Preface

Chemotaxis is the phenomenon in which bodily cells, bacteria, and other single-cell or multicellular organisms direct their movements according to certain chemicals in their environment. This is important for bacteria to find food by swimming towards the highest concentration of food molecules, or to flee from poisons. In multicellular organisms, chemotaxis is critical to early and subsequent phases of development, as well as in normal function. This new book discusses reserch in the study of chemotaxis including the cell migration signalosome, the role of chemotaxis in the association of the azospirillum brasilense plant, the role of CD46 in the control of chemotaxis of activated T cells in MS pathogenesis and the regulation of chemotaxis by heterotrimeric G proteins.

Chapter 1- Cell migration is an essential process involved in the development of the organism during embryogenesis and then throughout life in physiological and pathological events including inflammation, wound healing, vascular diseases and cancers. The urokinase receptor (uPAR), a glycosyl-phosphatidyl-inositol (GPI) anchored-protein, regulates a variety of important cellular processes such as pericellular proteolysis and cell migration. In fact, uPAR is involved in different kinds of cell migration including haptotaxis, diapedesis and chemotaxis. uPAR is an important motogenic receptor because it can directly regulate and coordinate not only the generation of the signal that actually starts the migration of the cell but also the degradation of the proteins of the extracellular matrix that opens the path to the migratory cells. In addition, uPAR has other unique properties resulting from its relationship with a wide array of membrane receptors such as seven-transmembrane domain receptors, tyrosine kinase receptors and integrins. By

initiating the formation of large signalling complexes, uPAR is the foundation stone of the cell migration signalosome primarily constituted by uPAR and the integrins. These latter compose a large family of membrane receptors that provides connection to the cell cytoskeleton, cell survival and adhesion. Thereby, the combination of uPAR and the integrins brings new essential properties to the migratory cell. Furthermore, the exceptionally wide array of ligands and membrane-bound partners of uPAR and the integrins consents to add new constituents, finely adjust the composition, and modulate the functions of the cell migration signalosome in order to meet the various cellular requirements of the migratory cell throughout its journey. These properties are perfectly suited for the spatial and temporal regulation of cell migration.

This chapter will discuss the molecular mechanisms of cell migration with a particular emphasize on the role of uPAR and uPAR-integrin interactions that lead to the formation of the cell migration signalosome.

Chapter 2- The genus *Azospirillum* belongs to the plant growth-promoting bacteria group, capable of positively influencing the growth and yield of numerous plant species, many of them with agronomic and ecological importance. Plant growth promotion is largely determined by the efficient colonization of the rhizosphere (soil influenced by roots and microorganisms). Root exudates constitute the most significant source of nutrients in the rhizosphere and seem to participate in the early colonization by inducing the chemotactic response of bacteria. Therefore, chemotaxis is considered an essential mechanism for the successful root colonization by *Azospirillum*.

In this chapter the authors present a background and new insights on *Azospirillum* chemotaxis, concerning the genetic aspects and its use for addressing biotechnological applications. First, the authors demonstrate that the genetic complementation of a mutant strain, impaired in surface motility, led to the identification of the gene *chsA* (chemotactic signaling protein). The deduced translation product, ChsA protein, contained a PAS sensory domain and EAL active site domain. The latter has phosphodiesterase activity (PDE-A) for the hydrolysis of c-di-GMP [cyclic-bis (3' –5') dimeric GMP], a compound known to function as a second messenger in different cellular processes, including motility, biofilm formation and cellular differentiation.

After cloning *chs*A, ChsA protein was expressed and purified by affinity chromatography. ChsA activity in presence of *bis-p*-nitro phenyl-phosphate was 0.59-ÂµM min^{-1} mg^{-1} protein, demonstrating that it displayed phosphodiesterase activity. This suggests that ChsA is a component of the signaling pathway controlling chemotaxis in *Azospirillum*. Then, the authors

propose that the redox state of the cell is sensed through the PAS domain and directly coupled to the transmitter EAL module, showing PDE-A activity.

Second, the chemotaxis of different strains of A. brasilense toward strawberry root exudates was investigated. The agar-plate assay was used, including two concentrations of exudates from three commercial varieties of strawberry, collected at different time intervals. To quantify the chemotactic response, the capillary method was used. In all cases, a positive chemotactic reaction was found, revealing higher responses in endophytic than in rhizospheric strains, being this strain-specific. Furthermore, the variation of the chemotactic response observed depended on the concentration and time to collect the exudates, as well as the total sugar content. Considering that A. brasilense possessses biotechnological application, addressing to a sustainable agriculture, determining the genes and mechanisms involved in chemotaxis response, as well as the level of activity of strains to root exudates may represent an initial step in selecting them for use as inoculants in different crops.

Chapter 3- Multiple Sclerosis (MS) is an autoimmune disease characterized by chronic inflammation of the brain. One of the main occurrences is the breach of the blood-brain-barrier, resulting in the entrance of inflammatory cells, which perpetuate the inflammation occurring in the brain. One of the mechanisms controlling cell migration is mediated by the release of small soluble molecules, called chemokines and by the expression of their corresponding receptors, the chemokine receptors. Hence, the conjoint expression of chemokine receptors and production of their relevant chemokines will direct the migration of cells towards the site of inflammation. Among the cells involved in the pathogenesis of MS, T cells have been shown to play an important role. Indeed, T cell activation is crucial for the immune homeostasis, notably through the balance of effectors T cells (Teff) and regulatory T cells (Tregs). In MS, defective Treg functions have been observed, which might partly explain the increased inflammation seen in MS. Among Tregs, Tr1 cells are characterized by the secretion of large amount of IL-10, an anti-inflammatory cytokine. The molecule CD46 is a regulator of complement activity. However, its activation also promotes T cell activation and differentiation toward Tr1 cells. This pathway is altered in MS, as the amount of IL-10 produced by CD46-activated T cells is largely reduced. This chapter will discuss preliminary evidence suggestive of a role of CD46 in the control of chemotaxis of activated T cells, which might play a role in MS pathogenesis.

Chapter 4- With the ability to mediate chemotaxis of inflammatory cells, chemoattractants have been shown to contribute to the development of inflammatory diseases, such as atherosclerosis and angiogenesis. Many chemoattractant receptors belong to the family of seven-transmembrane-domain G protein-coupled receptors (GPCRs) and elicit their effects through heterotrimeric ($\alpha\beta\gamma$) guanine nucleotide-binding proteins (G proteins). The three subunits form two functional compartments – the $G\alpha$ subunit and the stable $G\beta\gamma$ complex. Both the dissociated GTP-bound $G\alpha$ subunit and $G\beta\gamma$ complex can exert biological effects. G proteins are classified into four major subfamilies, G_s, G_i, G_q and G_{12}, according to the amino acid sequence homology and functional specialization of the $G\alpha$ subunit. Members in all G protein subfamilies are known to interact with chemoattractant receptors individually or simultaneously, which trigger the activation of multiple signaling molecules, leading to actin reorganization and subsequent cell mobilization. In this chapter, the authors will discuss the promiscuity of chemoattractant receptors in G protein coupling and examine their underlying molecular mechanisms in directed cell migration.

Chapter 5- The unicellular biflagellate alga *Chlamydomonas reinhardtii* is widely used as a simple model to study fundamental cellular processes, including chemotaxis. *C. reinhardtii* is attracted towards ammonium ions (NH_4^+) and peptide mixtures, such as a pancreatic digest of casein (tryptone). The sensitivity to NH_4^+ is transiently induced in vegetative cells by nitrogen deprivation, whereas the sensitivity to tryptone requires formation of mature gametes. The clock-controlled RNA-binding protein CHLAMY1 might be involved in regulation of the diurnal rhythm of chemotaxis to NH_4^+. The authors measured inhibition of rhodopsin-mediated photoreceptor currents by the chemoattractant tryptone as an indirect assay of early stages of chemosensory transduction in *C. reinhardtii* gametes. The results showed that the magnitude of the response to tryptone depended on the concentration of monovalent metal cations in the medium with the selectivity sequence $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$. It was inhibited by extracellular Ba^{2+} and Ca^{2+} in millimolar concentrations, and by dibutyryl-cAMP. These observations suggest the involvement of K^+ channels modulated by cyclic nucleotides in *C. reinhardtii* chemotaxis. In order to identify active ingredients, the authors subjected tryptone to fractionation by gel filtration and demonstrated that only fractions that contain individual amino acids and dipeptides were functionally active. However, none of the 18 tested individual amino acids fully mimicked the effect of tryptone. The authors hypothesize that a specific combination of

amino acids and/or dipeptides acts as a chemoattractant for *C. reinhardtii* gametes.

Chapter 6- The authors propose a new way for testing the biological action of silver nanoparticles. The nanoparticles (with sizes ranging from 3 to 16 nm) were obtained by the original method of biochemical synthesis in reverse micelles stabilized by anionic surfactant bis-(2-ethylhexyl) sodium sulfosuccinate (AOT). From micellar solution the nanoparticles were transferred into the water phase; water solutions of the nanoparticles were used for testing their biological activity. Our assay is based on negative chemotaxis, a motile reaction of cells to an unfavorable chemical environment. Plasmodium of the slime mold *Physarum polycephalum* used as an object is a multinuclear amoeboid cell with unlimited growth and auto-oscillatory mode of locomotion. Biocidal and repellent effects were compared for silver nanoparticles, Ag^+ ions, and AOT; the latter two reagents were introduced in the concentrations equal to those present in the nanoparticles' solution. All substances were tested in water solution and in the agar gel. The authors have revealed that in characteristics common for repellents, such as increase of the period of contractile auto-oscillations, decrease of the area of spreading on substrate, and substrate preference in spatial tests, silver nanoparticles proved to be substantially more effective than Ag^+ ions, AOT and the sum Ag^+ + AOT. The lethal concentration of the nanoparticles for macroplasmodium in water solution was found to be about 10 μg/ml, the concentrations effective for chemotaxis were 30 times lower. The chemotactic tests allow the quantitative estimation of the biological reaction and monitoring of its dynamics; in resolution, they are superior to the tests based on the lethal action of biocidal agents. It is shown also that the spatial chemotactic tests are sensitive enough to distinguish between the effectiveness of different nanoparticle preparations. In particular, the authors found that, at the equal silver concentration, the repellent activity is higher for the 5 nm than for the 9 nm particles.

The results obtained allow to conclude that the chemotaxis-based assay could be helpful for finding a proper balance between the efficiency of silver nanoparticles as antimicrobial drug and the risk of tissue damage during medical treatment, and, hence, for elaborating an optimal protocol of their clinical application.

Chapter 7- *Chlamydomonas* has long been one of the most successful unicellular organism for genetic and biochemical studies of the photosynthesis, organelle genomes and flagellar assembly. The availability of the new molecular genetic techniques is increasing interest in *Chlamydomonas* as a model system for research in areas like swimming behavior where it

previously has not been widely exploited. The swimming behavior of *Chlamydomonas reinhardtii* is influenced by several different external stimuli including chemical attractants. Chemotaxis of the green alga is altered during gametic differentiation. Gametogenesis results in the conversion of chemotactically active vegetative cells into chemotactically inactive gametes. This experimental system offers the opportunity to study cellular behavior and differentiation at the molecular level with use of a wide range of molecular genetic approaches, including gene tagging by insertional mutagenesis, quantitative PCR and RNA interference. In this chapter I discuss recent progress in the field of chemotaxis in *Chlamydomonas*. Emphasis is placed on the signal pathways by which the two environmental cues – ammonium and light control chemotaxis and gametic differentiation.