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REVIEW

Role of extracellular cations in cell motility, polarity, and chemotaxis

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Abstract: The concentration of cations in the aqueous environment of free living organisms and cells within the human body influence motility, shape, and chemotaxis. The role of extracellular cations is usually perceived to be the source for intracellular cations in the process of homeostasis. The role of surface molecules that interact with extracellular cations is believed to be that of channels, transporters, and exchangers. However, the role of Ca^{2+} as a signal and chemoattractant and the discovery of the Ca^{2+} receptor have demonstrated that extracellular cations can function as signals at the cell surface, and the plasma membrane molecules they interact with can function as bona fide receptors that activate coupled signal transduction pathways, associated molecules in the plasma membrane, or the cytoskeleton. With this perspective in mind, we have reviewed the cationic composition of aqueous environments of free living cells and cells that move in multicellular organisms, most notably humans, the range of molecules interacting with cations at the cell surface, the concept of a cell surface cation receptor, and the roles extracellular cations and plasma membrane proteins that interact with them play in the regulation of motility, shape, and chemotaxis. Hopefully, the perspective of this review will increase awareness of the roles extracellular cations play and the possibility that many of the plasma membrane proteins that interact with them could also play roles as receptors.

Keywords: extracellular cations, chemotaxis, transporters, calcium, receptors

Introduction

Cations in the aqueous environment play major roles in facilitating cell motility, chemotaxis, and polarity.[1–9] However, their role is perceived primarily as a source for intracellular cations in the process of homeostasis. Within this conceptual framework, the roles of proteins at the cell surface that interact with extracellular cations have been perceived as primarily that of channels, transporters, or exchangers. The idea that extracellular cations may function as signals at the cell surface, regulating cell behavior and physiology through interactions with coupled receptors, is not generally realized, and when it is, it is limited to Ca^{2+}.[10,11] The general perception that the role of extracellular cations is a passive one is rooted in the knowledge first that there are a large number of biochemical reactions and interactions within the cell that depend upon constant, optimum concentrations of particular cations,[12–16] and second that when cations function as signals, they do so intracellularly.[14,17–23] Therefore, most experimental biologists prepare solutions for cell and tissue cultures based on recipes sometimes formulated as long ago as 125 years for very specialized purposes, as is the case for Ringer's solution[24–30] without concern for optimization.[4]

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The idea that extracellular cations may alter cell function by interacting with surface molecules is emerging. It has been demonstrated that Ca^{2+}-binding molecules on the surface of a variety of cell types function as bona fide coupled receptors,[10,31,32] that specific threshold concentrations of extracellular cations are critical for cell motility, polarity, and chemotaxis,[4,5,33,34] and that gradients of extracellular cations can direct cell movement through the process of chemotaxis.[5,35–50] It therefore seems timely that the role of extracellular cations be explored within this new contextual framework.

Cations in environmental niches

When studying cell behavior in vitro, a scientist should be cognizant of the concentrations of cations in the natural environment to which that cell type has adapted. The ocean, in which life began, has undergone major changes in cation content. Presently, the ocean contains approximately 10 mM Ca^{2+}, 55 mM Mg^{2+}, 450 mM Na^{+}, and 10 mM K^{+} (Figure 1, Table 1).[51–56] During the Hadean period, approximately four billion years ago, when thermophilic archaebacteria lived in the ocean, the estimated concentrations of Ca^{2+}, Na^{+}, and K^{+} were far lower than they are today (Figure 1).[57,58] The major soluble anion was Cl^{-}, as it is now.[57,58] The concentrations of Ca^{2+}, Mg^{2+}, and Na^{+} increased to their present levels by the Mesoproterozoic era, approximately 1.5 billion years ago, well after the cyanobacteria had evolved and multicellular eukaryotes had appeared (Figures 1A and 1B). This coincided with a change from a reducing to an oxidizing atmosphere (Figures 1A and 1B),[59] a result of photosynthesis by cyanobacteria.[58–61] When O_2 accumulated in the atmosphere, the ocean changed from a "soda ocean" to a "halite ocean", and soluble Ca^{2+} accumulated due to the reduction in CO_2, and hence less $CaCO_3$ precipitation.[59] Thus, during the evolution of the ocean biota, there was a 200-fold increase in soluble Ca^{2+}, a six-fold increase in Mg^{2+}, and a five-fold increase in Na^{+} (Figure 1).

The changes in the cation concentrations might seem challenging in the evolution of cell surface mechanisms regulating homeostasis, but these changes probably occurred gradually, and in fact may have been far less challenging than the differences that face present day organisms that experience rapid changes in salinity.[62] Fresh water bodies contain about 10 times less Ca^{2+}, 100 times less Mg^{2+}, 1000 times less Na^{+}, and 100 times less K^{+} than the ocean (Table 1). The Atlantic salmon can move from a fresh water to salt water environment during the parr-smolt transformation,[63] as a result of developmental changes in the gills that include the expression of transport proteins.[64–71] Microorganisms and algae in fresh water are commonly faced with dramatic changes in salinity when the water becomes brackish, through evaporation or after rains (Table 1). Some species die when water rapidly becomes brackish,[69,70] while others adapt rapidly by adjusting their osmotic pressure[72] through aquaporins, cation transporters, and exchangers.[73–76]

Another major and important environmental niche for free living organisms is soil.[51,77–82] The concentrations of the major cations in soil are, on average, much higher than in fresh water bodies, but far lower than that in the ocean (Table 1). Cow, pig, and poultry manure, which are often added to farm soil as fertilizers, can be equal to or higher in Ca^{2+} and K^{+} than seawater, but lower in Mg^{2+} and Na^{+} (Table 1). Cow and pig manure can be, on average, far higher in K^{+} and Na^{+} than the soil.

What seems most striking in our survey of aqueous environments is the extreme differences in the concentrations of the major cations (Table 1). Therefore, no single culture solution would fulfill the cationic requirements for all free living organisms. While many experimental scientists have imitated the cationic composition of natural fluid, in many cases, in the interest of expediency, solutions have been substituted that are poor imitations. Most scientists studying salt water organisms have used either filtered or artificial seawater, straying from the natural composition of the ocean only for specific experimental purposes (Table 2). Interestingly, the culture media developed for protozoans such as *Tetrahymena* and colonial organisms like *Volvox* can contain Na^{+} concentrations far higher than that in streams and ponds, suggesting that normally these organisms may be better adapted to brackish water at the edges of ponds or soil (Table 2). In the case of *Dictyostelium discoideum*, a soil amoeba that has been used as a laboratory model for multicellular development and chemotaxis,[7,8,83–91] common buffer solutions contain Na^{+} and/or K^{+} at concentrations (Table 2) far higher than in fresh water bodies, but in the range found in calcareous soil, manures, and soil fertilized with manure (Table 1), ie, their natural habitats.[83,92–98] However, the majority of *D. discoideum* media do not contain Ca^{2+} as an additive, so the final concentration of Ca^{2+} is usually one order of magnitude below that of soil or manure (Table 2). The compositions of these media have recently been compared for their capacity to support motility and chemotaxis.[4] Most have been found optimum for chemotactic orientation, but suboptimum for motility.[4] In culturing chicken and human cells, the most common media that have been developed contain Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} at concentrations (Table 2) close to that found in human blood and interstitial fluid (Table 3).[24–28,99–102]

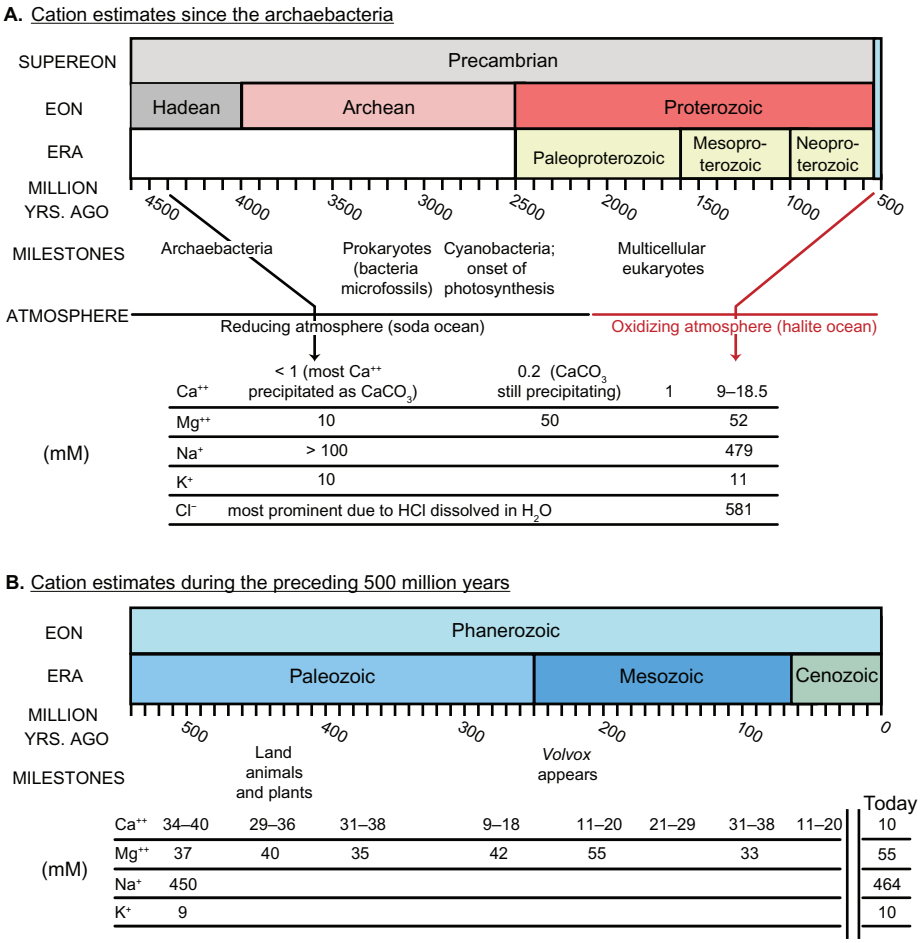

Figure 1 Estimated concentrations of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} during the evolution of the oceans.

Table 1 Concentrations of the major soluble cations in sea water, fresh water bodies, drinking water, soil, and manure

Source	Ca^{2+} [mM]	Mg^{2+} [mM]	Na^{+} [mM]	K^{+} [mM]	References
Seawater	9–10.4	55–59	439–464	10	51–56
Fresh water					
Lakes	0.5	0.3	0.1	0.1	229,230
Ponds	0.4	0.2	0.03	0.1	231
Rivers[a]	1.8	0.8	0.5	0.1	232
Brackish water	6.5	15	90	n.d.	233
	27.5	18.1	58	1.2	232
	5.8	5.5	43	7.7	234
Iowa City tap water	0.4	0.6	0.5	n.d.	Iowa City Water Division, 2010 (*personal communication*)
San Diego tap water	1.5[#]	~1.1	~4.2	~0.12	San Diego, Public Utility Dept. 2010 (*personal communication*)
Spanish bottled water	0.01–4.5	0.01–2.9	0.03–47	0.01–1.3	235
Calcareous soil	14	7	29	1	78,79,81
	12.5	3.4	1.4	1	80,236
Cow manure*	~19	~22	177	283	237
Poultry manure**	18	6.7	0.4	1.2	238
Pig manure	~8	~3.3	~28	~31	239

Notes: [a]Median of 1109 river sample sites; *Measured at saturation moisture content of the manure; **Determined as change of soil solution due to leaching from manure after surface application (0–5 cm) compared with control. The authors state in discussion that soluble salts accumulated included substantial amounts of Ca^{2+} and Mg^{2+} as well as K^{+} and Na^{+}, reflecting the generally high basic cation content of the poultry manure used (the content is given as g/kg manure); [#]reached 3.75 mM, as inferred by hardness.[240]

Table 2 Concentrations of major soluble cations in commonly used laboratory media

Organism	Medium	Ca^{2+} [mM]	Mg^{2+} [mM]	Na^{+} [mM]	K^{+} [mM]	References
Protozoa						
Dictyostelium discoideum	Tricine buffer	–	–	–	5	3,241
	20 mM Ca^{2+} solution	20			5	3
	40 mM K^{+} solution	–	2.5	5	40	3,242
	Bonner salts	2.7	–	10	10	243,244
	Developmental buffer	0.2	2.5	15	–	245,246
	Sorenson buffer	–	–	5.4	14	217,247
	KK2	0.1	2.5	–	24.3	248
	Phosphate buffer	–	–	8	7.4	249
	Developmental buffer (without divalent cations)	–	–	15	–	250
	Barber buffer	–	–	–	60	181
Tetrahymena pyriformis	*Tetrahymena pyriformis* strain 1630/1W media	0.5	1	120	7.5	251,252
	Mating Type II variety 1 medium	0.75	0.2	0.00001	5.7	253
Acanthamoeba	Neff's media	2	0.02	–	0.05	254
	Amoebae inorganic media	0.5	0.05	–	0.43	255,256
	Modified Neff's amoeba media	0.04	0.05	3.96	1	257
Protozoa/Metazoa transition						
Volvocales	*Volvocacean* media	0.5	0.08	2.2	0.98	258–261
	Modified *Volvox* media for axenic strains	0.5	0.2	4.2	0.7	260
Metazoa						
Tripneustes gratilla (sea urchin)	Filtered seawater*	~9.7	~57	~450	~10.1	262
Stronglyocentrotus purpuratus (sea urchin)	Calcium and magnesium free Millipore filtered seawater*	10 added	–	439–464	10–10.2	263
Marine culture medium (phytoplankton, sea urchin)	Artificial seawater	9.3	48.3	439	9	264–266
Xenopus laevis	Defined oocyte media	1	1	48	2.5	267
	Roswell Park Memorial Institute (RPMI)-1640 media	2.3	2.7	131	5	100,101,268
Gallus gallus	Medium 199	1.8	0.8	143	5.4	269,270
	Minimum Essential Media (MEM)	1.8	1	140	5	102,271,272
Homo sapiens	Ringer solution**	1.25	–	136	1.3	25,273
	Lactated Ringer solution	1.4	–	130	4	25,29,30
	Medium 199	1.8	0.8	143	5.4	269,270
	Minimum essential media (MEM)	1.8	1	140	5	102
	Leibovitz media (LM)	3.5	0.8	138	6	274
	Roswell Park Memorial Institute (RPMI) media	2.3	2.5	129	5	100
	Roswell Park Memorial Institute (RPMI)-1640 media	2.3	2.7	131	5	101

Notes: *Information from Table 1; **can only be approximated.[273]

Cations in human fluids

The most interesting and medically important cationic environments are within the human body. Although the vascular system provides an environment for a host of different cell types, including red blood cells, platelets, white blood cells, and endothelial cells, it is by no means the only extracellular fluid environment. Every organ and cavity contains fluids that act as soluble environments for a number of different cell types. In many cases, the fluids generated by organs are released into other regions of the body or from the body. It should first be realized that, although high in Na^{+}, human blood by no means mimics seawater. Blood (Table 3) contains approximately one-fourth the level of Ca^{2+}, one-third the level of Na^{+}, less than half the level of K^{+}, and seventy times less than the level of Mg^{2+} found in seawater (Table 1). Interstitial fluid, which bathes cells in tissues, mimics the

Table 3 Concentration of the major soluble cations in extracellular fluids

Extracellular tissue fluid	Ca^{2+} [mM]	Na^{+} [mM]	Mg^{2+} [mM]	K^{+} [mM]	References
Blood plasma	2.5	138	0.8	4.2	19, 113, 275–280
Interstitial fluid[a]	1.2	135	0.5	3.2	103
Sweat	2.5	65–82[$]	0.2–0.3[$]	5–8[$]	281, 282
Saliva	0.9–1.6	17–23	0.05–0.1	19.2–19.9	283, 284
Pancreatic fluid	0.1–0.2	140	0.1–0.2	5	276
Stomach	0.6	68	0.7	13.4	285
Duodenum	2–6	107–150	0.7	4.3–10.9	286–292
Jejunum	0.5–8.5	140	0.9	5–6	276, 285, 292–294
Ileum	2.5	138	0.9	6	293, 294
Bile	7	140	3.5	5–10	276
Prostatic fluid	30	157	16.7	66.8	295, 296
Cerebrospinal fluid	1.2–1.4	147	1.2–3.7	2.7–2.8	114, 297, 298
Bone	2–40**	145	0.8	25	104, 105, 299–301
Amniotic fluid	3.6–4.1[b]	128–134[b]	0.6–1.4[b]	3.9–4.6[b]	302–304
Scarpa's fluid (endolymph)	0.02	1.3	0.63	157	305, 306
Perilymph	1.3	148	0.85	4.2	306, 307
Follicular fluid	1.0	141	0.7	4.6	308
Synovial fluid	2.7	110	1.0	4	309, 310
Urine*	5	132	7	25	110, 311
Milk	7.5	4.7	1.4	15.2	312

Notes: [a]Measured as plasma hemodilution due to change of a position by healthy subjects; [b]Varies due to time course of gestation; [$]Reflects a variation due to exposure (to 100°F) time up to 16 days; *Data depends on nutritional status; **In vivo studies have been performed on human cell lines and on rabbit bone crypts. The range reflects the extracellular calcium concentration at resorbing and nonresorbing sites.
Abbreviation: HUVEC, human umbilical vein endothelial cells.

cationic concentrations in blood (Table 3).[103] The fluids in other body locations contain cation concentrations generally similar to that of blood, with exceptions (Table 3). The cationic contents of spinal fluid, cerebral fluid, synovial fluid, and urine are highly similar to that of blood and interstitial fluid (Table 3). Sweat and saliva, however, have much lower Na^{+} and Mg^{2+} concentrations, but higher K^{+} concentrations (Table 3). Pancreatic fluid has lower Ca^{2+} and Mg^{2+} concentrations, while prostate fluid contains approximately 30 times the Ca^{2+} concentration, 12 times the K^{+} concentration, and 10 times the Mg^{2+} concentration of blood (Table 3). Perhaps the most interesting and dramatic cationic fluctuations are those of Ca^{2+} in regions of bone resorption and formation. The Ca^{2+} concentration in osteopathic lacunae can rise from 2 to 40 mM during bone resorption in the crypts of the rabbit ear chamber.[104–106] There is evidence to suggest that similar extracellular fluctuations in Ca^{2+} concentration occur in humans.[107,108]

In humans, a variety of diseases affect the cation concentrations of body fluids. In patients with hyperkalemia, potassium levels in serum can increase to above 7 mM in severe cases.[109] The causes of hyperkalemia are varied, including dietary imbalances, rhabdomyolysis (rapid muscle breakdown following severe injury), tumor cell lysis, renal failure, and Addison's disease.[109] Individuals with prostatitis exhibit reduced levels of K^{+} in their urine and, over time, a rise in prostate fluid.[110] Individuals can also suffer from hypercalcemia.[111] Patients with cancer of the parathyroid can have life-threatening concentrations of serum Ca^{2+}.[112] The preceding examples represent only a fraction of diseases associated with defects in the regulation of cation concentrations in body fluid.[109,110,113–115]

A bona fide receptor

The transport classification of the International Union of Biochemistry and Molecular Biology groups channels, transporters, and exchangers into nine classes, each with three or more families (http://www.tcdb.org/). The classification is constantly updated and databases are available to compare transporter protein sequences between or among species and to predict structures[116–118] (http://www.membranetransport.org/, http://www-abcdb.biotoul.fr/; http://lab.digibench.net/transporter/). No distinction is made in this scheme as to whether a protein is capable of directly sensing extracellular ions[4,119] and, therefore, may have the potential to activate a signal transduction pathway, the cytoskeleton, or a membrane-associated protein.

Membrane proteins transporting extracellular cations can be activated by an intracellular signal (Figure 2A), a signal from a plasma membrane-associated protein (Figure 2A),

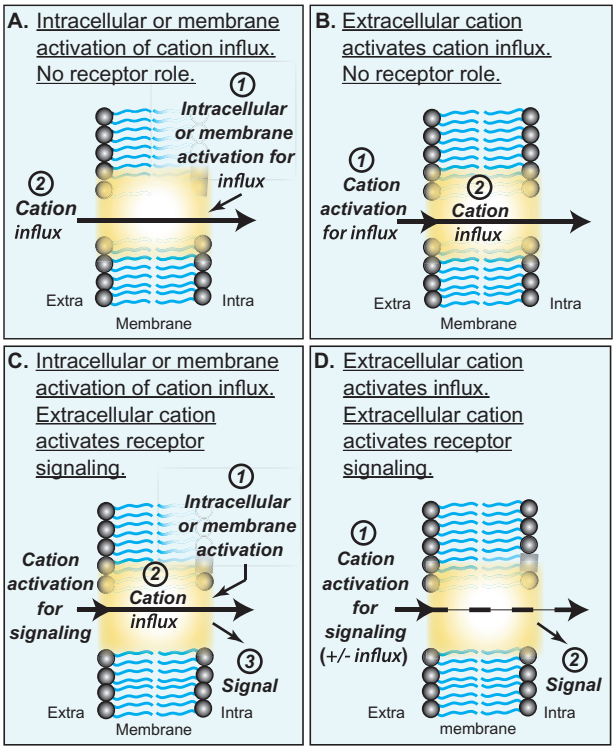

Figure 2 Models of how plasma membrane molecules may or may not function as cation receptors. **A**) An intracellular or plasma membrane-associated molecule activates the transporter in the plasma membrane, resulting in cation influx. In this case, the cation transporter does not act as a cation receptor for signaling. **B**) The cation is sensed by the transporter and transported into the cell. In this case, the transporter does not act as a receptor for signaling. **C**) An intracellular or membrane molecule activates the transporter, resulting in cation influx and cation activation of a signal. In this case, the transporter acts as a receptor for signaling. **D**) The cation is sensed by the transporter, which may or may not result in influx, but which does result in signaling. In this case, the transporter acts as a receptor for signaling.

or by the extracellular cation (Figure 2B).[4,12,18,21,120–125] None of these scenarios exclude the possibility that the activated membrane protein, upon interacting with the extracellular cation, then functions as a coupled receptor (Figures 2C and 2D). To be a bona fide extracellular cation receptor, a molecule must interact at the surface of the cell with a cation and through that interaction directly activate a signal transduction pathway, the cytoskeleton, or a membrane-associated protein (Figure 2). The cation may or may not be transported intracellularly.

A bona fide Ca^{2+} receptor

A bona fide Ca^{2+} receptor (CaR) unrelated to the channels studied earlier in muscle contraction and synapses,[126,127] was cloned from the parathyroid.[31,128] CaR was then identified in a wide array of other cell types.[11,32] Disease states correlating with mutations in the protein were then identified.[47,129–132] Although its function can be related to Ca^{2+} homeostasis,[133] it is also required for hematopoietic stem cell migration during development[36] and is involved in chemotaxis in Ca^{2+} gradients in a variety of cell types, including osteoclasts,[43] osteoblasts,[38,40] keratinocytes,[46] gonadotropin-releasing hormone neurons,[42,134] and monocytes and macrophages.[135] CaR appears to be the only family 3/C G protein-coupled calcium receptor that can activate signal transduction pathways solely by binding Ca^{2+}.[136] CaR can also modulate the activity of other receptors in the plasma membrane. For example, MacLeod et al[137] showed transactivation of the epidermal growth factor receptor by CaR. In addition, Huang et al[138] explored the hypothesis that CaR interacted with other ion channels in the regulation of electrolyte homeostasis. They showed that in human embryonic kidney cells, CaR interacted with two separate but related K^+ channels, Kir4.1 and Kir4.2, both of which are present along with CaR in the distal nephron of the kidney. Furthermore, measurements of currents across *Xenopus laevis* oocyte membranes indicated that interaction of CaR and K^+ channels inhibited currents through the latter. Ion flow was restored (ie, not inhibited) in oocytes expressing CaR mutations that abolished its ability to interact with the K^+ channels.

Biochemical assays of the properties of CaR revealed that it is a low affinity receptor, with a K_D of 4.2–18.8 mM,[139] containing multiple Ca^{2+} binding sites.[140] Mg^{2+} is also an agonist, although less potent. CaRs can also be activated or modulated by stimuli other than Ca^{2+}, including amino acids, polyamines,[141] polypeptides, and increased pH,[142] a subject reviewed in Magno et al.[133]

CaR is a member of the Group II family C G protein-coupled receptors, members of which have seven membrane domains.[11] The extracellular domains of these receptors form two lobes separated by a cleft[32,136] and hence are referred to as having a venus-fly trap structure that oscillates between an open and a closed state in the absence of ligand. It has been suggested that ligand-binding to both lobes is required to stabilize the trap in the closed conformation to activate the receptor.[136] The ligand-binding sites of CaR have not been precisely identified because the crystal structure has not been solved and no direct and quantitative method to measure Ca^{2+} binding exists.[130,133] The transmembrane helices of CaR are linked by intracellular and extracellular loops, as is the case for other members of the G protein-coupled receptor family.[97,136,143–145] Mutational analyses of the loops of CaR suggest that the second intracellular loop is required for G protein-mediated signaling.[146] CaR interacts with multiple G proteins, including $G\alpha_i$, $G\alpha_q$, $G\beta\gamma$, and $G\alpha_{12/13}$.[147–149]

The intracellular carboxy-terminal tail of CaR, which is the least conserved region of the protein,[150] plays an essential role in phosphoinositide metabolism.[138–140] Other regions of the tail have been shown to bind a number of additional accessory proteins, including filamin, an actin cross-linking protein.[151,152]

Ca^{2+} binding to CaR has been shown to initiate complex downstream pathways. Phospholipase C,[143,148] phospholipase A2,[153] and phospholipase D[149]are activated in some cases, and the synthesis of second messengers, including inositol triphosphate and diacylglycerol, are induced.[154] Several components of kinase cascade signal transduction pathways can also be activated, including ERK, c-Jun NH2-terminal kinase (JNK), and p38MAPK.[155] Recently, Boudot et al[43] provided evidence that the PI3 kinase/Akt signal transduction pathway is activated by extracellular calcium via CaR in the osteoclast precursor cell line RAW264.7, and that activation is required for optimal cell migration during Ca^{2+} chemotaxis. siRNA directed against the Ca^{2+} sensing receptor abolished phosphorylation of Akt, a PI3 kinase target. In addition, Ca^{2+}-stimulated migration was abolished in RAW264.7 cells expressing a kinase-dead point mutation in Akt.[43]

Therefore, CaR provides the first documented example of a coupled receptor that is directly activated by an extracellular cation, and provides a well established model for testing whether or not other molecules in the plasma membrane function as coupled cation receptors.

IP_3R, another potential Ca^{2+} receptor

There is also reason to believe that the inositol triphosphate receptor, IP_3R, may function as a Ca^{2+} receptor. IP_3R is a member of a diverse group of cation-permeable channels, ie, the transient receptor potential channels, which can be activated by a variety of stimuli. The first transient receptor potential gene, *Drosophila trp,* was cloned in 1989[156] following its initial discovery through analysis of a spontaneously occurring photoreceptor mutation.[157,158] Transient receptor potential channels are now considered a superfamily[159] defined by sequence homology and topology.[160] The numerous members of the seven subfamilies vary in regulatory mechanisms[161,162] as well as cation selectivity.[163,164] Many members are non-selective cation channels.[161] As a general rule, transient receptor potential channels are tetrameric structures, each monomer comprised of six transmembrane domains with a pore-associated region between domains 5 and 6.[162]

IP_3R is usually found in the endoplasmic reticulum,[165,166] and is not coupled to G proteins. Both the COOH and NH_2 termini are cytoplasmic (relative to the endoplasmic reticulum).[167–169] IP_3R was demonstrated to be required for Ca^{2+} release into the cytoplasm from endoplasmic reticulum stores following any signal that elevates its ligand, IP_3.[170] However, IP_3Rs have also been located in the plasma membrane.[168,171–173] Subcellular fractionation studies by Rossier et al[172] implicated actin filaments in maintaining the close association of IP_3Rs with the plasma membrane. Fadool and Ache[173] used electrophysiological measurements to describe receptors in the plasma membrane of *Panulirus argus* olfactory neurons that resembled, but were not identical to, classic IP_3Rs. Dellis et al[171] used whole-cell recordings in the presence or absence of specific inhibitors to analyze conductance of IP_3R, as well as IP_3R pore mutants in the plasma membrane of DT40 B cells in response to stimulation of the B cell receptor. They concluded that although a small number of IP_3Rs were localized in the plasma membrane, they contributed substantially to responses induced by B cell receptor ligand-binding.

In *D. discoideum* chemotaxis, Traynor et al[174] cloned a gene encoding an IP_3-like receptor, IplA. $^{45}Ca^{2+}$ uptake in response to the chemoattractant cAMP was abolished in an *iplA* null mutant. However, these cells still underwent chemotaxis in a spatial gradient of cAMP, leading the authors to conclude that intracellular Ca^{2+} signaling was not essential for cAMP chemotaxis.[174] Subsequently, Schaloske et al[175] found that although an agonist-induced Ca^{2+} entry pathway remained intact in *iplA* null cells, store-operated calcium entry channels did not function. Fache et al[176] showed that *iplA*$^-$ cells were defective in calcium stimulated motility under high shear stress conditions, suggesting IplA may be a mechanoreceptor. Recently, it was discovered that in addition to serving as a mechanoreceptor, *iplA* serves as the receptor for Ca^{2+} chemotaxis in *D. discoideum* (Lusche et al, in preparation). The recently described Ca^{2+} chemotaxis system for *D. discoideum*[5] will be described in a subsequent section of this review.

Other potential Ca^{2+} receptors

There are additional plasma membrane proteins with CaR-like characteristics. The neurotransmitter, glutamate, is the ligand for metabotropic glutamate receptors (mGluRs) which are present in the central nervous system.[136] A subset of these receptors (mGluRs 1–8) is also activated by calcium.[177,178] Tabata and Kano[179] reported that sensitivity to glutamate was enhanced by calcium in Purkinje cells. Gamma aminobutyric acid (GABA) B receptors bind the inhibitory neurotransmitter

GABA. Calcium binds the venus-fly trap domain of these receptors next to the binding site for GABA.[180] Ca^{2+} appears to control the affinity of these receptors for GABA.[136] Therefore, extracellular Ca^{2+} can directly interact with a receptor to affect activation by a more specific ligand.

Monovalent cation receptors

Although some monovalent cation channels, transporters, and exchangers may sense extracellular cation concentrations, there has been no demonstration of a bona fide monovalent cation receptor in the plasma membrane for the major ions, Na^+ and K^+. However, there have been hints. For instance, the putative monovalent cation/H^+ exchanger, Nhe1, of *D. discoideum*[181] mediates enhancement of cAMP chemotaxis by 15 mM K^+ or Na^+, and the facilitation of motility by 40 mM K^{+4}. ClustalW sequence alignment[182] as well as a Blast search[5] gave the best alignment with the Nhe8 isoform of a number of other species, including humans. Homologies of domains of Nhe1 to a number of receptors in other species suggested that it may serve as an extracellular K^+ receptor. *D. discoideum* Nhe1 falls into a family of proteins known to exchange Na^+ or K^+ for protons without directly altering membrane potential,[119,183,184] a characteristic of G protein-coupled receptors, including those that sense divalent cations.[185,186] In addition, a search of the Conserved Domain Database revealed that *D. discoideum* Nhe1 contains a KefB domain,[4] which is known to interact with K^+ in a variety of transport proteins.[187–190] The first 30 amino acids of the N-terminal domain of Nhe1 contain a signal peptide common to the first transmembrane domain of a class of trimeric G protein-coupled receptors found in higher eukaryotes.[191,192] A 150-amino acid region is homologous to a similarly positioned region in a *Caenorhabditis elegans* family of chemosensory receptors.[193,194] Finally, Patel and Barber[181] demonstrated that Nhe1 was localized in the plasma membrane and showed, as Lusche et al[4] did subsequently, that the null mutant was defective in polarity, motility, and chemotaxis in buffer solutions containing a concentration of K^+ that facilitates these attributes in wild-type cells. Most importantly, the *nhe1*$^-$ mutant when compared with its parent strain did not exhibit a major difference in the concentration of cytosolic H^+ in buffer,[181] suggesting it may function more as a receptor than as a monovalent cation/H^+ exchanger, although its effect could still be on subtle changes in intracellular pH.[181]

Examples of monovalent cation channels that may prove to have receptor functions include the P2X-ATP-gated channels,[195] the ligand-gated ion channels[196,197] and the glutamate-gated ion channels.[198–204]

Extracellular cations facilitate cell motility and polarity

Although the concentration of an extracellular cation can increase rapidly in the environment of a cell or form a transient spatial gradient, it is likely that most of the time the concentration is relatively constant, and cells function at an optimal concentration. In support of this hypothesis, in vitro studies of the motility of a variety of cell types have revealed optimum concentrations of key cations, most notably Ca^{2+}, Na^+, and K^+ (Table 4). In studies of the facilitation of motility by Ca^{2+}, the optimum concentrations were found to be 1–10 mM for cyanobacteria, 9 mM for sea urchin sperm, and 10–20 mM for *D. discoideum* amoebae (Table 4). These optima may seem high (Table 4), but they are actually within the concentration range of the natural environments of these organisms (Table 1). For a variety of human cells, including bronchial epithelial cells, prostate carcinoma cells, human keratinocytes, human umbilical vein endothelial cells, fibroblasts, and melanoma cells, the optimum concentrations of Ca^{2+} range between 0.4 and 5 mM (Table 4), a range that encompasses the Ca^{2+} concentration of blood and interstitial fluid (Table 3). For the motility of murine macrophages and keratinocytes, the optimum Ca^{2+} concentrations are 1.6 mM and 0.5 mM, although one study found the relatively high concentration of 9.2 mM to be optimum for murine fibroblasts (Table 4). The optimum Mg^{2+} concentrations for a variety of cell types was measured by Grzesiak and Pierschbacher [205] to be 1.4 mM, which is close to the concentrations in blood and interstitial fluid. Therefore, it appears that most human migratory cells require divalent cation concentrations close to that of blood and interstitial fluid for maximum motility. One can only wonder how defective cell motility might be in environments in the body, such as in bile, prostate fluid, areas of bone resorption, and milk, where Ca^{2+}, and in some cases Mg^{2+}, can reach extremely high concentrations (Table 3).

The optimum concentration of monovalent cations is also quite interesting. For ocean cyanobacteria and at least two species of *Vibrio*, maximum motility is reached at concentrations well below that in the ocean, but motility remains maximum as concentrations are raised higher (Table 4). This suggests that there is tolerance after the optimum concentration is attained. The fact that cyanobacteria are found in all known aquatic environments may explain such tolerance. Perhaps the biggest enigma is that the optimum

Table 4 Cationic enhancement of cell motility

Cell type	Cation	Optimal concentration	Assay	References
Human bronchial epithelial cells	Ca^{2+}	5 mM	Scratch wound assay	313
Human keratinocytes	Ca^{2+}	1.6 mM	Boyden chamber	205
HUVEC	Ca^{2+}	1.6 mM	Boyden chamber	205
Human lung fibroblasts	Ca^{2+}	1.6 mM	Boyden chamber	205
Melanoma cells	Ca^{2+}	4 mM	Boyden chamber	314
Murine fibroblasts	Ca^{2+}	9.2 mM	Cells filmed in culture flasks	206
Murine macrophages	Ca^{2+}	1.6 mM	Boyden chamber	205
Murine keratinocytes	Ca^{2+}	0.5 mM	Boyden chamber	46
Sea urchin spermatozoa	Ca^{2+}	9 mM	Cells filmed on slides	315
Cyanobacterium *Synechococcus*	Ca^{2+}	1–10 mM	Cells filmed on slides	316
Dictyostelium discoideum	Ca^{2+}	10–20 mM	Sykes-Moore perfusion chamber	3
Human keratinocytes	Mg^{2+}	1.4 mM	Boyden chamber	205
HUVEC	Mg^{2+}	1.4 mM	Boyden chamber	205
Human lung fibroblasts	Mg^{2+}	1.4 mM	Boyden chamber	205
HUVEC	Mg^{2+}	0.1 mM	Boyden chamber	44
Murine macrophages	Mg^{2+}	1.4 mM	Boyden chamber	205
Bovine capillary endothelial cells	Mg^{2+}	2.4 mM	Boyden chamber	317
Murine fibroblasts	Na^{+}	56 mM	Cells filmed in culture flasks	206
Rat spermatozoa	Na^{+}	40 mM	Cells filmed on slides	318
Cyanobacterium *Synechococcus* strain WH8113	Na^{+}	150–250 mM	Cells filmed on slides	50
Marine species of *Vibrio*	Na^{+}	50 mM	Cells filmed on slides	319
Vibrio cholerae	Na^{+}	200 mM	Cells filmed on slides	320
Marine species of *Halomonas*	Na^{+}	400 mM	Cells filmed on slides	321
Melanoma cells	K^{+}	30–54 mM	Boyden chamber	314
Human T cells	K^{+}	30 mM	Boyden chamber	322
Human PMN	K^{+}	125 mM	Cells filmed on slides	323
Murine fibroblasts	K^{+}	0.5–73 mM	Cells filmed in culture flasks	206

Abbreviations: PMN, polymorphonuclear leukocytes; HUVEC, human umbilical vein endothelial cells.

concentration of K^+ for human cell motility can be 10 times that in blood and interstitial fluid (Table 4). For murine fibroblasts, but not spermatozoa, these high K^+ optima are close to that for Na^+ (Table 4). Since the studies to assess K^+ optima were performed in low Na^+, what may have been identified is the optimum for either Na^+ or K^+, cations that can interchangeably interact with surface molecules. This has been demonstrated for the monovalent cation/H^+ exchanger Nhe1 in *D. discoideum*.[181] Gail et al[206] demonstrated that although mouse fibroblast motility exhibits high tolerance for extreme changes in the extracellular concentrations of Na^+ and K^+, but requires a more precise optimum Ca^{2+} concentration, Lusche et al[3,4] further demonstrated that the motility of *D. discoideum* could be similarly facilitated by either extracellular K^+ at 40 mM or Ca^{2+} at 10–20 mM, but through different surface molecules. Hence the optimization of motility can be complex and can be facilitated by different cations with different valances, and through different molecules on the same cell surface.

Many types of animal cells must polarize to translocate at maximum velocity. Hence cell polarization also requires optimum concentrations of cations. Optimum Ca^{2+} concentrations have been identified for the polarization of *D. discoideum* amoebae,[2–4] and human polymorphonuclear leukocytes,[207] stratification of keratinocytes,[208] spreading of human umbilical vein endothelial cells,[44] and detachment of intercellular contacts by Madin-Darby canine kidney cells[209] (Table 5).

Additional transporters that may prove to be receptors

We have already discussed how the Nhe1 of *D. discoideum* mediates both the Na^+/K^+ requirement for chemotaxis and the K^+ requirement for motility and polarity.[4] There is no clear evidence that Nhe1 performs this role simply as an exchanger, as a receptor, or both. Because Ca^{2+} can substitute for K^+ or Na^+ to facilitate the same cellular changes,[3,4] and does so through a different cell surface molecule that appears to be a

Table 5 Cationic regulation of cell shape

Cell type	Cation	Optimal concentration	Effect	References
Human PMN	Ca^{2+}	1–5 mM	Required for polarization	207
HUVEC	Ca^{2+}	1 mM	Reduced spreading of cells	44
Rat osteoclasts	Ca^{2+}	20 mM	Reduced cell size	324
MDCK	Ca^{2+}	<5 mM	Cells contracted	209
Murine keratinocytes	Ca^{2+}	1.2 mM	Required to stratify, keratinize normally	208
Dictyostelium discoideum	Ca^{2+}	10 mM	Elongation and suppression of lateral pseudopods	3
MDCK	Mg^{2+}	<5 mM	Cells contracted	209
Rat vascular smooth muscle cells	Mg^{2+}	1.2 mM	Cells contracted	325
Human PMN	K^+	125 mM	Induced polarization	323

Abbreviations: PMN, polymorphonuclear; MDCK, Madin-Darby canine kidney; HUVEC, human umbilical vein endothelial cells.

receptor (Lusche et al, in preparation), it has been proposed that Nhe1 acts as a receptor, and that the Ca^{2+} receptor and Nhe1 function independently upstream of the same signal transduction pathway.[4] Observations on the channel requirements of neutrophil chemotaxis relate to these observations on *D. discoideum* chemotaxis. Neutrophil chemotaxis to fMLP is dependent upon transient receptor potential and chloride channels,[210–213] as well as an Nhe channel,[214] the latter from the same family of which Nhe1 of *D. discoideum* is a member.[215] Neutrophils respond to the rapid addition of fMLP by an influx of calcium. The rise in cytosolic calcium is necessary for migration.[216] However, Kindzelskii et al[213] also found that the transient receptor potential channels were involved in chemotaxis and localized in lipid rafts at the leading edge in fMLP-stimulated cells. This may be related to the possible formation of Ca^{2+} gradients in the cytosol of neutrophils undergoing chemotaxis up FMLP gradients.[5,35,38,45,213,217] Ritter et al[214] demonstrated that the addition of an Nhe inhibitor (HOE694, HOE642, or EIPA) disrupted motility during chemotaxis in a Boyden chamber.

deHart et al[218] showed that the K^+ channel, Kir4.2, is involved in the migration of microvascular endothelial cells and mouse embryonic fibroblasts. They showed that GFP-α9β1 integrin colocalized with mCherry-tagged Kir4.2 at focal adhesion sites along the leading edge during migration and that migration could be inhibited by a small hairpin RNA knockdown of Kir4.2. Because the activity of these channels could not be measured during migration, the authors speculated that local K^+ efflux, mediated by Kir4.2, regulated the rate of migration through integrin-mediated modulation of cell extensions, possibly by inhibition of lateral protrusions. However, colocalization experiments also suggested that the Kir4.2 channel may function as a receptor, interacting directly with integrin at focal adhesion sites.

A last example worth considering is transient receptor potential melastatin 8 (TRPM-8), a channel that primarily mediates Na^+ and Ca^{2+} influx.[219] It is localized apically in epithelial secretory prostate cells.[220] Because this channel is upregulated in the early stages of prostate cancer and downregulated during progression to the invasive stage,[221] it has been considered a potential target for new cancer therapies.[222] Gkika et al,[223] using whole-cell patch clamp measurements, showed that treatment of human embryonic kidney-TRPM8-inducible cells increased Ca^{2+} entry via activated TRPM-8 channels. In addition, cell surface biotinylation experiments revealed that treatment with prostate serum antigen enhanced the expression of TRPM-8 channels in the plasma membrane of human embryonic kidney-TRPM8 cells, as well as in cells of the PC3 prostate cancer cell line. Inhibitor studies indicated that prostate serum antigen activated TRPM-8 via the bradykinin 2 receptor, a classic 7 transmembrane G protein-coupled receptor. Interestingly, migration of PC3 cells was reduced by prostate serum antigen-induced TRPM-8 activation. The authors concluded that prostate serum antigen activation of TRPM-8 channels may inhibit metastasis in the early stages of cancer, but the gradual loss of TRPM-8 leads to an invasive cellular phenotype. Although, there was no indication that human embryonic kidney-TRPM8 played a role as a receptor, the complexity of the human embryonic kidney-induced response makes one wonder if TRPM-8 plays a role as a Ca^{2+} channel receptor as well. Ion channel activity has also been implicated in the progression of numerous other cancers,[224] including those of the cervix[225] and brain.[226] The correct experiments to test this hypothesis have not been performed, given the pervasive assumption that the role of channels, transporters, and exchangers is solely to move cations in or out of cells.

Chemotaxis in cation gradients

In previous sections of the review, we have focused primarily on the "global" effects of extracellular cations on cell behavior. However, cations can form soluble gradients

through diffusion if released from a source. Although we have very little direct proof for the generality of such gradients, their existence has been demonstrated in such events as bone resorption, in which the release of Ca^{2+} has been measured.[104] We can also speculate on their existence by the behavior of cells in cation gradients generated in vitro. Here, we review the literature on the possible role of chemotaxis up cation gradients, primarily Ca^{2+} gradients, during development, tissue maintenance, angiogenesis, disease states, and *D. discoideum* aggregation.

Development

Ca^{2+} chemotaxis has been implicated in the homing of newly formed immune cells. Hematopoietic stem cells give rise to cells of both myeloid and lymphoid lineages in the fetal liver. Hematopoietic stem cells then migrate from the fetal liver to the bone marrow, where they reside in the endosteal niche, an area of active bone remodeling. The expression of CaR on the surface of murine hematopoietic stem cells was confirmed by flow cytometry and by reverse transcription-polymerase chain reaction, lending support to the idea that extracellular Ca^{2+} played a role in targeting of hematopoietic stem cells.[36] Histological analyses revealed a striking absence of hematopoietic stem cells in $CaR^{-/-}$ mice.[36] Hematopoietic stem cells from $CaR^{-/-}$ mice engrafted into irradiated wild-type mice failed to migrate to the endosteal niche. These studies suggested that the defect was due to an inability of the $CaR^{-/-}$ hematopoietic stem cells to detect extracellular Ca^{2+} gradients.

Ca^{2+} also acts as a possible second chemoattractant in the migration of gonadotropin-releasing hormone neurons from their point of origin in the anterior nasal compartment to the hypothalamus during development. The role of Ca^{2+} gradients was studied in vitro using olfactory-derived (GN11) and hypothalamus-derived (GT1-7) murine cell lines.[42] Cells of both lines chemotaxed up Ca^{2+} gradients in Boyden chambers. Both cell lines also underwent chemotaxis up gradients of the monocyte chemoattractant protein-1. Elevated levels of Ca^{2+} induced the production of monocyte chemoattractant protein-1 in GT1-7 cells, but not in GN11 cells, indicating that extracellular Ca^{2+} may promote gonadotropin-releasing hormone neuronal migration to the hypothalamus through several mechanisms. The Ca^{2+} effects appear to be CaR-mediated, because both chemotaxis and the increased production of monocyte chemoattractant protein-1 in high Ca^{2+} were attenuated in cells expressing a dominant-negative form of CaR. Numerous, less detailed observations on the effects of Ca^{2+} and other cations on motility and chemotaxis during development, suggest that cation chemotaxis may indeed play a more general role and this role may involve cation receptors.

Tissue maintenance

The process of bone remodeling, which is a balance between bone resorption and bone formation, is carried out by osteoclasts and osteoblasts, respectively. It is one of the most interesting examples of cationic chemotaxis. Osteoclast precursors, which are mononuclear cells recruited from the circulation, enter the bone and fuse to form multinucleated osteoclasts that degrade bone. The concentration of extracellular Ca^{2+} in the area of bone resorption reaches 40 mM. A murine mononuclear osteoclast precursor cell line was used as a model to determine if Ca^{2+} gradients attract mononuclear cells to the bone.[43] Boyden chamber assays revealed that the osteoclast precursor cells migrate up Ca^{2+} gradients. The chemotactic effect of Ca^{2+} is mediated through CaR, because migration is inhibited when siRNA is used to knock down CaR expression.[43] A murine osteoblast-like cell line, also expressing CaR, undergoes chemotaxis in a Ca^{2+} gradient.[38,40] This story would obviously be complete if both a Ca^{2+} gradient and migrating cells could be visualized simultaneously in vivo, but a refined method for performing this has not been developed to date.

The homing of immune cells to sites of injury and infection is an essential part of the host defense response, a process generally attributed to the induction of cell migration by gradients of chemokines. Extracellular fluids at these sites have been reported to have elevated levels of Ca^{2+}.[227,228] Human monocytes express CaR. Monocytes chemotax up Ca^{2+} gradients,[39] and chemotaxis is enhanced in combined gradients of monocyte chemotactic protein-1 and Ca^{2+}, indicating that Ca^{2+} gradients may function alongside chemokine gradients to enhance the chemotactic response. These results were confirmed by in vivo studies where mice were injected at homing sites with 5 mM Ca^{2+}, monocyte chemotactic protein-1, or a combination of both, and monocyte infiltration was quantified by immunostaining of tissue sections.[39]

Ca^{2+} chemotaxis has also been implicated in angiogenesis. This process is important in the progression of wound healing, tissue remodeling, and tumor formation. Multiple cell types are involved in this process, including endothelial progenitor cells from blood and bone marrow, which possess stem cell-like qualities similar to those of hematopoietic stem cells.[35] Because extracellular Ca^{2+} regulates homing of hematopoietic stem cells to endosteal niches,[36] Aguirre et al[35] investigated the effects of extracellular Ca^{2+} on endothelial progenitor cells derived from rat bone marrow. Expression

of CaR on the surface of endothelial progenitor cells was demonstrated by immunofluorescent staining with two antibodies against different receptor epitopes. Endothelial progenitor cells underwent chemotaxis in a gradient of extracellular Ca^{2+}. Lapidos et al[44] also reported that human umbilical vein endothelial cells underwent chemotaxis in Mg^{2+} gradients. At the lower concentration of 0.1 mM, Mg^{2+} had a chemokinetic effect. It is unclear why similar effects were not observed for Ca^{2+} in this particular study, given that others have reported that human umbilical vein endothelial cells exhibit increased motility when stimulated with millimolar concentrations of Ca^{2+} in the range of those measured in wound fluid,[205] and both Ca^{2+} and Mg^{2+} levels increase after wounding. These few examples suggest that cation gradients and cation receptors may play roles in other aspects of tissue maintenance.

Disease states

Recently it was demonstrated that Ca^{2+} may be involved in macrophage chemotaxis.[135] When tested in Ca^{2+} gradients in Boyden chambers, macrophages and macrophage-derived foam cells, the lipid-sequestering cells found in atherosclerotic plaques, underwent chemotaxis, but Ca^{2+} concentrations in the source of a Boyden chamber higher than 1 mM inhibited cell motility. This is lower than the normal range for Ca^{2+} chemotaxis in Boyden chambers (Table 6). These results suggest that high calcium might generate gradients to recruit macrophages and foam cells to the developing lesions, but once the cells migrate into a lesion, the high Ca^{2+} concentration suppresses motility, leading to the retention of those cells in the atherosclerotic plaque.

Cationic chemotaxis has also been implicated in cancer metastasis. Breast cancer cells have a propensity to metastasize to bone. This preference is believed to be due to the release of several chemotactic factors during bone resorption, one of which is Ca^{2+}. Saidak et al[47] found that, in Boyden chamber assays, cells of the highly metastatic line, MDA-MB-231, exhibited a strong chemotactic response to Ca^{2+}, cells of the moderately metastatic lines, MCF7 and T47D, exhibited a more moderate response, while cells of the nonmetastatic line, BT474, cells did not respond.[47] Knockdown of CaR by siRNA inhibited chemotaxis up Ca^{2+} gradients in the responding cell lines. Because CaR was expressed in all of the cell lines, the authors concluded that CaR was necessary, but not sufficient, for Ca^{2+} chemotaxis, and that additional factors were at play.[47]

D. discoideum chemotaxis

Finally, chemotaxis of *D. discoideum* amoebae up Ca^{2+} gradients has been demonstrated using microfluidic chambers.[5] *D. discoideum* underwent chemotaxis in gradients in which 10 mM Ca^{2+} was at the source, roughly the same concentration effective in the source well of Boyden chambers for a number of cell types (Table 6). A parallel gradient

Table 6 Chemotaxis to cations

Cell type	Cation	Optimal concentration	Methods	References
Human MDA-MB-231 MCF7	Ca^{2+}	5 mM	Boyden chamber and scratch wound assay	47
Human monocytes	Ca^{2+}	5 mM	Boyden chamber	39
Human monocytes	Ca^{2+}	3–10 mM	Boyden chamber	40
Murine hematopoietic stem cells	Ca^{2+}	n/a	In vivo engraftment of $CaR^{-/-}$ cells into wild-type mice	36
Rat bone marrow endothial progenitor cells	Ca^{2+}	3–10 mM	Boyden chamber	35
Murine RAW 264.7	Ca^{2+}	10 mM	Boyden chamber	43
Murine monocytes	Ca^{2+}	5 mM	Boyden chamber	39
Murine monocytes	Ca^{2+}	5 mM	In vivo injection of Ca^{2+} and subsequent quantitation of infiltrating monocytes	39
Murine GnRH neurons	Ca^{2+}	7.5 mM	Boyden chamber	42
Murine macrophage derived foam cells	Ca^{2+}	1 mM	Boyden chamber	135
Murine macrophages	Ca^{2+}	1 mM	Boyden chamber	135
Murine MC3T3-E1 Osteoblast-like	Ca^{2+}	5 mM	Boyden chamber	38
Murine MC3T3-E1 Osteoblast-like	Ca^{2+}	3–10 mM	Boyden chamber	40
Murine MC3T3-E1 Osteoblast-like	Ca^{2+}	4.8 mM	Boyden chamber	326
Dictyostelium discoideum	Ca^{2+}	10 mM	Microfluidic chamber	5
Bracken fern spermatozoids	Ca^{2+}	2.5 mM	Capillary	48
HUVEC	Mg^{2+}	10 mM	Boyden chamber	44
Rat bone marrow endothial progenitor cells	Mg^{2+}	10 mM	Boyden chamber	35

Abbreviations: HUVEC, human umbilical vein endothelial cells; GnRH, gonadotropin-releasing hormone; CaR, Ca^{2+} receptor.

of the chemoattractant cAMP enhanced chemotaxis in a Ca^{2+} gradient, the same result obtained with monocytes in parallel gradients of Ca^{2+} and MCP-1.[39] An opposing gradient of cAMP overrode chemotaxis to Ca^{2+}, suggesting that a cAMP gradient presented a stronger chemotactic signal than a Ca^{2+} gradient.[5] A high global cAMP signal has been shown to induce the release of Ca^{2+}, suggesting that as cAMP is released through a population, it causes a transient, coordinated release of Ca^{2+} and the formation of transient Ca^{2+} gradients. Ca^{2+} may function as a second chemoattractant that finetunes cellular movement in the front of each cAMP wave.[5] The fact that many of the animal cell types that undergo chemotaxis to Ca^{2+} also undergo chemotaxis to cell type-specific signals raises the possibility that Ca^{2+} chemotaxis is ancient and conserved, and that cell types have added other cell type-specific chemotaxis systems later in evolution.[5] In contrast with the results on Ca^{2+} chemotaxis, there are no definitive studies demonstrating that monovalent cations serve as chemoattractants, rather than simply chemokinetic agents, for animal cells. Attempts to obtain chemotaxis up K^+ gradients with *D. discoideum* using microfluidic chambers have failed (unpublished data). However, these failures do not exclude the possibility that monovalent cation gradients exist, that chemotaxis to these gradients occurs, and that they occur through monovalent cation receptors.

Conclusion

It is hoped that this review will engender an interest in the possibility that in addition to sources of intracellular cations, extracellular cations may play roles as signals in cell motility, polarity, and chemotaxis. Cations have the potential to interact with plasma membrane proteins, which in turn activate signal transduction pathways, other membrane proteins, or the cytoskeleton. There is no question that Ca^{2+} plays such a role. We have reviewed just how different the extracellular cationic milieu is for the biota of different organisms and cell types within an organism, and emphasized that organism and cell type must have adapted to these cationic environments. We have also reviewed the literature demonstrating that Ca^{2+} gradients direct chemotaxis through bona fide cation receptors.

Because many of the surface molecules that transport cations must interact with the cations, we believe they represent easy targets to which signaling events could have been coupled during evolution. Hence, we would not be surprised to find that many cationic transporters, exchangers, and channels, upon cationic interaction, regulate other molecular processes through direct interaction. Hopefully, this review will heighten the awareness of such possibilities.

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