorphous insulins [12] were detectable in the chemotactic behavior of these cells.

All of these experimental data gained on *Tetrahymena* point to that molecular backgrounds of induction swimming behavior could be very well conserved on the side of ligand and chemotaxis receptors too. A strong support of this hypothetical analysis was the comparative study of consensus sequences of amino acids as a metric tool of molecular phylogeny of these essential building blocks of structure and signal molecules in unicellular organisms, and the chemotactic responsiveness of *T. pyriformis* as a representative unicellular fossil of the process studied. As shown in Fig. 3.4, there was a close relationship between the appearance of chemoattractant amino acids (Gly, Glu, Pro, Ala) and their chemotaxis inducer ability, which suggests that the inducibility of chemotaxis was one of the most significant and fundamental functions, even at the very initial phase of biomolecular evolution [32].

More evidence which supports that there are highly conserved mechanisms concerning chemotactic signaling is that the optimal chemotactic concentrations of

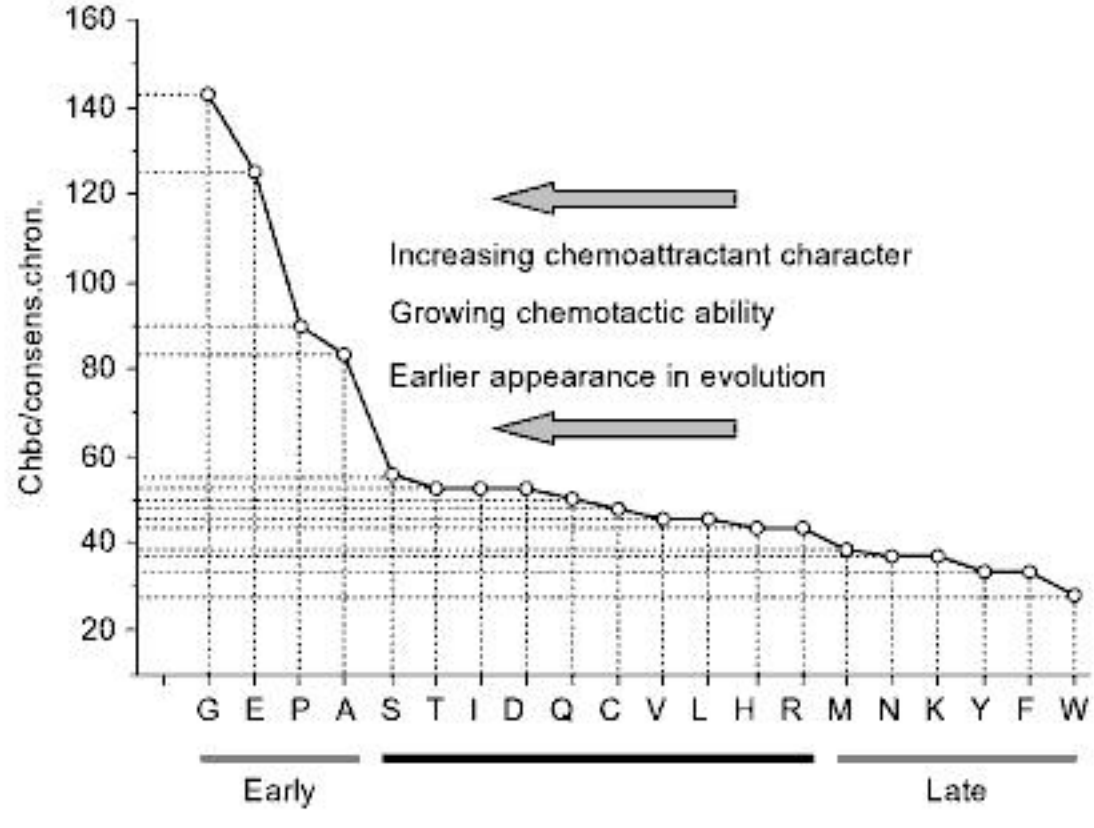


FIGURE 3.4

Correlation of phylogenetical appearance of amino acids in primordial soup and their chemotactic activity in *Tetrahymena pyriformis* GL.

vertebrate type signal molecules (e.g., vasoactive peptides, pituitary hormones) in *Tetrahymena* chemotaxis are identical to the concentrations (range, 10^{-12}–10^{-9} M) measured in the circulatory system of vertebrates/human [34]. These data also support the possibility that the non-professional chemoattractant substances (e.g., peptide hormones and cyclodextrin carrier associated steroid hormones) may be capable of a wide range of induction of chemotaxis, although this has yet to be investigated.

3.4 Specific signaling pathways

It is obvious that majority of the aforementioned migratory responses of *Tetrahymena* is elicited via specific signaling pathways. Investigation of these processes was more detailed in the case of chemotaxis, where groups of professional signal molecules were identified. Among others, formyl methionine containing di-, tri-, or oligopeptides (e.g., fMLF) [37], derivatives of soluble cytokine receptors (SEWS) [38], and chemokines (e.g., IL-8) [33] proved to be strong chemoattractants, while aromatic amino acids (Phe, Tyr, Trp) [32] or some biogenic amines (e.g., serotonin) [39] are referred to as chemorepellents in *Tetrahymena* cells. High sensibility of *Tetrahymena* chemotaxis receptors was demonstrated also by species-alien pheromones (Er-1 and Er-2) of *Euplotes raikovii* which molecules are structurally close homologues and thought to act via highly specific receptors. While Er-1 had a wide range

(10^{-12}–10^{-6} M) chemorepellent effect, Er-2 elicited characteristic chemoattractant responses [40]. Relevant responsiveness to other pheromones (tricosene and bornyl acetate) was also previously detected by Csaba and Kovács [41].

A wide range of other, non-professional chemotaxis inducer ligands were described in *Tetrahymena*. Inorganic ions [42], amino acids [32], as well as oligopeptides [43], volatile oils [35], insulin [12], IL-6 [28], melatonin [44], and vasoactive peptides (e.g., atrial natriuretic peptide [45] and endothelins [46]) are working as chemotactic substances. High chemotactic sensitivity of *Tetrahymena* was proved by slight modifications, i.e., amidation or formylation of the ligands, resulting an altered signaling character embodied in migration [47]. In respect of practical significance of chemotactic moieties, it should be mentioned that some dyes and secretagogues were referred as chemorepellent in ciliate. Alcian blue, cibacron blue, and oxidant NBT were found to be repellent in a wide concentration range in *Tetrahymena*; nevertheless, this activity was not accompanied with cytotoxic effects [48].

In general, the most frequently described signaling pathways are starting from the trimer G-protein linked membrane-receptors. The cytoplasmic transmitters are cascades of cyclic nucleotides (cAMP, cGMP) and protein kinases (PKA, PKG), while the cytoskeleton (e.g., basal body of cilia) specific end-point activation is carried out by a variety of enzymes (Fig. 3.5A). Over the above described "classical" way of signaling, the ciliary membrane itself and the sub-membranous molecular complexes including basal bodies represent a motion specialized functional unit (Fig. 3.5B). First signal transmitters of transmembrane receptors of cilia are cAMP, cGMP, and Ca^{2+}; however, receptors can induce tyrosine phosphorylation cascade in a direct way too. Next level signaling targets are protein kinases that have also a significant role in determining the direction of swimming (e.g., PKA – forward swimming, PKG – backward swimming). In the group of implementer proteins, the dominant members are centrin, calmodulin, TCBP 23/25, kinesins (Kin1/2/5), and dyneins. The presence of molecular links in signaling between receptors and motor proteins of cilia provides the possibility of orchestrated interaction of cytoskeletal and signal transmitter proteins [49–51].

As previously mentioned, receptors and the downstream signaling network, identical to those of vertebrates, were demonstrated by tools of molecular genetics as significant in evolving chemotactic responses of *Tetrahymena*. Membrane-receptors of insulin [52] or formyl-peptides [37], the inducibility of second messenger systems as cAMP [53], Ca^{2+}-calmodulin [54], or IP3 [55] and the metabolic pathways [10, 56] are all adequate to the higher ranks of phylogeny. Rapid and structure dependent chemotactic effects elicited by water soluble steroids (e.g., testosterone or progesterone) were also demonstrated in this model cell, in which data has proven the chemotactic moiety of steroid membrane-receptors for the first time in literature [57].

While sensitive screening of the environment and chemotaxis is one of the most essential reactions of the free-swimming cells, the chemotactic responses of these ciliate model cells are considered rather physiological, too. These reactions are triggered not only by substances listed above but other inorganic and organic parameters were

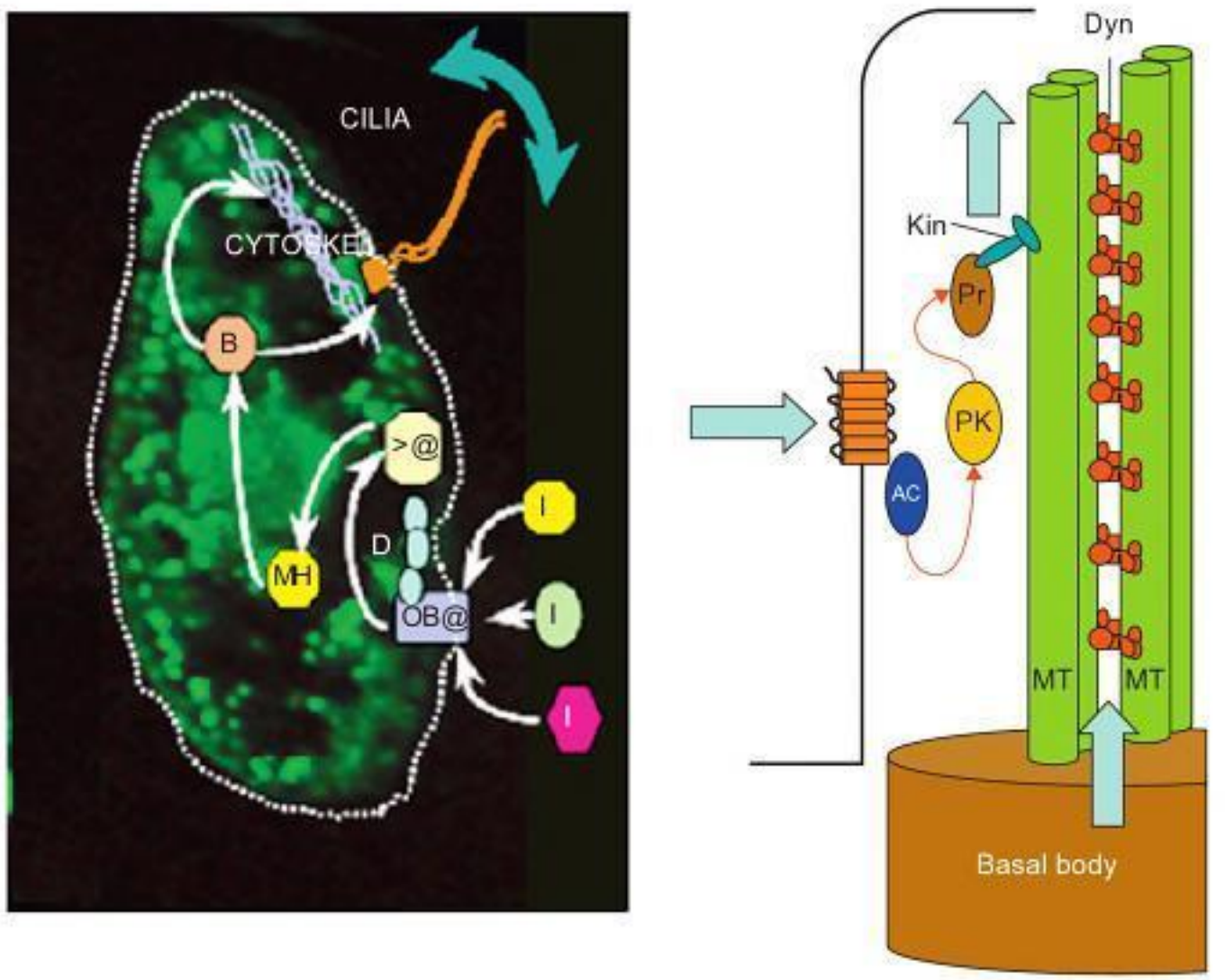


FIGURE 3.5

Main signaling pathways in *Tetrahymena* swimming. A – ligand (L), receptor (REC), trimer G-protein (G), adenyl cyclase (AC), protein kinases (PK), modulator protein(Pr), target enzymes (E); B – microtubule (MT), kinesin (Kin), dynein (Dyn).

also reported as modulators of migratory responses in *Tetrahymena* and other ciliates. Availability of nutrients, cell density, ATP/ADP ratio, pH, and oxygen tension are the factors influencing the amplitude of chemokinetic behavior in *Tetrahymena*, although its chemokinetic responsiveness proved to be a constant quality [58].

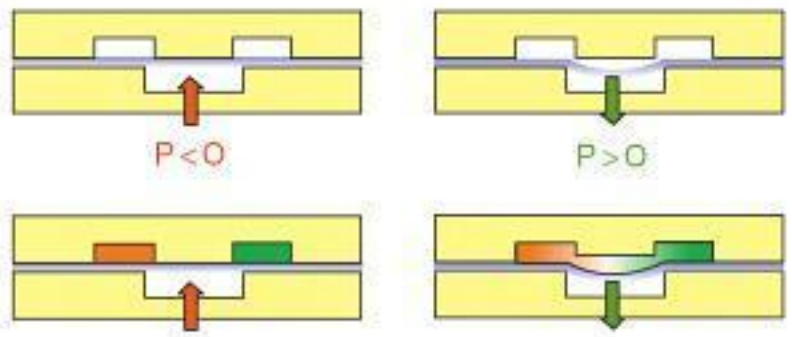
3.5 Microbiorobotics in *Tetrahymena*

The toolbox of microbiorobotics has shown a spectacular gain in the recent years. Microneedle, optical and magnetic tweezers, magnetic twisting cytometry, atomic and high-resolution force microscopy, and a wide variety of tracking techniques were developed to characterize cell and molecular mechanics [59]. The majority of the techniques listed above are dedicated to test individual cells or cell cultures growing on surfaces and it is hard to adapt them to free-swimming cells like bacteria or

Tetrahymena. Nevertheless, data in literature show that following certain scientific and technical considerations, it is possible to apply also these cell types.

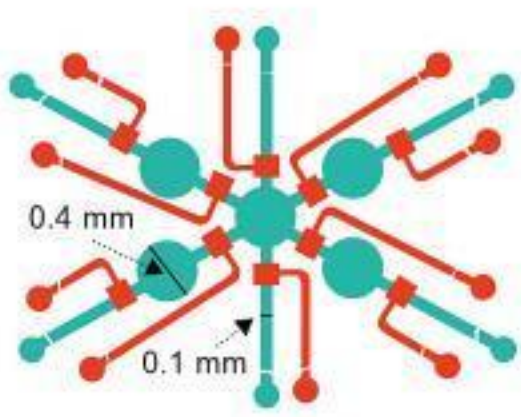
In the case of chemotaxis or other migratory responses elicited by chemical signals, microrobotics provides also a wide scale of tools. Among others PDMS-based biological sensor platforms are most frequently used. The variety of these platforms is very large and a detailed description exceeds the volume of the present chapter. Therefore, only one of the most imaginative tool the pneumatic valve-controlled device and choices of its development will be described here (Fig. 3.6A).

As the upper panel of the figure shows the main element of the device is the pneumatically controlled valve that connects two microchambers filled with cells and the test substance. While microchambers are also connected to containers of solvent and test substance via valve operated junctions, increasing or decreasing profiles



A well-controlled concentration gradient develops between the two chambers connected by pneumatic valves

(A)



(B)

FIGURE 3.6

Application of pneumatic valves in high-tech chemotaxis assay systems. (A) Operation of pneumatic valves in preformed channels of PDMS; (B) Potential applications (design and complete system) of pneumatic valve operated multichannels for detection responsiveness of more cell cultures or testing more substances at the same time (Figure and design in panel B are presented on the basis of collaboration between Nam, S. and Park, S. – Division NanoSciences, EHWA Womans University, Seoul, Republic of Korea and Kőhidai, L. – Department Genetics, Cell & Immunobiology, Semmelweis University, Budapest, Hungary).

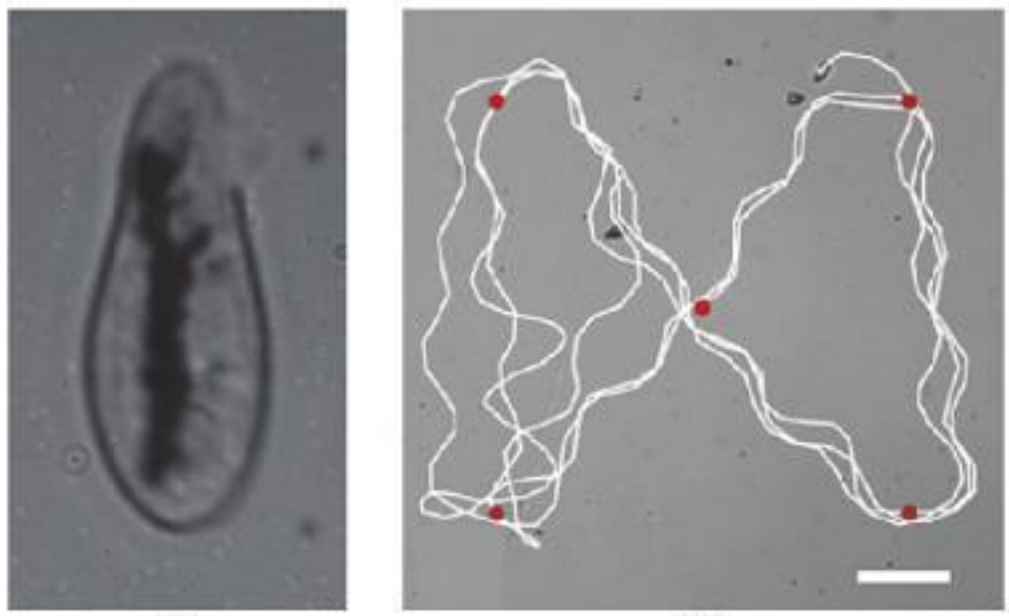
of concentration gradients are developed and controlled easily. The time dependent (2–60 min) stability of the gradient shows that the device is comparable to the other assays/arrays used in chemotaxis research. Sensitivity of the technique is shown by the chemotactic responsiveness of *Tetrahymena* detected even at very low concentrations (10 pM Gly-Pro) by this technique [60], while in other setups, the effect of the chemoattractant N-methyl-D-aspartate and the blocker effects on PLC and PI3K has proven the usability of the new chemotaxis chip [61]. Single and multiple setups (on the theoretical basis of PP-chamber) of this kind of valve-controlled systems are available, which makes it possible to test chemotactic responsiveness of different cell cultures or chemoattractant moiety of more substances/concentrations in parallel (Fig. 3.6B).

Investigations on bacteria (e.g., *S. marcescens* or *E. coli*) had a pioneering role in the introduction of *Tetrahymena* to this field of research. Fabrication of microdevices/microbarges covered with whole bacterial cells or cell fragments furnished with flagellum (microstructures) can be used as mechanical actuators. Results showed that even relatively large particles are controllable and environmental stimuli like magnetic fields or UV light can guide them on longer paths or time periods [62]. The technique described above was also applied to cover walls of PDMS microchannels with "bacterial carpets." In a low-Reynolds number fluidic environment, these surfaces furnished with active beating flagella were used to develop bacteria powered pumps (linear microchannels) or mixing performance (Y-junction microchannels), which were sensible to the chemical (e.g., glucose, pH, oxygen) or thermal (in range 15°C–35°C) modifications of the environment [63, 64].

To develop pumps of mixing performances using surface fixed *Tetrahymena* cells or ghosts theoretically also given; however, this kind of channel was not reported until now due to some technical difficulties, e.g., it is hard to attach cells to the wall. In other areas, nevertheless, swimming of *Tetrahymena* cells was successfully guided with different extracellular effects. One of the most spectacular phenomena is the galvanotaxis induced by using parallel carbon electrodes connected to a DC power supply. While in no electric field (resting phase), the swimming pattern of cells was random, application of 5 V/cm electric field could induce the unidirectional swimming toward the cathode in the majority of cells. Guiding cells with the polarity of the electric field is very effective in the case of *Tetrahymena* as repetitive inversion of the anode and the cathode resulted in an immediate change in swimming direction. Evaluation of swimming shows also that there is no significant change in the swimming velocity (400–450 μm/s) compared to that of the resting phase. *Tetrahymena*'s responsiveness to changes in the polarity of the electric field is fast and closely aligned at 180°, which should be the result of well-organized synergism of opposite forces generated by hundreds of peritrich cilia covering the cell. In molecular levels, this kind of responsiveness is produced by hyperpolarization and activation of voltage-dependent Ca^{2+} channels on the anodal part and ciliary reversal resulted from the depolarization and activation of voltage-dependent K^+-channels of the cell. As cilia of cells responds diversely to currents flowing into (slow down) and flowing out (speed up) which results a self-aligning torque in linear swimming.

Similar to magnetic twisting cytometry, application of ferromagnetic nanoparticles provides the possibility for magnetotactic motion control in *Tetrahymena* too [65]. In this case, *Tetrahymena* is loaded by magnetite particles via physiological pathways (see Fig. 3.1A). The particles are stored in the cytoplasm in membrane bound condition for a long time ($t > 10$ min), then they are magnetized by a rectangular magnet with a surface field 1964 G/1 min. There is no detectable interference of treatment with physiological activity of cells; however, 1 h after magnetization, the magnetic dipoles developed in particles is still present and distribution of vesicles loaded with particles shows a characteristic linear arrangement in the cytoplasm. There are more ways of magnetotactic motion control of cells. Similar to galvanotaxis discussed above, the application of magnetic field (2 mT) along "*x*" or "*y*" axes can determine swimming direction of cells. A significant difference to galvanotaxis is that, in this case, the velocity of cells could significantly exceed the average values (786 μm/s vs. 400 μm/s), which shows that this kind of environmental control can increase swimming characteristics without any fatal effect. There are much more complex movement patterns, which could be forced to make by *Tetrahymena* loaded by the magnetic particles (Fig. 3.7). Real-time feedback control using a tracking algorithm was used to determine the path through five sequential points. As Fig. 3.7 shows, *Tetrahymena* cells were repeatedly directed through a five-point pattern. The cells' velocity (448 μm/s) was more easily controlled than the aforementioned linear swimming. These results described above were the first proving *Tetrahymena* as a good candidate of unicellular microrobot guided by magnetotactic influences.

Another choice to interfere with migratory behavior of ciliates is when light or schedules of lighting are applied. Elicited responses are phototaxis, photokinesis in



(A) (B)

FIGURE 3.7

Magnetotactic control of *Tetrahymena* swimming. (A) Cells loaded with magnetite (50 nm iron oxide) particles and magnetized for 1 min; (B) Magnetotactic feedback control of swimming *Tetrahymena* (The scale bar is 250 μm).

the case of respecting the light and photophobicity resulting stops or reverse motion. In some protozoa, photoreceptor molecules were described like rhodopsin (*Fabrea salina*), blepharismin (*Blepharisma japonicum*), or stentorin (*Stentor coeruleus*). *Tetrahymena* has no such preformed structures for light detection; however, there are more evidences about its light sensibility: (i) some porphyrin-like end-products of its lipid metabolism can influence the fluidity of surface membrane [66]; (ii) membrane receptors, e.g., insulin have lighting dependent responsiveness [67]; (iii) synthesis and release of bioactive molecules, e.g., melatonin also make *Tetrahymena* sensible to lighting conditions [29].

Here, two recent results are discussed about phototactic responses of *Tetrahymena*. A phototaxis-like phenomenon described by Kim and et al. [68] documents that a white light composed of wide range varying intensities and wavelengths of 250–700 nm was applied as a spot light ($\emptyset \sim 100\,\mu m$) to illuminate *Tetrahymena* cells cultured in a Petri-dish. The short exposure time ($t < 10\,s$) had a capturing effect on the majority of individual cells which was accompanied by a short-term, in-plane counterclockwise rotational motion. This transient motion pattern was followed by the typical corkscrew swimming and resulted the escape of *Tetrahymena* from the light-spot. The detailed explanation of the phenomenon is not yet known; nevertheless, there are more possible explanations. As capturing phenomenon was observed only in a low wavelength range, it is possible that the UV components of light have a significant role as an inducer. It is also conceivable that a rapid change in the phospholipid metabolism takes place in which products are acting as regulators of membrane potential. This transient change in membrane potential results the changes in synchrony of ciliary beating. We should also consider the possibility that slight thermal effects of illumination are responsible for the transient change of the swimming behavior.

The new lighting equipment (*Cell-LED*®, Hungary) was also applied to analyze phototactic responsiveness in *Tetrahymena*. The equipment provides the possibility to test the effect of illumination, generated by LED lamps ($\forall = 15°$) in the range of 400–660 nm, on the motion of Tertahymena. Because the system is vertical, the setup of experiments allows us to evaluate both positive and negative phototactic responses of cells. As Fig. 3.8 shows, in a setup testing photophobic effects of light (bottom-up illumination) both UV (400 nm) and longer wavelengths (586 and 660 nm) light have photophobic effects on *Tetrahymena*. In contrast, the 465 nm wavelength light proved to be phototactic. These results confirm the assumption about the wavelength dependent induction or inhibition of metabolic processes of the light. Since the cell size shows a good correlation with the actual state of the cell cycle, it seems remarkable that, among high-responders, we could distinguish more distinct populations (e.g., 400 or 465 nm), indicating that cells representing different stages of the cell cycle (S or post-M phases) show different responsiveness to the light [69].

Concluding this part of the chapter, there is a novel way of evaluation the migratory moiety of *Tetrahymena* which has a close relation to the surface-linked migratory responses (e.g., ameboid movement). It is well known that ciliates, including *Tetrahymena*, have no surface dependent migration in classical way. However, these cells

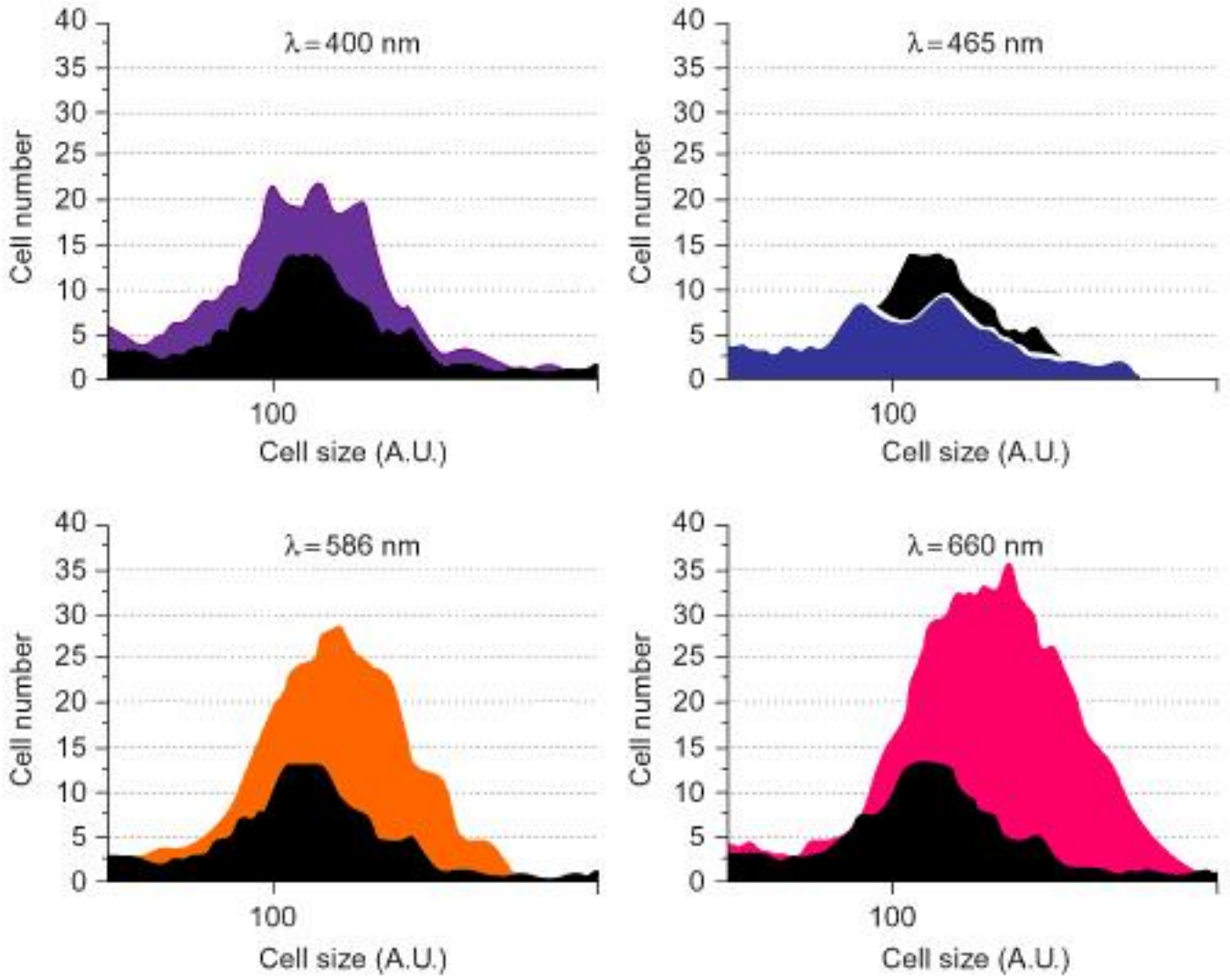


FIGURE 3.8

Phototactic responses of *Tetrahymena* induced by different wavelengths of illumination generated in Cell-LED®. Histograms shown in black represent responsiveness of *Tetrahymena* cultures without illumination [69].

develop transient connections with the bottom or other surfaces as they are covered with biofilms of bacteria or other advantageous substances to the ciliates. This temporary connection to the surface and moving on it are the creeping which is followed in consecutive cycles by swimming phases. Evaluation and measurement of this activity of cells were almost impossible due to the absence of proper equipment. The impedancemetry (electric cell-substrate impedance sensing – ECIS)-based new equipment [70] has opened new opportunities on this field. It provides a real-time measurement of transient and standard contacts developed between cells and the measuring electrode. As cells are essentially insulators, their adhesion to the electrode is measured as an increase of impedance (Z) and the value of Z has a good correlation with the total number attached at the moment. Sensing parameters of ECIS (Applied BioPhysics, USA) are high enough to detect not only cells attached to the electrodes (e.g., monocyte, fibroblast) but also their micro-motions, and, in the case of *Tetrahymena*, the creeping of cells is also recorded (Fig. 3.9) [71].

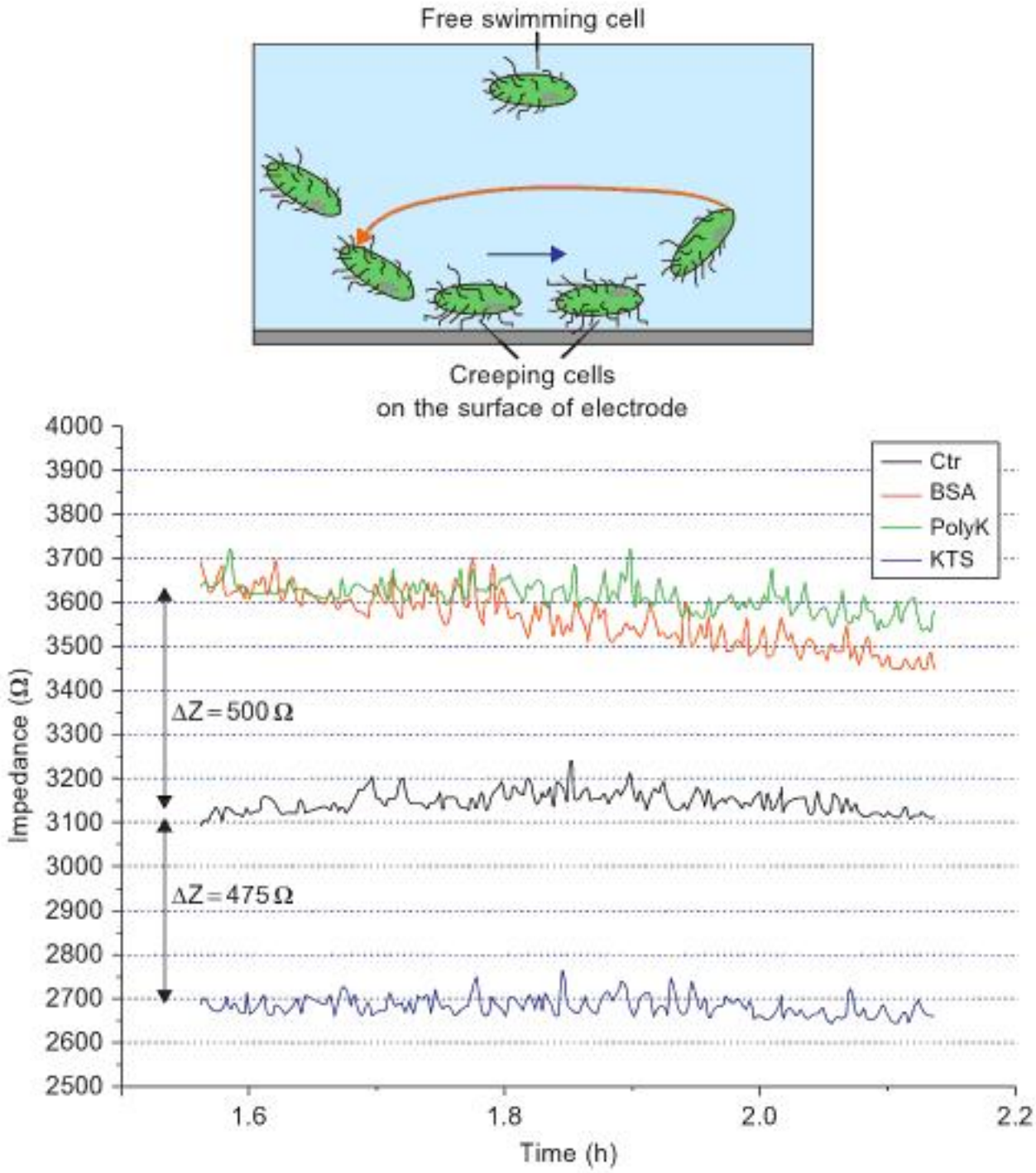


FIGURE 3.9

Recording of creeping of *Tetrahymena* by ECIS technique.

The advantage of this technique is that not only the surface dependent swimming behavior is detectable by the new application but also it detects whether cells distinguish coating substances. As the results on Fig. 3.9 show, a significant difference was detected in the number of creeping *Tetrahymena* cells. While the KTS disintegrin, a potent and selective inhibitor of alpha1beta1 integrin, could decrease ($\Delta Z = 450\Omega$) the number of creeping cells, polyK and BSA had inducer effect ($\Delta Z = 500\Omega$) on this adhesion-like activity of *Tetrahymena* cells. Besides the total number of cells attached to electrodes at a moment the technique provides the possibility to analyze identical creeping activities by e.g., MATLAB-based programs.

3.6 Migration-specific phenomena

Investigations of *Tetrahymena* chemotaxis were also essential as a set of general and migration-specific phenomena described by the help of experiments focused on this basic cell physiological activity.

Chemotactic selection (Fig. 3.10) describes the possibility to select sub-populations of cells by the help of chemical signals. As sub-populations represent groups of cells expressing higher chemotactic responsiveness to a ligand, it is feasible that the positive preference to the ligand is coded on different levels of the signaling systems in the cell. In the case of *Tetrahymena* cultures, passing 7–10 days (70–100 generations) are far enough to distinguish increased responses due to membrane level acute interactions (short-term) and genetically conserved (long-term) mechanisms [33, 72].

While in the first case, an ad hoc association of surface membrane components is most probably responsible for the increased chemotactic responses, the second, long-term variant develops regularly on the basis of a long-term selection of genetically adequate cells possessing preformed receptors. Furthermore, in addition to the presence of different types of receptors, diverse groups of ligands can also influence the efficiency of chemotactic selection as there is a tight matching of receptors and ligands. The variety of molecular structures suitable to act as a long-term selector is rather wide (Table 3.2). The diversity of ligands suggests that chemotactic signaling in respect of the selection is also very sensible and, in some cases, very small structural alterations of the ligand (steroids, bradykinins, etc.) result in chemotactic selection via different mechanisms.

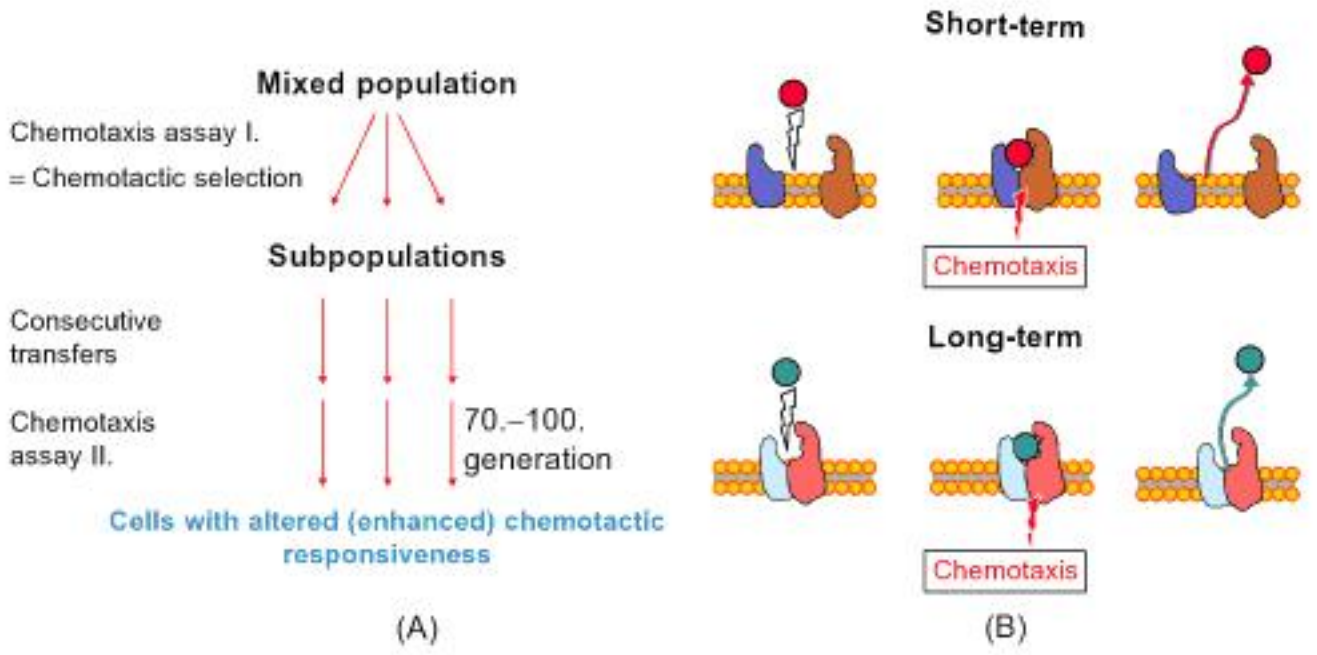


FIGURE 3.10

Chemotactic selection. (A) Flow-chart of chemotactic selection with its theoretical and practical approaches; (B) Hypothetical scheme of receptor dynamics of short- and long-term chemotactic responsiveness.

Table 3.2 Long- and Short-Term Selector Chemotactic Ligands

	Long-Term Selector	Short-Term Selector	Ref.
Biogenic amines – hormones	Histamine Di-iodo-tyrosine Insulin		72
Chemokines	IL-8 TNF-a	RANTES	33
Vasoactive peptides	Bradykinin 1–9; 1–8	Bradykinin 1–7; 2–8; des-Pro^2 Endothelin-1; –3	11 46
Lectins	Helix Con-A	Lens	73
SXWS peptides	SAWS SEWS	SKWS SDWS	38
ECM peptides	Elastin (VGVAPG)$^{3\text{-}4}$		75
Other peptides		Polylysines	74
Water soluble steroids (with beta-cyclodextrin carrier)	Testosterone Hydrocortisone Dexamethasone	Progesterone Estradiol	57

Author feels to underline that besides the strong theoretical background of chemotactic selection it requires to introduce new technologies as a part of the practical implementation.

The second phenomenon which was described at first using *Tetrahymena* chemotaxis is the *chemotactic range fitting (CRF)* [32]. Investigations of chemotactic properties of amino acids and correlations between the amplitude of chemoattractant or chemorepellent character and physicochemical characteristics of the ligands were the first where differences in effective ranges were described. According to the description of the phenomenon, the range of effectiveness is significantly wider for chemoattractant ligands than for chemorepellent ones (Fig. 3.11). The validity of this theory is supported by a decreased pK (–COOH), an increased pK (–NH2), and a decrease in solvent exposed areas and hydropathy indexes in chemoattractant amino acids compared to chemorepellent ones. Several evolutionary more "recent" ligands (e.g., soluble cytokine receptor motif SXWS, formyl peptides) have been corresponded to the physicochemical requirements of CRF; however, the phenomenon was described at first on amino acids which are considered the oldest organic substances of the prebiotic evolution.

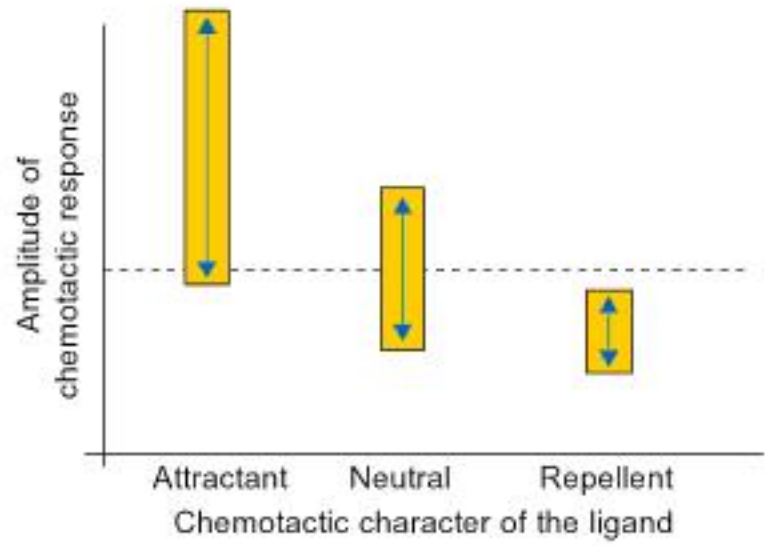


FIGURE 3.11

Schematic representation of chemotactic range fitting as a difference in chemotactic responsiveness elicited by chemoattractant and chemorepellent ligands.

The last migration-specific discovery is the *chemotactic drug-targeting* (*CDT*), which represents both a theoretical progress and an innovation on the varicolored field of drug-delivery systems. *Tetrahymena* was the eukaryotic model of the first and still several ongoing model experiments [76]; however, the future application of CDT is clinical. The theory of CDT itself is a reciprocal form of the classical drug-delivery systems. All other delivery systems work in a way that drugs have to reach the target cells crossing more barriers via enteral/circulatory systems and apparent volumes of distribution. This inevitably entails with several side effects as (i) the drug has to encounter with much more tissues and cells than required for therapeutic effect; (ii) the duration in the circulatory system is much longer than required; and (iii) degradation products of the drug are also spread in an unnecessarily large space of tissues. Contrary to the delivery mechanisms described above, chemotactic drug-targeting applies the drug in a conjugate composed of four main components: (i) carrier protein; (ii) chemotactic ligand; (iii) drug; and (iv) an enzyme labile spacer sequence by which release of the drug could be scheduled (Fig. 3.12). The strategy of the novel drug delivery is that by a purposeful selection of chemotactic ligand(s) the fate of target cell could be pre-determined. Application of chemoattractant ligands of cells to be treated with the drug will work as a biologically advantageous signal and cells will accumulate in the close proximity of the CDT conjugate administered which will significantly multiply the internalization to the target cells, while other non-targets remain practically intact in the treatment. The application of chemorepellent ligands still has biological/clinical significance as these conjugates can be used in cases when the nature of the drug containing the conjugate is compromised by a cell population which could be selectively kept away by the chemorepellent signal. Besides the appropriate selection of the chemotactic ligand, the well-chosen carrier molecule has also high significance in targeting as carriers can contribute to the fast

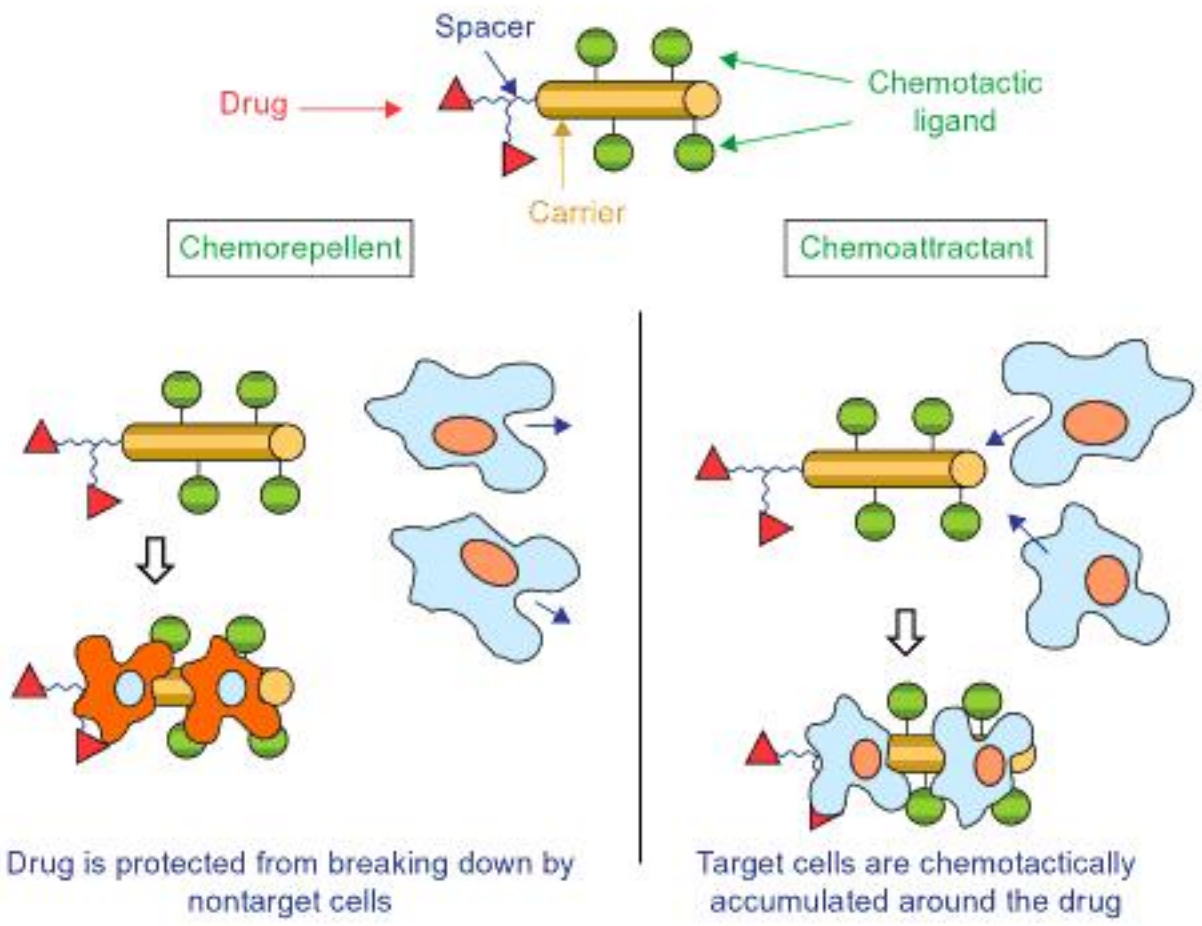


FIGURE 3.12

Theoretical scheme of chemotactic drug-targeting.

targeting by facilitating internalization of the conjugate. Internalization of CDT conjugates based on oligotuftsins is increased as tuftsins are inducers of phagocytosis, while polylysine carriers can contribute to the migration of the cells by enhancing the phase adhesion.

Investigations of CDT were successful and convergent in *Tetrahymena* and leukemic monocyte cell lines (e.g., J774 or THP-1). Experiments with the oligotusftsin carrier (OT20) labeled with fMLF or fNleLF targeting residues and carrying the cytotoxic folate antagonist methotrexate showed that the chemoattractant ligands could elicit higher chemotactic responses in conjugate with the formyl tripeptides and free of drug, while the chemotactic responses induced by the whole conjugate were highly sensible to the position of the drug. In OT20:For-NleLF conjugates with Mtx at the α or γ position only the latter has retained the chemoattractant moiety, together with its cytotoxic character which was elicited only after internalization [77].

CDT with the required peptide design itself conforms to the requirements of modern nanomedicine; however, there are more choices to develop it by innovations of microbiorobotics. In guiding CDT, conjugates or collecting positive responder cells, magnetotactic or galvanotactic mechanisms are feasible new approaches with relative low risk to the patients. In pilot experiments of these developments, investigations on *Tetrahymena* motion is still a promising approach.

3.7 Strategies in migration assays in *Tetrahymena*

The author feels that it is rather hard to understand theoretical and practical commentaries about motion of *Tetrahymena* and its significance in research of microbiorobotics without a short review on the technical backgrounds. Therefore in the last part of the chapter, a short review is given about those techniques which could be used as basics in implementation of biorobotics focused on migration of *Tetrahymena*.

In general, assays available to detect migration in ciliates like *Tetrahymena* are fundamentally different from techniques applied in cells migrating on solid or semisolid surfaces (Fig. 3.13). The most frequently used assays of amoeboid movement-based chemotaxis are carried out via surfaces furnished by pores (e.g., polycarbonate or nitrocellulose filters in Boyden chamber, transwell system, or CIM plate of xCELLigence DP) which makes the direction of movement unidirectional and irreversible. In contrast, the free-swimming ciliates like *Tetrahymena* prefer the open liquid systems where responder cells are not captured by a well-defined space and concentration of the ligand but in a reversible system, they are allowed to move free and, therefore, they can determine their position by a servo mechanism like adaptation to the environment.

This free type of locomotion has a dominant character in respect of the assay especially since chemotaxis assays are very different in the stability of the concentration gradient which value has to be correlated with the velocity of the model cell. Table 3.3 shows the velocities of free-swimming unicellular models vary in a wide range. While maximum speed of prokaryotic cells reaches the bottom line of the eukaryotic ciliates (200 μm/s), eukaryotic flagellate are in the same range of bacteria, which data indicate phagocytosis, as an essential drive of chemotaxis has an effect even on the values of velocity. Average velocity of *Tetrahymena* is rather low; nevertheless, it could be even doubled (see galvanotaxis results referred above).

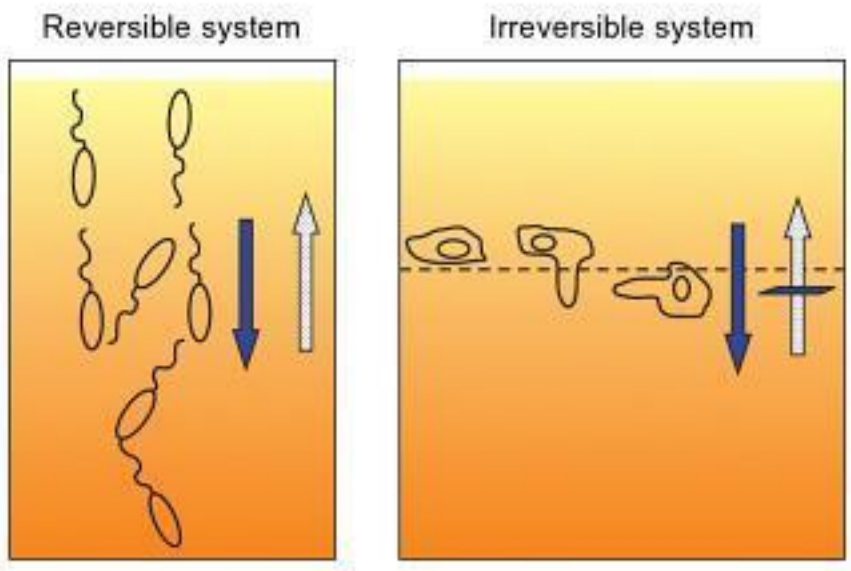


FIGURE 3.13

Differences of techniques used to measure chemotactic responsiveness in ciliates and cells migrating by amoeboid movement.

Table 3.3 Swimming Velocity of Prokaryotic and Eukaryotic Cells Used as Models in Biology

Taxon	Velocity (μm/s)	Ref.
Bacterium	6-15	80
Vibrio	200	78
Salmonella	20	78
Spirillum	50	78
Beggiatoa	2	78
Eukaryotic ciliates	300–2000	79
Paramecium	1000	80
Tetrahymena	**400**	81
Blepharisma	300	85
Eukaryotic flagellates	20–200	79
Dunaliella	40	83
Euglena	100	84
Ameba	5	79
Spermium	68–162	82

Considering the above mentioned limiting factors, there are three main categories of migration/chemotaxis assays which are accepted and provide good technical basis in innovation at the same time.

Agar plate assays belong to the oldest techniques developed [86] (Fig. 3.14). Their chief character is that preformed wells far from each other are used as containers of test substances and cells to be tested. The relatively long distance between the cells and the chemicals provide the possibility to develop a high resolution in concentration gradient. The positive chemotactic responder cells are also well isolated from the increased chemokinetic responders and non-responders. In a variant of the assay, the cells are migrating under the agar layer. In these assays, it is essential to take care on the thinness of the agar layer; otherwise, it will negatively impact the oxygen environment of cells. Another method to develop agar-based chemotaxis assays is to cut thin parallel channels, which are connected by a third cut (PP-chamber) [87]. In the long channel, also, a detailed concentration gradient can also develop which will guide the positive responder cells along the channel. Evaluation of the positive responder cells in agar plate assays is rather easy. Following fixation or decreasing the temperature which can immobilize the ciliates simple counting of native or stained cells under light microscope is offered. Nevertheless, more sensible techniques, e.g., detection of cells labeled by isotope or vital fluorescent dyes are also available.

Dealing with the free-swimming character of *Tetrahymena*, *capillary and T-maze techniques* were also proven to be reliable to measure chemotaxis (Fig. 3.15A). While the capillary technique covers a large field of assays, here, we present one of the most sophisticated, quantitative technique that applies high-volume precision multichannel micropipettes (8 to 12 channels). In the two-chamber technique, there are outer chambers (8 to 12 wells of a microtitration plate) filled with cells and identical

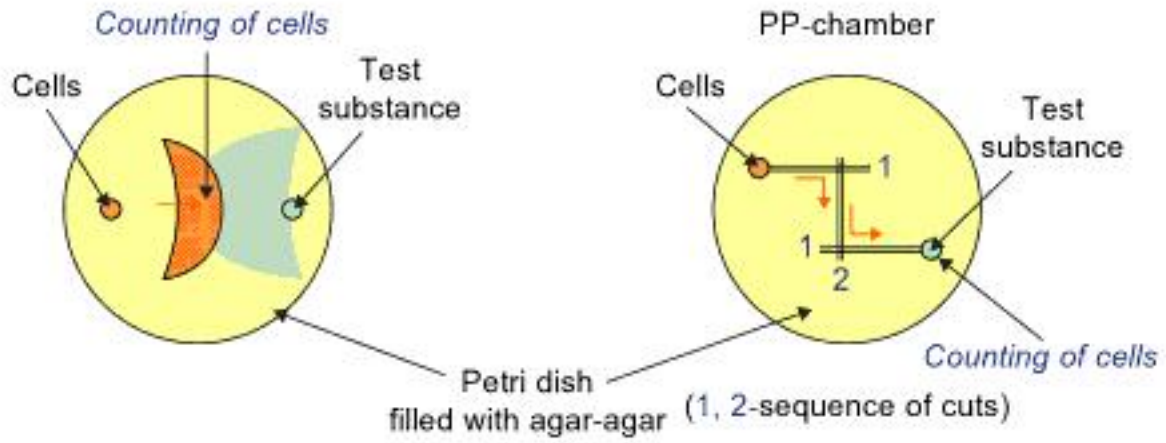


FIGURE 3.14

Agar plate chemotaxis assays.

inner chambers (tips of the micropipette) filled with the test substance [35]. Positive responder cells will migrate in maximal numbers into the capillary filled with the optimal concentration of chemoattractant. Mathematical modeling of this capillary assay has revealed that a time-delayed patchy environment model gives the optimal description of chemotactic phenomena across capillaries in *Tetrahymena* [88]. The other technique to test migratory responses is the T-maze assay, which is regularly performed in a more macroscopic, inverted T-shaped tool (Fig. 3.15B – bottom). This assay has microsized adaptations also which provide the possibility to evaluate 12–50 samples in parallel [89] (Fig. 3.15B – top). The essence of the method is that the cells are placed between two containers filled with different test substances and the assay provides information about the substance, preferred by the cells. Both assays are dedicated for concentration course experiments and selecting sub-populations of high- or low-responder cells (e.g., chemotactic selection)

Two special chemotaxis techniques deserve mentioning as they are considered strange in some respects (Fig. 3.16). *Dunn-chamber* seems to be a simple two-chamber tool at first sight. The horizontal arrangement of the two circular chambers and the bridge which allows communication between the two chambers has more practical value. Gravitational forces in the chambers and on the surface of the bridge are equalized, which have an advantageous influence on the stability of concentration gradient developed on the bridge where chemotaxis itself takes place under a cover-slip. In contrast, other assays where the stability of the gradient is between 0.5 and 4 h, in Dunn-chamber, it was measured to be 10 to 30 h depending on the molecular weight of the substance investigated. Therefore, the chamber is dedicated to analyze migratory responses of prokaryotic and eukaryotic cells in quantitative details [90]. An entirely different method is the *opalescence probe* which is based on the transparency (OD value) of cell-free fluid being significantly lower than fluid containing cells in different densities [91]. The vertical system is regularly built in cuvettes of a photometer, and cell cultures are layered onto a cell-free test substance containing culture medium or other buffers. When the substance assayed has a chemoattractant moiety, the boarder of cell culture is shifted to the cell-free part. This change is quantitatively detectable in a photometer. The only limiting factor of measurements

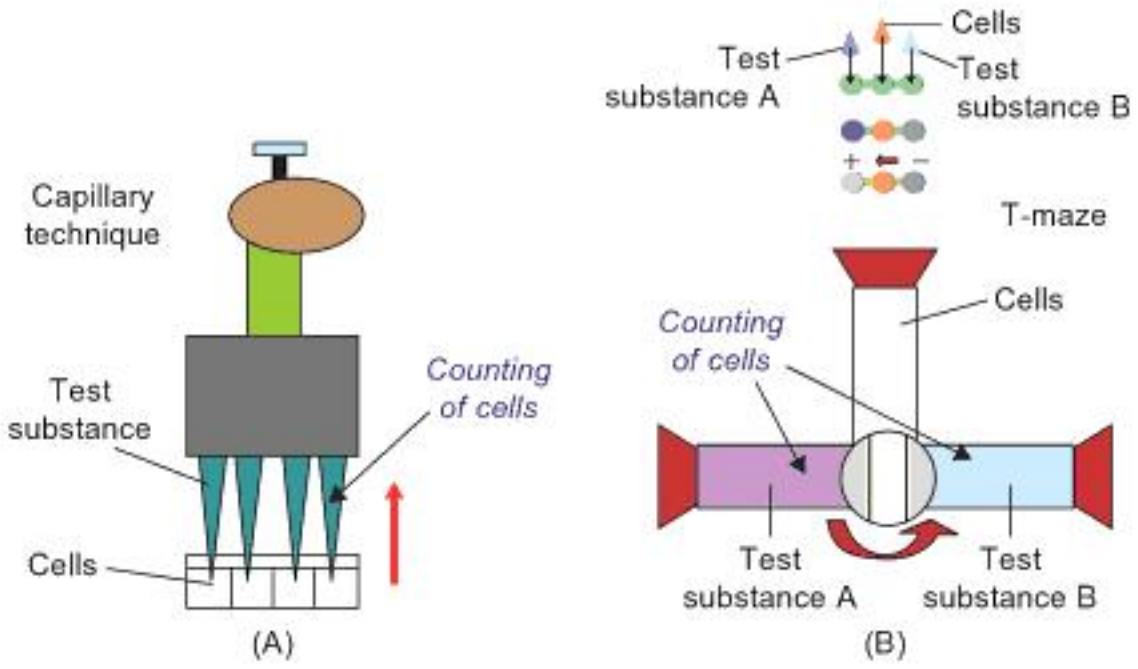


FIGURE 3.15

Chemotaxis assays dealing with cells applied in free fluid phases. (A) Setup of two-chamber capillary assay; (B) Macrosize and microsize T maze assays.

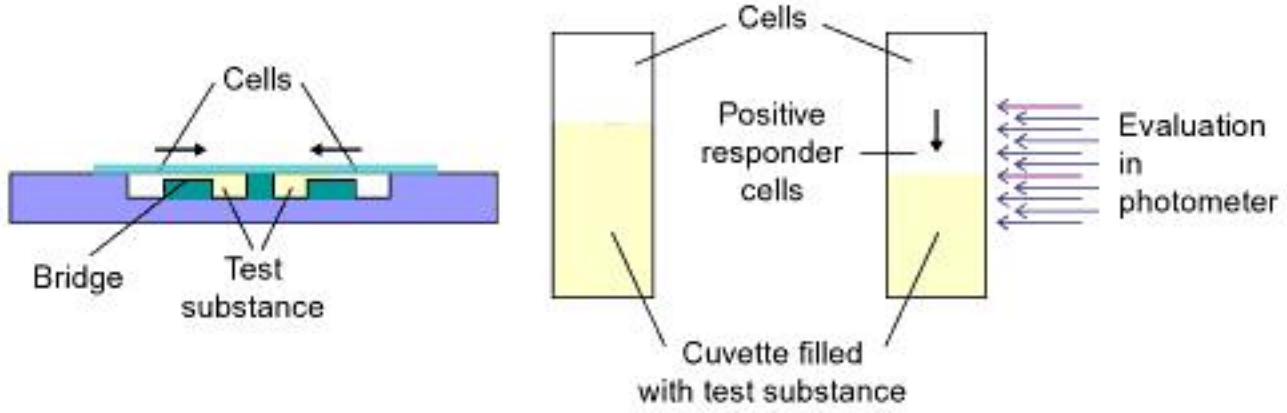


FIGURE 3.16

Special chemotaxis assays—Structure of Dunn-chamber and working mechanism of opalescence probe (Solid black arrows – direction of cell migration when test substance is a chemoattractant).

is that it is hard to compare results gained on aerobic and anaerobic taxa and that metabolic processes influenced by the test substance can also interfere with the shifts of cells into the deeper, oxygen-poor layers.

3.8 Concluding remarks

The main purpose of this section was to present the eukaryotic model cell *Tetrahymena*, with special regard to its, perhaps, most fundamental activity, the cell motility, and to show how is this migratory behavior influenced by endogenous or external factors of microbiorobotics. The author is confident that both the presented cell

itself and the main guiding strategies will convince the reader about the suitability of *Tetrahymena* as a proper target of research and its theoretical and practical suitability to develop more techniques. Our future prospect is that these unicellular organisms start a prosperous decade in 2011 and their migration-specific applications in medicine, nanoscience, cellular levels of environment protection, etc., will bring more Nobel prizes and other appreciation in science and practical life.

Acknowledgements

The author wishes to express his gratitude to all colleagues who have allowed and facilitated his *Tetrahymena* cell-based experiments in the past 30 years. Thanks in particular to his mentors Prof. György Csaba and Dr. Péter Kovács and the members of the Chemotaxis Research Group, GCI-SU, Budapest, Hungary.

References

[1] Christensen ST, Guerra CF, Awan A, Wheatley DN, Satir P. Insulin receptor-like proteins in *Tetrahymena thermophila* ciliary membranes. Curr Biol 2003;13:R50–2.

[2] Csaba G, Inczefi-Gonda A, Fehér T. Induction of steroid binding sites (receptors) and presence of steroid hormones in the unicellular *T. pyriformis*. Comp Biochem Physiol A Comp Physiol 1985;82:567–70.

[3] Schultz JE, Schönborn C. Cyclic AMP formation in *Tetrahymena pyriformis* is controlled by a K(+)-conductance. FEBS Lett 1994;356:322–6.

[4] Kőhidai L, Barsony J, Roth J, Marx SJ. Rapid effects of insulin on cyclic GMP location in an intact protozoan. Experientia 1992;48:476–81.

[5] Suzuki Y, Nagao S, Abe K, Hirabayashi T, Watanabe Y. *Tetrahymena* calcium-binding protein is indeed a calmodulin. J Biochem 1981;89:333–6.

[6] Kovács P, Csaba G. Effect of phorbol 12-myristate 13-acetate (PMA) on the phosphoinositol (PI) system in *Tetrahymena*. Study of the 32P incorporation and breakdown of phospholipids. Cell Biochem Funct 1995;13:85–9.

[7] Kőhidai L. Chemotaxis: the proper physiological response to evaluate phylogeny of signal molecules. Acta Biol Hung 1999;50:375–94.

[8] Kovács P, Sundermann CA, Csaba G. Investigations of receptor-mediated phagocytosis by hormone-induced (imprinted) *Tetrahymena pyriformis*. Experientia 1996; 52:769–73.

[9] Christensen ST. Insulin rescues the unicellular eukaryote *Tetrahymena* from dying in a complete, synthetic nutrient medium. Cell Biol Int 1993;17:833–7.

[10] Kőhidai L, Csaba G. Effects of insulin and histamine in themselves and in combination on the glucose metabolism of *Tetrahymena*. Acta Biol Hung 1985;36:281–5.

[11] Kőhidai L, Kovács K, Csaba G. Direct chemotactic effect of bradykinin and related peptides—Significance of amino- and carboxyterminal character of oligopeptides in chemotaxis. Cell Biol Internat 2002;26:55–62.

[12] Csaba G, Kovács P, Kőhidai L. *Tetrahymena* cells distinguish insulins according to their amorphous and crystalline condition or their bovine and porcine origin. Study

of imprinting in aspects of hormone binding and chemotaxis. Microbios 1994;80: 215–221.
[13] Csaba G. Phylogeny and ontogeny of hormone receptors: the selection theory of receptor formation and hormonal imprinting. Biol Rev Camb Philos Soc 1980;55:47–63.
[14] Csaba G. The unicellular *Tetrahymena* as a model cell for receptor research. Int Rev Cytol 1985;95:327–77.
[15] Gibbons IR, Rowe AJ. Dynein: a protein with adenosine triphosphatase activity from cilia. Science 1965;149:424–6.
[16] Berger JD. Riding the ciliate cell cycle—A thirty-five-year perspective. J Euk Microbiol 2001;48:505–18.
[17] Fan Q, Yao M. New telomere formation coupled with site-specific chromosome breakage in *Tetrahymena thermophila*. Mol Cell Biol 1996;16:1267–74.
[18] Yao MC, Chao JL. RNA-guided DNA deletion in *Tetrahymena*: An RNAi-based mechanism for programmed genome rearrangements. Annu Rev Genet 2005;39: 537–59.
[19] Ren Q, Gorovsky MA. H2A.Z acetylation modulates an essential charge patch. Mol Cell 2001;7:1329–35.
[20] Thazhath R, Liu C, Gaertig J. Polyglycylation domain of beta-tubulin maintains axonemal architecture and affects cytokinesis in *Tetrahymena*. Nat Cell Biol 2002;4:256–9.
[21] Rabl J, Leibundgut M, Ataide SF, Haag A, Ban N. Crystal structure of the eukaryotic 40S ribosomal subunit in complex with initiation factor 1. Science 2011;331:730–6.
[22] Hegyesi H, Kovács P, Falus A, Csaba G. Presence and localization of histidine decarboxylase enzyme (HDC) and histamine in *Tetrahymena pyriformis*. Cell Biol Int 1998;22:493–7.
[23] Csaba G, Kovács P. Effect of hormones and hormone-induced imprinting on the serotonin level in *Tetrahymena*: immunocytochemical studies. Microbios 1994;80:155–63.
[24] LeRoith D, Shiloach J, Roth J, Lesniak MA. Evolutionary origins of vertebrate hormones: substances similar to mammalian insulin are native to unicellular eukaryotes. Proc Natl Acad Sci USA 1980;77:6184–8.
[25] LeRoith D, Liotta AS, Roth J, Shiloach J, Levis ME, Pert CB. ACTH and beta endorphin-like materials are native to unicellular organisms. Proc Natl Acad Sci USA 1982;79:2086–90.
[26] Schwabe C, LeRoith D, Thompson RP, Shiloach J, Roth J. Relaxin extracted from protozoa (*Tetrahymena pyriformis*). Molecular and immunologic properties. J Biol Chem 1983;258:2778–81.
[27] Kőhidai L, Csaba G. Effects of the mammalian vasoconstrictor peptide, endothelin-1, on *Tetrahymena pyriformis* GL, and the immunocytological detection of endogenous endothelin-like activity. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1995;111:311–6.
[28] Kőhidai L, Kovács P, Lázár-Molnár E, Csaba G. Presence, uptake and localization of an immunoreactively interleukin 6 (IL-6)-like molecule in *Tetrahymena pyriformis*. Cell Biol Int 2000;24:749–55.
[29] Kőhidai L, Vakkuri O, Keresztesi M, Leppäluoto J, Csaba G. Melatonin in the unicellular *Tetrahymena pyriformis*: effects of different lighting conditions. Cell Biochem Funct 2002;20:269–72.
[30] Hawkins PT, Stephens LR, Suire S, Wilson M. PI3K signaling in neutrophils. Curr Top Microbiol Immunol 2010;346:183–202.
[31] Ragot R. Negative necrotaxis. Blood Cells 1993;19:81–8.

[32] Kőhidai L, Láng O, Csaba G. Chemotactic-range-fitting of amino acids and its correlations to physicochemical parameters in *Tetrahymena pyriformis*–Phylogenetical consequences. Cell Mol Biol 2003;49:OL487–95.
[33] Kőhidai L, Csaba G. Chemotaxis and chemotactic selection induced with cytokines (IL-8, RANTES and TNFα) in the unicellular *Tetrahymena pyriformis*. Cytokine 1998;10: 481–6.
[34] Kőhidai L, Csaba G. Different and selective chemotactic responses of *Tetrahymena pyriformis* to two families of signal molecules: lectins and peptide hormones. Acta Microbiol Immunol Hung 1996;43:83–91.
[35] Kőhidai L, Lemberkovits É, Csaba G. Molecule dependent chemotactic responses of *Tetrahymena pyriformis* elicited by volatile oils. Acta Protozool 1995;34:181–5.
[36] Kőhidai L, Soós P, Csaba G. Effects of dipeptides containing the amino acid, proline on the chemotaxis of *Tetrahymena pyriformis*. Evolutionary conclusions on the formation of hormone receptors and hormones. Cell Biol Internat 1997;21:341–5.
[37] Kőhidai L, Török K, Illyés E, Tamási J, Sebestyén F, Láng O, et al. Characterization of chemotactic ability to peptides containing N-formyl-methionyl residue in *Tetrahymena* model–for-Met-Leu-Phe (f-MLP) as a targeting ligand. Cell Biol Int 2003;27:695–700.
[38] Illyés E, Hudecz F, Kőhidai L, Láng O, Szabó P, Sebestyén F. Synthesis of oligopeptides with SXWS sequence and their chemotactic effect on a ciliated protozoon *Tetrahymena pyriformis*. J Peptide Sci 2002;8:13–22.
[39] Kőhidai L, Karsa J, Csaba G. Effects of hormones on the chemotaxis in *Tetrahymena* – Investigations on receptor memory. Microbios 1994;77:75–85.
[40] Kőhidai, L, Gál, G, Banchetti, R. Interspecific effect of Er-1 and Er-2 *Euplotes pheromones* in *Tetrahymena*. Acta Zool Sin 2007;52:1125–32.
[41] Csaba G, Kovács P. Pheromone and insulin induced chemotaxis in *Tetrahymena*. Microbios 1993;76:35–9.
[42] Tanabe H, Kurihara K, Kobatake Y. Changes in membrane potential and membrane fluidity in *Tetrahymena pyriformis* in association with chemoreception of hydrophobic stimuli: fluorescence studies. Biochemistry 1980;19:5339–44.
[43] Leick V. Chemotactic properties, cellular binding and uptake of peptides and peptide derivatives: studies with *Tetrahymena thermophila*. J Cell Sci 1992;103:565–70.
[44] Kőhidai L, Vakkuri O, Keresztesi M, Pállinger É, Leppaluoto J, Csaba G. Impact of melatonin on the cell division, phagocytosis and chemotaxis of *Tetrahymena pyriformis*. Acta Protozool 2002;41:85–9.
[45] Kőhidai L, Csaba G, Karsa J. Effect of atrial natriuretic peptide /ANP/ on the unicellular model *Tetrahymena pyriformis*. Microbios 1995;82:27–40.
[46] Kőhidai L, Tóth K, Ruskoaho H, Csaba G. Effect of vasoactive peptides in Terahymena–Chemotactic properties of endothelins (ET-1, ET-2, ET-3, fragment 11-21 of ET-1 and big endothelin)–a short-term inducible signaling mechanism of chemotaxis. Cell Biol Internat 2001;25:1173–7.
[47] Kőhidai L, Bősze Sz, Soós P, Illyés E, Láng O, Mák M, et al. Chemotactic activity of oligopeptides containing EWS motif on *Tetrahymena pyriformis*. The effect of amidation of the C-terminal residue. Cell Biochem Funct 2003;21:113–20.
[48] Francis JT, Hennessey TM. Chemorepellents in *Paramecium* and *Tetrahymena*. J Eukaryot Microbiol 1995;42:78–83.
[49] Awan A, Bernstein M, Hamasaki T, Satir P. Cloning and characterization of Kin5, a novel *Tetrahymena* ciliary kinesin II. Cell Motil Cytoskelet 2004;58:1–9.
[50] Guerra C, Wada Y, Leick V, Bell A, Satir P. Cloning, localization, and axonemal function of *Tetrahymena* centrin. Mol Biol Cell 2003;14:251–61.

[51] Seetharam RN, Satir P. Coordination of outer arm dynein activity along axonemal doublet microtubules. Cell Motil Cytoskelet 2008;65:572–80.
[52] Christopher GK, Sundermann CA. Isolation and partial characterization of the insulin binding sites of *Tetrahymena pyriformis*. Biochem Biophys Res Commun 1995;212: 515–23.
[53] Csaba G, Lantos T. Effect of cyclic AMP and theophylline on phagocytotic activity of *Tetrahymena pyriformis*. Experientia 1976;32:321–2.
[54] Schultz JE, Klumpp S. Calcium/calmodulin-regulated guanylate cyclases in the ciliary membranes from *Paramecium* and *Tetrahymena*. Adv Cyclic Nucleotide Protein Phosphorylation Res 1984;17:275–83.
[55] Kovács P, Csaba G. Involvement of the phosphoinositol (PI) system in the mechanism of hormonal imprinting. Biochem Biophys Res Commun 1990;170:119–26.
[56] Connett RJ, Blum JJ. Metabolic pathways in *Tetrahymena*. Estimation of rates of the tricarboxylic acid cycle, glyoxylate cycle, lipid synthesis, and related pathways by use of multiple labeled substrates. J Biol Chem 1972;247:5199–209.
[57] Kőhidai L, Katona J, Csaba G. Effects of steroid hormones on five functional parameters of *Tetrahymena*: evolutionary conclusions. Cell Biochem Funct 2003;21: 19–26.
[58] Koppelhus U, Hellung-Larsen P, Leick V. Physiological parameters affecting the chemosensory response of *Tetrahymena*. Biol Bull 1994;187:1–7.
[59] Van Vliet KJ, Bao G, Suresh S. The biomechanics toolbox: experimental approaches for living cells and biomolecules. Acta Materialia 2003;51:5881–905.
[60] Nam SW, Van Noort D, Yang Y, Park S. A biological sensor platform using a pneumatic-valve controlled microfluidic device containing *Tetrahymena pyriformis*. Lab Chip 2007;7:638–40.
[61] Nam SW, Kim ST, Lee KM, Kim SH, Kou S, Lim J, et al. N-Methyl-D-aspartate receptor-mediated chemotaxis and Ca(2+) signaling in *Tetrahymena pyriformis*. Protist 2009;160:331–42.
[62] Steager E, Kim C-B, Patel J, Bith S, Naik C, Reber L, et al. Control of microfabricated structures powered by flagellated bacteria using phototaxis. Appl Phys Lett 2007;90:263901.
[63] Kim MJ, Breuer KS. Microfluidic pump powered by self-organizing bacteria. Small 2008;4:111–8.
[64] Kim, MJ, Breuer KS. The use of bacterial carpets to enhance mixing in microfluidic systems. J Fluids Eng 2007;121:319–24.
[65] Kim DH, Cheang UK, Kőhidai L, Byun D, Kim, MJ. Artificial magnetotactic motion control of *Tetrahymena pyriformis* using ferromagnetic nanoparticles A tool for fabrication of microbiorobots. Appl Phys Lett 2010;97:173702–3.
[66] Ruben L, Lageson J, Hyzy B, Hooper AB. Growth cycle-dependent overproduction and accumulation of protoporphyrin IX in *Tetrahymena*: effect of heavy metals. J Protozool 1982;29:233–8.
[67] Kőhidai L, Darvas Zs, Csaba G. The effect of varying illumination on imprinting of *Tetrahymena* by insulin. Acta Microbiol Hung 1987;34:179–85.
[68] Kim DH, Casale D, Kőhidai L, Kim MJ. Galvanotactic and phototactic control of *Tetrahymena pyriformis* as a microfluidic workhorse. Appl Phys Lett 2009;94: 163901–3.
[69] Kőhidai Zs, Lajkó E, Igaz A, Láng O, Köhidai L. Chemotactic effects of different wave lengths (400-660nm) of light and schedules of illumination detected with Cell-LED® in *Tetrahymena pyriformis* GL cultures. J Photochem Photobiol B (submitted)

[70] Giaever I, Keese CR. Monitoring fibroblast behavior in tissue culture with an applied electric field. Proc Natl Acad Sci USA 1984;81:3761–4.
[71] Kőhidai L, Láng O, Sáfár O, Szabó R, Mező G, Muto Y. Novel, ECIS-based technique to evaluate of slow migratory elements (creeping) of the swimming behavior of *Tetrahymena pyriformis*. Kemotaxis Workshop, Budapest, Semmelweis University; 2007, p.2.
[72] Kőhidai L, Schiess N, Csaba G. Chemotactic selection of *Tetrahymena pyriformis* GL induced with histamine, di-iodotyrosine or insulin. Comp Biochem Physiol 2000; 126C:1–9.
[73] Kőhidai L, Bánky Cs, Csaba G. Comparison of lectin induced chemotactic selection and chemical imprinting in *Tetrahymena pyriformis*. Acta Protozool 2003;42:91–7.
[74] Szabó, R, Mező, G, Hudecz, F, Kőhidai, L. Effect of the polylysine based polymeric on the growth and chemotaxis of *Tetrahymena pyriformis*. J Bioact Compat Polymers 2003;17:399–415.
[75] Kőhidai L, Kun L, Pállinger É, Csaba G, Mihala N, Majer Z, et al. Cell-physiological effects of elastin derived (VGVAPG)n oligomers in a unicellular model system. J Pept Sci 2004;10:427–38.
[76] Láng O, Török K, Mező G, Hudecz F, Kőhidai L. Chemotactic conjugates: New aspects in drug-targeting. Cellular Transport Strategies for Targeting of Epitopes, Drugrs and Reportes Molecules, Budapest, Eötvös Loránd University; 2003, p.118.
[77] Mező G, Láng O, Jakab A, Bai KB, Szabó I, Schlosser G, et al. Synthesis of oligotuftsin-based branched oligopeptide conjugates for chemotactic drug targeting. J Pept Sci 2006;12:328–36.
[78] Bacterial motility. McGraw-Hill encyclopedia of science and technology. New York: McGraw Hill; 1960. p. 63.
[79] McNeill AR. The Invertebrates. London: Cambridge University Press; 1979.
[80] Bray D. Cell Movements. New York: Garland; 1992.
[81] Hellung-Larsen, P, Leick, V, Tommerup, N. Chemoattraction in *Tetrahymena*: on the role of chemokinesis. Biol Bull 1986;170:357–67.
[82] Katz DF, Dott HM. Methods of measuring swimming speed of spermatozoa. J Reprod Fertility 1975;45:263–72.
[83] Chengala AA, Hondzo M, Troolin M, Lefebvre PA. Kinetic responses of *Dunaliella* in moving fluids. Biotechnol Bioeng 2010;107:65–75.
[84] Hall A. Phototaxis in Euglenoids. MSTA J 2005;50:35–44.
[85] Fornshell JA. Swimming dynamics and vertical distribution of the ciliate *Blepharisma persicinum* Perty in laboratory cultures. J Exp Marine Biol Ecol 1984;82:53–8.
[86] Nelson RD, Quie PG, Simmons RL. Chemotaxis under agarose: a new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. J Immunol 1975;115:1650–56.
[87] Kőhidai L. Method for determination of chemoattraction in *Tetrahymena pyriformis*. Current Microbiol 1995;30:251–53.
[88] Sáfár O, Köhidai L, Hegedüs A. Time-delayed model of unbiased movement of Tetrahymena pyriformis. Period Math Hung 2011; 63:215–25.
[89] Van Houten J, Hansmal H, Kung Ch. Two quantitative assays for chemotaxis in *Paramecium*. J Comp Physiol 1975;A 104:211–23.
[90] Zicha D, Dunn GA, Brown AF. A new direct-viewing chemotaxis chamber. J Cell Sci 1991;99:769–75.
[91] Koppelhus U, Hellung-Larsen P, Leick V. An improved quantitative assay for chemokinesis in *Tetrahymena*. Biol Bull 1994;187:8–15.