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Signaling Pathways for Transduction of the Initial Message of the Glycocode into Cellular Responses

Key Words
Apoptosis
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Signal transduction
Tyrosine kinase receptors

Abstract
The sugar units of glycan structures store information and establish an alphabet of life. The language of the oligosaccharide coding units is deciphered by receptors such as lectins and the decoded message can be transduced by multiple signaling pathways. Similar to glycoconjugates, these receptors can exhibit pronounced changes in quantitative and qualitative aspects of expression, as attested by a wealth of lectin and immunohistochemical studies. Since histochemistry provides a static picture, it is essential to shed light on the mechanisms of how a recognitive protein-carbohydrate interplay can be transduced into cellular responses. Their consequences for example for cell morphology will then be visible to the histochemist. Therefore, basic signaling routes will be graphically outlined and their trigger potential will be explained by selected examples from the realm of glycosciences.

Introduction

Molecular recognition governs all aspects of cellular activities. These crucial interactions comprise various types of biochemical rendezvous, including the intimate association of oligosaccharides with proteins [Quiocho, 1989; Imberty et al., 1993; Lemieux, 1996; von der Lieth et al., 1996, 1998; Bundle, 1997; Siebert et al., 1997; Gabius, 1998]. Since plant lectins have a long tradition as biochemical and immunological tools, it is well appreciated that specific interactions can be established by sugars as docking points for lectins and that this binding can reliably trigger cellular responses such as lymphocyte proliferation [Rüdiger, 1997, 1998]. Beyond the descriptive cataloguing of oligosaccharide structures and lectins, the documentation

Abbreviations used in this paper:
AA = Arachidonic acid; AC = adenylyl cyclase; ADP-RC = ADP-ribosyl cyclase; $[Ca^{2+}]_c$ = cytosolic free Ca^{2+} concentration; cADP-rb = cyclic ADP-ribose; CaM = calmodulin; CaM-PK II = calmodulin-dependent protein kinase II; cGC = cytosolic guanylyl cyclase; CIF = Ca^{2+} influx factor; CRE = cAMP response element; CREB = CRE-binding protein; 1, 2-DAG = 1, 2-diacylglycerol; EGF = epidermal growth factor; EGFR = epidermal growth factor receptor; Eph receptor = erythropoietin-producing human hepatocellular carcinoma cells receptor; ERK = extracellular signal-regulated kinase; FAK = focal adhesion kinase; Grb2 = growth factor receptor-bound protein 2; Ins-1, 4, 5-P_3 = inositol-1, 4, 5-triphosphate; JNK/SAPK = c-Jun N-terminal kinase/stress-activated protein kinase; L = ligand; LT-C_4 = leukotriene C_4; MAPK = mitogen-activated protein kinase; MAPKK = MAPK kinase; MAPKKK = MAPK kinase kinase; mbGC = membrane-bound guanylyl cyclase; MEK = MAP/ERK kinase; MEKK = MEK kinase/SAPK kinase kinase; MKK4 = SAPK kinase 4; PAK = p21-activated kinase; P-CaM = phosphocalmodulin; PDGF = platelet-derived growth factor; PKA = cAMP-dependent protein kinase; PKB = protein kinase B; PKC = protein kinase C; PLA_2 = phospholipase A_2; PLCγ = phospholipase Cγ; PtdIns = phosphatidylinositol; PtdIns3K = phosphatidylinositol-3-kinase; PtdIns4K = phosphatidylinositol-4-kinase; PtdIns-4-P = phosphatidylinositol-4-phosphate; PtdIns4P5K = phosphatidylinositol-4-phosphate-5-kinase; PtdIns-4, 5-P_2 = phosphatidylinositol-4, 5-biphosphate; PtdIns-3, 4, 5-P_3 = phosphatidylinositol-3, 4, 5-triphosphate; SH2 domain = Src homology 2 domain; Sos = Son of sevenless; TNF = tumor necrosis factor; TPA = 12-O-tetradecanoylphorbol-13-acetate.

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of these interactions has clearly pointed to an actual physiological role of tissue sugar receptors, where plant agglutinins can merely act as substitutes for animal lectins.

The demonstration of the presence of diverse families of endogenous lectins in animal tissues has opened a new branch in the field of glycosciences and engenders a host of questions in regard to their actual functions in cells and organs in normal and disease states [Ashwell and Harford, 1982; Barondes, 1984; Gabius, 1988, 1991, 1997a, b; Drickamer and Taylor, 1993; for a collection of recent reviews, see Gabius and Gabius, 1997; Kaltner and Stierstorfer, 1998; Zanetta, 1998]. Obviously, animal tissue lectins are superseding the commonly employed plant lectins as laboratory tools due to their availability, offering the advantage by their testing to infer actual mechanisms of physiological relevance. To comprehend how lectins deliver their physiological messages it is important to experimentally identify their primary binding targets, their signaling pathways and the mechanisms which are operative during ensuing cellular responses. Although some lectins are known to harbor additional non-sugar-binding sites [Barondes, 1988; Gabius, 1994, 1997a], their definition as a carbohydrate-binding protein which neither is an antibody nor a sugar-specific enzyme calls for a receptor site for a sugar structure. Figure 1A depicts common lectin targets, among which soluble glycoproteins, different types of membrane-bound or matrix-associated glycoproteins, proteoglycans and glycolipids, such as glycosphingolipids, cerebrosides and gangliosides as carriers for the lectin-reactive sugar determinants are predominant. Whereas other articles in this issue deal with the structural and biosynthetic aspects of the ligands and their location [Brinck et al., 1998; Brockhausen et al., 1998; Danguy et al., 1998; Geyer and Geyer, 1998; Hakomori, 1998; Mann and Waterman, 1998; Plendl and Sinowatz, 1998; Sharon, 1998; Zschäbitz, 1998], this review will focus on describing ways of signal transmission downstream from the binding event.

In principle, two major mechanistic modes of action could account for the observed signaling processes. As shown in figure 1B, lectins can bind to the glycopart of plasma membrane-associated receptors eliciting the generation of intracellular signals, and conversely plasma membrane-bound lectins specifically interact with glycoligands like a hormone receptor, thereby also initiating signaling processes. As also depicted in figure 1B, it is suggestive to assume that the most common mechanism of action for a lectin with at least two binding sites is mediated by lectin-induced receptor clustering leading to receptor dimerization [Metzger, 1992]. Indeed, plant and animal lectins have widely proven their potency to act as cross-linking devices

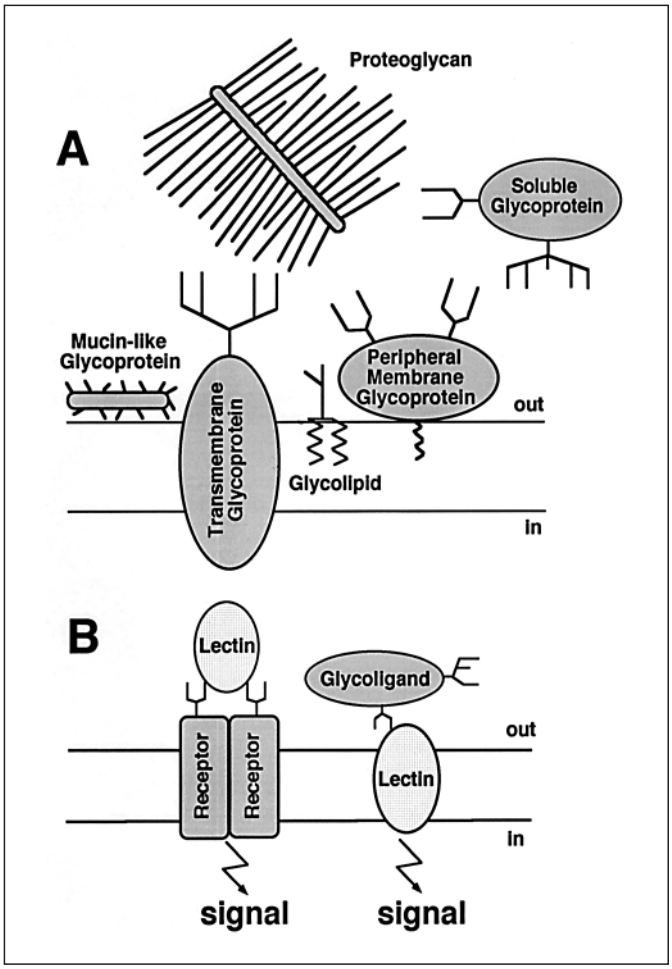


Fig. 1. Lectin ligands and different systems for signaling by and through lectins. **A** An adequate binding partner, i.e. the target carbohydrate structure, can be presented by different types of carrier molecules. An extracellular soluble glycoprotein, a peripheral membrane glycoprotein with a lipidic anchor, a glycoprotein with a transmembrane domain, a mucin-like glycoprotein, a proteoglycan, and an integral membrane glycolipid are depicted. **B** Lectins are able to initiate a signaling cascade upon binding and cross-linking plasma membrane receptors with a biantennary N-linked sugar chain which thereby are dimerized (left). These lectins are not necessarily soluble, but can well be an integral part of an adjacent cell or an extracellular matrix (not shown). The glycobiological interplay can also be operative with the lectin being the effector after interaction with an appropriate glycoligand (right). These glycoligands could also be part of an adjacent cell or extracellular matrix (not shown).

which can even lead to the generation of lattice-like receptor patches [Mandal and Brewer, 1993; Brewer, 1996].

To avoid any misinterpretation it is fair to add at this point that not all lectins will mediate a signal through plasma membrane-bound receptors, and not all plasma

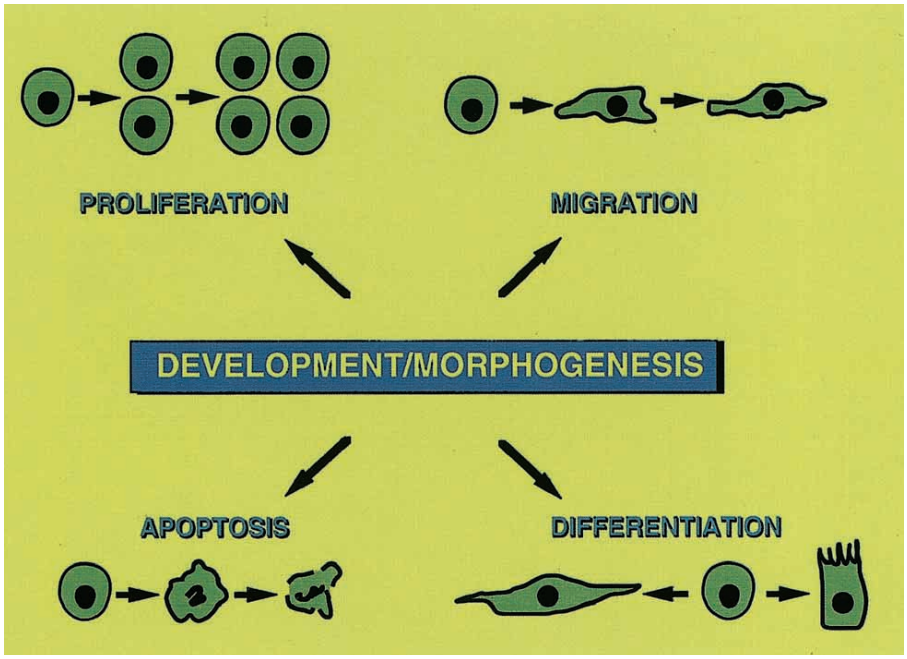


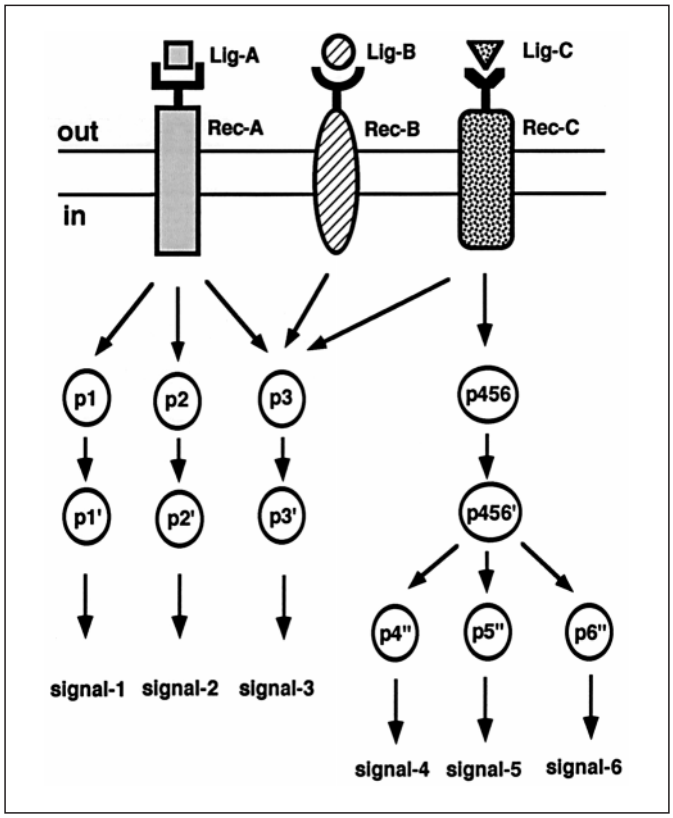
Fig. 2. Major cellular events responsible for development/morphogenesis. During development and morphogenesis of multicellular organisms, cells undergo multiple changes among which proliferation, migration, differentiation and apoptosis are the most relevant.

membrane-bound lectins will evoke signals that are transmitted to the cellular interior. Other non-signaling functions of lectins include the routing of glycoproteins destined for cell-specific uptake and their place in the innate immune system to label foreign cells for complement attack [Gabius, 1987a, 1997a,b]. Furthermore, any signal elicitation inevitably requires the stepwise cooperation of various activities in a defined space-temporal organization, whose disruption will silence the cascade. This possibility of transient interference with a signaling pathway should not be considered as deleterious, but as a valuable handle to augment the extent of plasticity in the communication network [Klein et al., 1989].

Up to this stage, we have exclusively focused our strictly schematic discussion on lectin ligands at the plasma membrane or the extracellular space. It should not be neglected, however, that the presence of glycoproteins is not restricted to these sites. Indeed, nuclear and cytoplasmic occurrence of glycoproteins has been demonstrated, raising evidence for a probable extension of the concept of the glycobiology of signal initiation and transmission [Hart, 1997; Sharon and Lis, 1997; Villalobo et al., 1997a]. The term 'signaling' is here succinctly defined in its broadest sense. Any more elaborate explanations require attention to be turned to individual pathways of signal transduction. To avert the notion of an inscrutable network of highly complicated and confusing reaction cascades, we will offer,

Fig. 3. Convergence, divergence and interconnection of signaling pathways. Ligand A (Lig-A) binds to receptor A (Rec-A) activating three different signaling pathways which elicit three distinct signals (signal-1, signal-2 and signal-3). Ligands A, B and C (Lig-A, Lig-B and Lig-C) bind to their respective receptors (Rec-A, Rec-B and Rec-C) all of which signal by a common pathway eliciting an identical signal (signal-3). Ligand C binds to its receptor (Rec-C) and elicits signaling through a pathway that is thereafter branched into three different subpathways which elicit three distinct signals (signal-4, signal-5 and signal-6). The components (p, p′ and p″) of the different signaling pathways which refer to mediator-generating enzymes such as kinases or phospholipases are placed in categories defined by the number of the eventually elicited signal. Binding of lectins to glycoligands frequently signals through elaborated and interconnected transduction networks as represented in this cartoon.

Fig. 4. Monodirectional and bidirectional cellular signaling. **A** Monodirectional signaling (see arrows) is achieved upon activation of a receptor by three modes. A soluble ligand interacts with a receptor during endocrine, paracrine or autocrine stimulation (left). A matrix-associated ligand interacts with a receptor during juxtacrine stimulation (center). A neighbor cell-associated ligand interacts with a receptor during transcellular stimulation (right). **B** Bidirectional signaling (see arrows) is achieved upon activation of two receptors each located in a different cell. Homotypic interactions by two identical molecules (left) and heterotypic interactions by two distinct molecules (right) are depicted. Monodirectional and bidirectional signaling can be achieved by membrane-bound lectins upon interaction with membrane-bound glycoligands.



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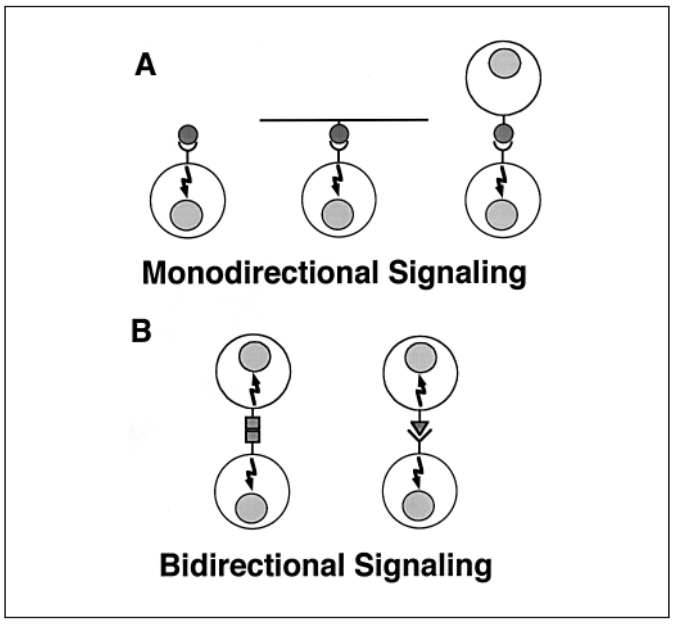

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aided by deliberately simplified illustrations, a primer to such mechanisms with relevance to cellular reactions important for development and morphogenesis, in which cellular proliferation, migration, differentiation and apoptosis are major constitutive events (see fig. 2).

General Features of Cellular Signaling

Signaling by and through lectins uses the signal transduction pathways present in the cell to send precise bits of information from the extracellular medium to the cell interior or to enable communication between different intracellular compartments. Our brief overview of the major signaling pathways and their interconnections serves the purpose of familiarizing the reader with the principles of such reactions. The variety of biochemical pathways and our continuously improving level of understanding unquestionably precludes an in-depth description of each single series of reactions within the given space of this article. Therefore, we shall place emphasis on selected cell signaling systems which have relevance beyond glycobiology. The involvement of the pathways effecting the functions of endogenous lectins and also the exemplary description of the impact of exogenous lectins on signaling reactions will be discussed thereafter. Special attention will be devoted to those mechanisms supposedly relevant for development and morphogenesis.

The molecular organization of complex organisms necessitates an intricate signal elaboration network. It is therefore possible that certain recognitive events will not automatically transmit an identical message under any conceivable circumstance. On the contrary, the space-temporal context should be able to modulate the actual meaning of the message, opening the way for a combinatorial level of communication [Sporn and Roberts, 1988]. The flow of information can thus be guided into different directions from a single starting point, which has potential for a vivid cross talk adding a remarkable level of sophistication to these systems. As an advantageous by-product, functional redundancy is achieved, which can compensate for the loss of individual components of the whole system. To illustrate the principles of the mentioned cross-talk, a reasonable scheme for the convergence and divergence of interconnected signaling pathways derived from three distinct receptors and associated ligands is shown in figure 3. Moreover, it is conceivable that the pairing between ligands and receptors in some instances might not be too specific, thereby allowing for a certain degree of promiscuity in their interactions.

In general, ligands and receptors are distinct molecular entities and the signal travels in one direction upon their interaction (fig. 4A). The ligand for a given receptor could be a soluble molecule (fig. 4A, left), a matrix-associated molecule (fig. 4A, center) or a molecule associated with

the plasma membrane of another interacting cell (fig. 4A, right). However, the functional division line between both entities artificially separated by the nomenclature can be blurred. Explicitly, two molecules, each located in the plasma membrane of different cells, can be able to generate bidirectional signals affecting both cells upon interaction between each other (fig. 4B). Therefore, each part of the recognition system could be considered at the same time as a ligand and a receptor, in this instance questioning the value of a strict use of the nomenclature. An illustrative example of homotypic molecular interaction yielding a bidirectional signaling is given by cadherins (fig. 4B, left). These cellular adhesion molecules appear to induce cytoskeletal reorganization and gene transcription in both interacting cells, employing intracellular proteins named catenins as mediators after association [Huber et al., 1996; Yamada and Geiger, 1997]. Moreover and as an example of heterotypic molecular interactions eliciting bidirectional signaling, the erythropoietin-producing human hepatocellular carcinoma cell (Eph) receptors and ephrin-B ligands [Eph Nomenclature Committee, 1997] can be referred to (fig. 4B, right), since both types of molecules will transduce signals to cells harboring appropriate components of this recognition system [Orioli and Klein, 1997; Pasquale, 1997].

Besides this set of transmembrane signaling pathways, direct communication between the cytosolic compartments of adjacent cells in multicellular organisms is achieved by the gap junction channels. The gating function of these channels is accurately regulated, and the formed pores allow the passage of metabolites, ions and second messengers of <1 kDa, such as Ca^{2+}, cAMP, cGMP and inositol-1,4,5-triphosphate (Ins-1,4,5-P_3), between both cytosolic compartments. Thus, they play an important role in the synchronization of cellular responses as well as in the electrotonic coupling among the cells forming tissues [Loewenstein, 1987; Bruzzone et al., 1996].

In general, cells employ specific intracellular second messenger molecules and ions in the different pathways to transduce and to amplify the incoming signals. The pathways in which these second messengers are involved are often interconnected. As emphasized, we prefer to deal with individual cascades separately to introduce the reader to recurring themes, taking Ca^{2+}, cyclic nucleotides and phosphoinositides as examples.

Calcium-Mediated Signaling

The availability of Ca^{2+} has multiple ways of influencing cellular behavior, since this cation is an integral functional component of diverse enzymes and other protein families. In lectinology, the family of C-type animal lectins is defined by the presence of a recognition domain in which this essential cation can directly contact hydroxyl groups of the glycoligand [Sharon, 1993; Rini, 1995; Weis and Drickamer, 1996; Gabius, 1997a].

Calcium mobilization is a common mechanism used by the cell to transduce incoming signals from the extracellular or intracellular media, and from other cells. Thus, the range of calcium-mediated signaling affects multiple processes such as fertilization, cell proliferation, differentiation, apoptosis, cell adhesion, morphogenesis, muscle contraction-relaxation, cytoskeletal dynamics, endoexocytosis and secretion, transport mechanisms, electrophysiological events and neuronal communication.

In order to precisely regulate the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$), cells possess a number of transport systems involved in the uptake and release of Ca^{2+} from the extracellular medium, and from internal Ca^{2+} stores, such as the endoplasmic reticulum and the mitochondria, into the cytosol and vice versa (see fig. 5). These transport systems are used to accurately control the $[Ca^{2+}]_c$ at low levels (approximately 10^{-8} *M*) during the resting state and to allow its rapid increase (up to approximately 10^{-6} *M*) upon cellular stimulation.

The increase in $[Ca^{2+}]_c$ mainly occurs upon the opening of different types of Ca^{2+} channels located either in the plasma membrane or in the endoplasmic reticulum of the cell (see fig. 5). The sequential organization of events leading to the opening of Ca^{2+} channels and the activation of the transport systems that counterbalance their action (see fig. 5) may even result in oscillatory changes in the $[Ca^{2+}]_c$ [for reviews, see Berridge, 1990; Dupont et al., 1991; Fewtrell, 1993; Sneyd et al., 1995]. These oscillations are paramount events to exert the regulatory role of this cation on cellular functions.

Similar to the modulation of electromagnetic wave transmission in telecommunication, changes in the $[Ca^{2+}]_c$ offer a wide range of possibilities for information transfer. The reader can easily imagine that the signals elicited by calcium mobilization can be widely different depending on the frequency, amplitude and/or duration of the oscillatory changes in the $[Ca^{2+}]_c$. It is noteworthy in this context that the physiological relevance of the control of the frequency of the $[Ca^{2+}]_c$ oscillation was first recognized less than two decades ago [Rapp and Berridge, 1981]. Recently, it has been shown that the amplitude of this $[Ca^{2+}]_c$ signal also plays a prominent role. In detail, the activation of the transcription factors NFκ-B and JNK by high transient increases in $[Ca^{2+}]_c$ and the activation of nuclear factor of activated T cells by low and sustained transient increases in $[Ca^{2+}]_c$ has been demonstrated in B lymphocytes [Dol-

metsch et al., 1997]. The transcriptional apparatus is not the only object of this versatile modulatory mechanism. Having underscored the importance of changes of this parameter, the answer to the question of how such alterations are actually produced can now be outlined.

Activation of tyrosine kinase mitogenic receptors, such as the epidermal growth factor (EGF) receptor and the platelet-derived growth factor (PDGF) receptor, results in a transient increase in the $[Ca^{2+}]_c$. Remarkably, a concomitant transient alkalinization of the cytosol by activation of the Na^+/H^+ exchanger and an increase in phosphoinositide turnover are also seen upon mitogenic stimulation [Moolenaar et al., 1986; Rozengurt, 1986; Pandiella et al., 1988]. The integration of this Ca^{2+} signal is explicable in biochemical terms, starting with the dimerization of the growth factor receptors upon ligand binding. The binding of ligands leads to conformational changes responsible for the transphosphorylation and the concomitant activation of the receptor tyrosine kinase followed by the phosphorylation of a series of target proteins, as shown in figure 6.

These target proteins can vary for different growth factor receptors with intrinsic tyrosine kinase activity. In the case of the EGF receptor, and relevant for the generation of the Ca^{2+} signal, its transphosphorylation results in the activation of both phospholipase Cγ and phospholipase A_2, as illustrated in figure 6. The former hydrolyzes phosphatidylinositol-4,5-biphosphate (PtdIns-4,5-P_2) into Ins-1,4,5-P_3 and 1,2-diacylylycerol (1,2-DAG). As will also be outlined in the section Polyphosphatidylinositide-Mediated Signaling, Ins-1,4,5-P_3 activates Ca^{2+} channels in the endoplasmic reticulum, and 1,2-DAG activates protein kinase C. Concerning phospholipase A_2, its activation results in the formation of arachidonic acid from the hydrolysis of phospholipids with the final aim to open Ca^{2+} channels, thereby increasing the $[Ca^{2+}]_c$ (fig. 6). This unsaturated fatty acid is a substrate for its enzymatic transformation into leukotriene C_4 (LT-C_4) via the 5-lipoxygenase pathway, and LT-C_4 can open Ca^{2+} channels located in the plasma membrane. Thus, Ca^{2+} from both internal stores and the extracellular medium contributes to the generation of the mitogen-induced increase in the $[Ca^{2+}]_c$. In addition to these effectors, whose principal functions are emphasized in figure 6, at least one additional factor deserves attention. This is a messenger molecule denoted Ca^{2+} influx factor (CIF), which stimulates Ca^{2+} influx from the extracellular medium when intracellular Ca^{2+} stores are exhausted [Randriamampita and Tsien, 1993] (fig. 6). Incidentally, phytohemagglutinin was originally utilized as a stimulator to deprive internal Ca^{2+} stores and to induce the expression of CIF by Jurkat cells. Therefore, this lectin was an important tool to identify this new modulator that regulates the fluctuation of the $[Ca^{2+}]_c$ [Randriamampita and Tsien, 1993].

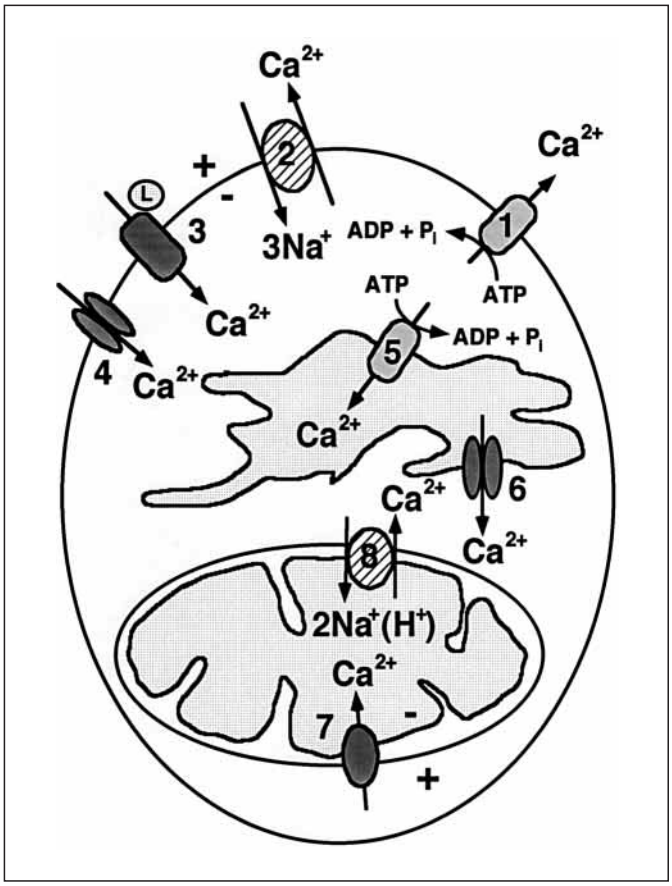

Fig. 5. Major cellular Ca^{2+} transport systems. Calcium ions are exported to the extracellular medium by the calmodulin-regulated plasma membrane Ca^{2+}-ATPase (1) and the Na^+/Ca^{2+} exchanger (2). Calcium uptake is achieved either by receptors with Ca^{2+} channel activity (3) for different extracellular ligands (L), or Ca^{2+} channels (4) regulated by voltage or intracellular ligands. Calcium uptake by the endoplasmic reticulum is carried out by the endoplasmic reticulum Ca^{2+}-ATPase (5) and calcium release from this organelle uses Ca^{2+} channels (6) regulated by inositol-1,4,5-triphosphate or cADP-ribose. Electrogenic calcium uptake by mitochondria is achieved by Ca^{2+} channels (7) and calcium release from this organelle is carried out either by a 2 Na^+/Ca^{2+} exchanger or a 2 H^+/Ca^{2+} exchanger depending on the cell type (8). Intracellular and extracellular calcium homeostasis are essential requirements for the activity of Ca^{2+}-dependent lectins.

To name another remarkable player in this game, mitochondria deserve explicit reference. Indeed, these organelles are also able to participate in the overall control of the $[Ca^{2+}]_c$ and to intervene in the regulation of Ca^{2+}-mediated signaling processes [Ichas et al., 1997]. To reach this end,

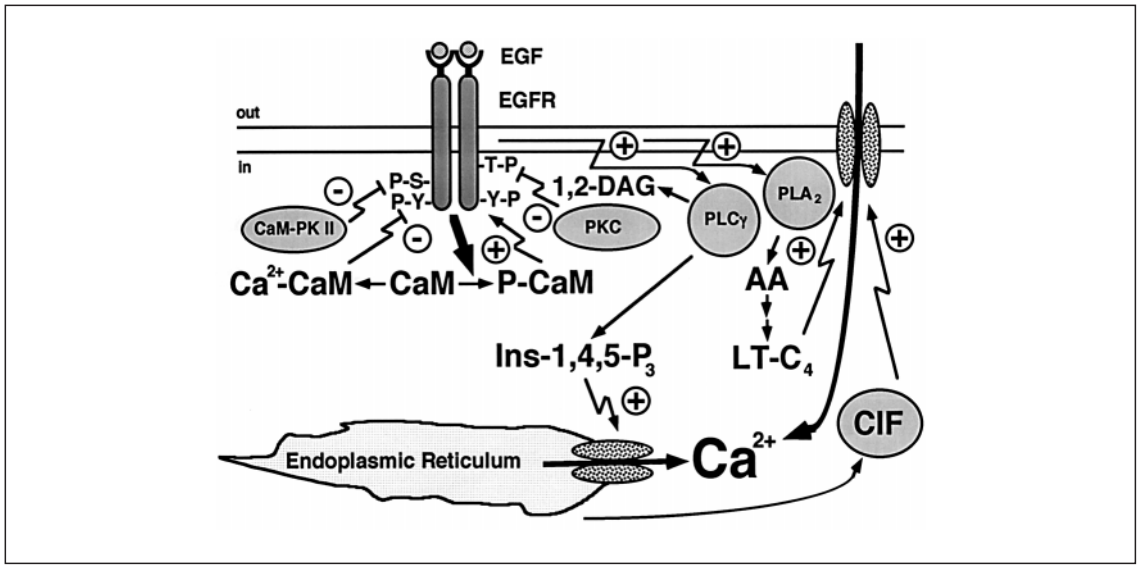

Fig. 6. Calcium signals generated by the EGF receptor and the retroregulation of its activity. Binding of the EGF to its receptor (EGFR) results in its dimerization and the transphosphorylation of tyrosine residues (Y-P) in its intracellular C-terminal tail. Among the substrates of the activated receptor is calmodulin (CaM), and the resulting phosphocalmodulin (P-CaM) appears to further activate (+) the EGFR tyrosine kinase. The EGFR also activates (+) both phospholipase Cγ (PLCγ) and phospholipase A_2 (PLA_2). PLCγ forms diacylglycerol (1,2-DAG), an activator of protein kinase C (PKC), and inositol-1,4,5-triphosphate (Ins-1,4,5-P_3), an activator (+) of Ca^{2+} channels in the endoplasmic reticulum. When the calcium stores from the endoplasmic reticulum are depleted, Ca^{2+} influx factor (CIF) is released activating (+) plasma membrane Ca^{2+} channels. PLA_2 forms arachidonic acid (AA) which is enzymatically metabolized to leukotriene C_4 ($LT\text{-}C_4$). This then activates (+) plasma membrane Ca^{2+} channels. The increase in the concentration of cytosolic Ca^{2+} results in the inhibition (–) of the EGFR tyrosine kinase activity by a triple mechanism: (1) phosphorylation of a threonine residue (-T-P) by PKC, (2) phosphorylation of two serine residues (-S-P) by calmodulin-dependent protein kinase II (CaM-PK II), and (3) direct interaction of the Ca^{2+}-CaM complex with the receptor. The intrinsic tyrosine kinase activity of the EGFR can also be differentially regulated by lectins recognizing distinct parts of the sugar chains of the receptor [see Zeng et al., 1995].

several transport systems participate in the import and export of Ca^{2+} between the mitochondrial matrix and the cytosol (see fig. 5).

A summary of events that result in calcium mobilization upon activation of the EGF receptor is given in figure 6. The schematic representation also includes the depiction of the role of CIF, when the Ca^{2+} stores from the endoplasmic reticulum are deprived upon sustained cell stimulation. To avoid any misunderstanding, we explicitly indicate that figure 6 should not be considered to reflect the spectrum of all possible EGF receptor-mediated regulatory mechanisms eventually controlling the $[Ca^{2+}]_c$ and the negative feedback control of the receptor by this messenger.

Signals mediated by Ca^{2+} mobilization are transduced by a plethora of Ca^{2+}-binding proteins among which calmodulin plays a prominent role [Means and Dedman, 1980]. Of special interest for development and morphogenesis is the fact that the Ca^{2+}-calmodulin complex regulates the cell cycle [Lu and Means, 1993]. In this context, it is relevant to mention that Ca^{2+} and calmodulin have also been implicated in the regulation of the tyrosine kinase activity of the EGF receptor by different mechanisms. As shown in figure 6, calmodulin-dependent protein kinase II has been described to phosphorylate two serine residues in this receptor, thereby inhibiting its tyrosine kinase activity [Countaway et al., 1992]. Likewise, protein kinase C, another Ca^{2+}-dependent kinase, phosphorylates a threonine residue in the receptor, also modulating its tyrosine kinase activity [Hunter et al., 1984] (fig. 6). Moreover, the Ca^{2+}-calmodulin complex directly interacts with the EGF receptor, similarly leading to the inhibition of its tyrosine kinase activity [San José et al., 1992; Martín-Nieto and Villalobo, 1998]. These redundant regulatory mechanisms underscore the importance of the Ca^{2+} signal in the feed-back regulation of the EGF receptor, which are integrated and presented in the upper left part of figure 6.

In addition, calmodulin is subjected to posttranslational modification which adds a further possibility for conceivable regulation. Thus, calmodulin is phosphorylated by the EGF receptor at tyrosine-99 [Benguría et al., 1994; De Frutos et al., 1997], and the resulting phosphocalmodulin appears to act as an additional activator of the EGF receptor tyrosine kinase [Benguría et al., 1995; Villalobo et al., 1997b]. In summary, phosphocalmodulin and nonphosphorylated calmodulin have opposite regulatory effects on the EGF receptor tyrosine kinase (see fig. 6).

It is of interest to mention that the purine nucleotide triphosphates ATP and GTP serve as donors for the phosphorylation of hydroxyl groups exposed on the surface of proteins, the hallmark of reversible posttranslational modification as exemplified above and discussed in more depth in the section Phosphorylation-Mediated Signaling. But in addition to this type of reaction, these compounds can be substrates for regulable cyclases forming intramolecular phosphodiesters, thereby forming cyclic nucleotides of special interest in signaling, as will be described below.

Cyclic Nucleotide-Mediated Signaling

Similar to fluctuations in the $[Ca^{2+}]_c$, changes in the level of metabolites which are biosynthesized in response to extracellular signals exert important regulatory functions. As already referred to, several cyclic nucleotides play prominent roles in cell physiology as second messengers. Among these, the most relevant are cAMP and cGMP. In addition, due to its recent description the cyclic diphosphonucleotide derivative cADP-ribose has entered the stage to enlarge the size of the group of signaling molecules in this category.

The synthesis of cAMP is mediated by adenylyl cyclases. The activity of these enzymes is regulated by heterotrimeric G proteins. The information input reaching these coupling factors originates from a family of G protein-coupled receptors which have the presence of seven transmembrane segments in common [Gilman, 1987; Birnbaumer et al., 1990]. Consequently, ligand binding to certain G protein-coupled receptors changes the concentration of this type of second messenger, as schematically shown in figure 7.

Receptor activation by ligand binding initiates a conformational change in the cytosolic domains of the receptor. This signal is conveyed to trimeric G proteins and results in the exchange of GDP for GTP in its Gα subunit. Subsequently, the Gα subunit dissociates from the trimer and travels to suitable signal receivers, leaving the complex with the Gβγ dimer behind (fig. 7). Both the GTP-bound Gα subunit and the Gβγ dimer independently play important regulatory roles. Signal-dependent acquisition of GTP transforms α-subunits into versatile modulators of target enzyme activity. The activated Gα subunit of stimulatory G proteins (G_s) enhances the enzymatic activity of certain adenylyl cyclases, whereas the GTP-loaded Gα subunit of inhibitory G-proteins (G_i) negatively affects their product generation, illustrating the basic principle to capitalize on the same mechanism for transferring signals with opposite effects. In addition, the Gβγ dimer can control the activity of various adenylyl cyclases as well as other enzymes such as phospholipase A_2. As outlined in the preceding section, this enzyme liberates arachidonic acid, which is an essential intermediate for effector production in Ca^{2+} mobilization (fig. 6). The Gβγ dimer also enhances the activity of phospholipase Cβ [Clapham and Neer, 1993], which synthesizes 1,2-DAG, as shown in figure 6 for its cousin phospholipase Cγ. The functional trimeric G protein cycle is ended by the hydrolysis of GTP bound to the Gα subunit by its intrinsic GTPase activity, and its reassociation with the Gβγ dimer reconstituting the Gαβγ trimer. This process is facilitated by a family of RGS proteins [Dohlman and Thorner, 1997]. It is informative in this context to highlight a veritable relevance of the modification of these proteins in causing symptoms of diseases in certain bacterial infection. ADP ribosylation of the Gα subunit by cholera toxin and pertussis toxin has dramatic consequences, since the arrest of the activated GTP-containing form of this subunit by the inhibition of the intrinsic GTPase activity causes a continuous stimulation of the downstream effectors.

Having explained the pathway for cAMP biosynthesis, further considerations can focus on the mission of this second messenger. The synthesized cAMP binds to the inactive tetrameric protein kinase A, thereby initiating the dissociation of their regulatory subunits from the active catalytic subunits (fig. 7). The active enzyme then turns to phosphorylate multiple target proteins. Furthermore, and in addition to short-term effects that result from the phosphorylation of their target proteins, the increase in the concentration of intracellular cAMP also induces long-term effects. Thus, the phosphorylation of a series of transcriptional activators such as cAMP response element-binding protein (CREB), cAMP response element modulator τ and activating transcription factor 1 induces, in cooperation with CREB-binding protein, the subsequent turn-on of the transcription of the genes under the control of cAMP response element. Finally, the inducible cAMP early repressor protein, whose expression is modulated by these transcriptional activators, and their dephosphorylation by phosphatases eventually switch off the positive transcriptional signal [Sassone-Corsi, 1995; Montminy, 1997]. Interestingly, the cAMP-dependent transcriptional machinery main-

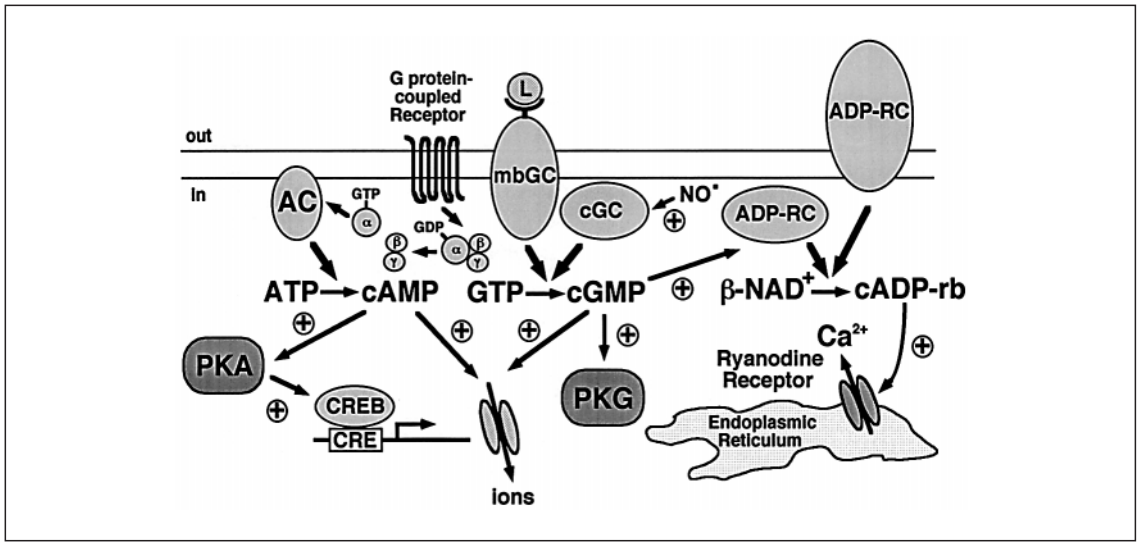

Fig. 7. Role of different cyclic nucleotides as second messengers. On the left, the synthesis of cAMP from ATP is achieved by adenylyl cyclases (AC) which are regulated by seven-transmembrane-domains receptors coupled to trimeric G proteins (α, β, γ). cAMP activates (+) the phosphorylation of target proteins via cAMP-dependent protein kinases (PKA) and regulates gene expression under the control of cAMP response elements (CRE) via cAMP response element-binding proteins (CREB). In the center, the synthesis of cGMP from GTP is carried out by membrane-bound guanylyl cyclases (mbGC) under the control of a variety of ligands (L), i.e. guanylin/uroguanylin and different types of natriuretic peptides, and by cytosolic guanylyl cyclases (cGC) under the positive control (+) of nitric oxide (NO*). cGMP activates (+) cGMP-dependent protein kinases and some ADP-ribosyl cyclases. Both cAMP and cGMP activate (+) different ion channels. On the right, the synthesis of cADP-ribose (cADP-rb) from β-NAD^+ is catalyzed by different ADP-ribosyl cyclases (ADP-RC). In turn, cADP-ribose activates (+) ryanodine receptors from the endoplasmic reticulum whose functionality leads to an increase of the concentration of free cytosolic calcium. As an example of the role of a cyclic nucleotide in glycobiology, it could be mentioned that the activation of the adenylyl cyclase markedly increases the activity of a branching enzyme for N glycans in cell surface glycoproteins [see Sultan et al., 1997].

tains an intensive cross talk with the DAG-dependent transcriptional system which utilizes protein kinase C to phosphorylate the transcriptional elements of TPA-responsive element to control gene expression [Sassone-Corsi, 1995].

Similar to the case of cAMP, another important cyclic nucleotide involved in signal transduction systems is cGMP, which is likewise incorporated into figure 7. The synthesis of cGMP is carried out by guanylyl cyclases, an extensive family of enzymes. Individual members are found as membrane-bound proteins or as soluble proteins which are localized in the cytosol [Wedel and Garbers, 1997]. The best characterized soluble guanylyl cyclases have a prosthetic heme group and one of its natural activity modulators is nitric oxide [Ignarro, 1990]. The localization of heme oxygenases in the brain, where it is rather unlikely to predominantly serve in the breakdown of hematoporphyrins, has lent credit to the assumption that also carbon monoxide can regulate the cGMP levels [Wedel and Garbers, 1997]. Nitric oxide is a free radical gas with a short half-life which is synthesized upon demand from *L*-arginine in mammalian cells. Interestingly, the nitric oxide synthases are regulated by the Ca^{2+}-calmodulin complex [Marletta, 1994; Nathan and Xie, 1994]. Nitric oxide is involved in the regulation of multiple physiological functions such as vascular relaxation, synaptic transmission, cellular growth and cytotoxic immune response. Thus, this compound can be considered as a second messenger capable to be reactive in the producing cell and in cells in the immediate vicinity. As can be expected from a messenger molecule with eminent duties, aberrations of the production levels deviating from the desirably normal values are either causal for or aggravate certain pathological processes such as vascular hypertension, dysfunction in penis erection or hypotension associated with septic shock [Gros and Wolin, 1995].

Of special interest in this review is the fact that nitric oxide also regulates cell proliferation. Having introduced the EGF receptor in the preceding paragraphs, it is illustra-

tive to mention another example in which different pathways can directly interact with each other. In this context, it has been described that the S-nitrosylation of the EGF receptor by nitric oxide causes the reversible inactivation of the receptor tyrosine kinase activity [Estrada et al., 1997].

The regulation of the membrane-bound guanylyl cyclases is very different from their soluble counterparts. Hence, the known extracellular ligands for these membrane-bound enzymes are guanylin/uroguanylin and different types of natriuretic peptides, although the nature of the physiological ligands of many isoforms of this enzyme is not known at present [Wedel and Garbers, 1997]. Besides functions relating to the modulation of enzyme activities, both cGMP and cAMP also regulate cyclic nucleotide-gated ion channels from olfactory receptor cells and retinal photoreceptors [Zagotta and Siegelbaum, 1996] (see fig. 7). Glycohistochemical characteristics of the olfactory system are discussed in this special issue [Plendl and Sinowatz, 1998].

Having introduced the main components of this group of signaling molecules, it is now appropriate to document once more the already mentioned interconnections of signaling pathways. It could be expected that the abundance of these messengers can be affected by oscillations in the concentration of cytosolic free Ca^{2+}. Indeed, the metabolism of the second messengers cAMP and cGMP is intimately connected to calcium homeostasis and the functionality of its receptor protein calmodulin. Notably, Ca^{2+}/calmodulin-dependent cyclic nucleotide phosphodiesterases participate in their degradation to temporarily limit the action of these effectors.

In addition to the already mentioned cyclic nucleotides, cyclic ADP-ribose, another family member of this group of signaling molecules, is depicted in figure 7. This second messenger is synthesized from β-NAD^+. The ADP-ribosyl cyclase activity is ascribed to CD38, an ecto-enzyme of the plasma membrane, to a cytoplasmic ADP-ribosyl cyclase and another intracellular enzyme activated by cGMP. As shown in figure 7, the targets for cyclic ADP-ribose encompass ryanodine receptors located in the endoplasmic reticulum of nonexcitable cells. These receptors are Ca^{2+} channels that participate in the increase of the $[Ca^{2+}]_c$ mediated by the Ca^{2+}-induced Ca^{2+} release mechanism, in which calmodulin is also involved [for a review, see Lee, 1996].

As a summary, the systems which participate in the synthesis of cAMP, cGMP and cADP-ribose and a selection of the cellular targets of these second messengers are illustrated in figure 7.

Polyphosphatidylinositide-Mediated Signaling

Lipids provide storage systems for messengers such as arachidonic acid derivatives or 1,2-DAG, as referred to in figure 6. Moreover, inositol-containing phospholipids undergoing enzymatic cleavage by phospholipases (fig. 6, 8) serve as precursors for another class of mediators, which will be discussed in this section. Evidently, and not at all surprisingly, its turnover is not an isolated entity. The metabolism of the polyphosphoinositides is coupled to agonist and growth factor receptors [Monaco and Gershengorn, 1992; Pike, 1992].

The sequential phosphorylation of phosphatidylinositol by phosphatidylinositol-4-kinase and phosphatidylinositol-

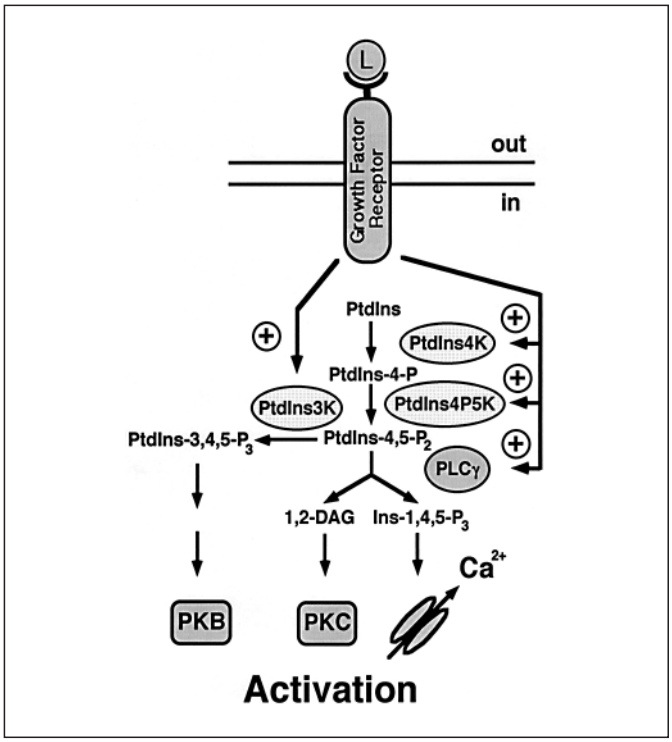

Fig. 8. Role of different phosphatidylinositol-derived compounds as second messengers. Phosphatidylinositol (PtdIns) is sequentially phosphorylated by phosphatidylinositol-4-kinase (PtdIns4K), phosphatidylinositol-4-phosphate-5-kinase (PtdIns4P5K) and phosphatidylinositol-3-kinase (PtdIns3K) to yield phosphatidylinositol-4-phosphate (PtdIns-4-P), phosphatidylinositol-4,5-biphosphate (PtdIns-4,5-P_2), and phosphatidylinositol-3,4,5-triphosphate (PtdIns-3,4,5-P_3). PtdIns-4,5-P_2 is hydrolyzed by phospholipase Cγ (PLCγ) to yield 1,2-diacylglycerol (1,2-DAG), an activator of protein kinase C (PKC), and inositol-1,4,5-triphosphate (Ins-1,4,5-P_3), an activator of calcium channels located in the endoplasmic reticulum. PtdIns-3,4,5-P_3 activates a protein kinase cascade which results in the activation of protein kinase B (PKB). Growth factor receptor tyrosine kinases recruit and activate (+) the enzymes involved upon ligand (L) binding. As an example of the involvement of these pathways in lectinology, it is relevant to mention that PtdIns-4,5-P_2 synthesis increases upon cell stimulation with the immunomodulatory mistletoe lectin [see Gabius et al., 1992, 1995].

4-phosphate-5-kinase yields PtdIns-4,5-P_2, a precursor molecule for several second messengers (fig. 8). This substance is produced at a 2-fold increased rate, when monocytic cells are exposed to the immunomodulatory mistletoe lectin at a concentration which effectively enhances cytokine synthesis and secretion, although the net result of cytokine availability should not be solely considered to be beneficial due to its potential stimulation of proliferation of responsive tumor cells such as melanoma, kidney tumor or myeloma cells [Gabius et al., 1992, 1995]. Both phosphatidylinositol4-kinase and phosphatidylinositol-4-phosphate-5-kinase bind to and are activated by the EGF receptor [Cochet et al., 1991], again underscoring the intimate relation between different signaling routes. The hydrolysis of PtdIns-4,5-P_2 by phospholipase Cγ forms Ins-1,4,5-P_3 and 1,2-DAG (fig. 8). As mentioned earlier, the former is an activator of Ca^{2+} channels in the endoplasmic reticulum and the latter is an activator of certain protein kinase C isoforms. Moreover, PtdIns-4,5-P_2 is phosphorylated to phosphatidylinositol-3,4,5-triphosphate (PtdIns-3,4,5-P_3) by phosphatidylinositol-3-kinase, which is recruited and activated by growth factor receptor tyrosine kinases (fig. 8). This example clearly underlines the intertwined nature of signaling pathways which can only formally be separated for didactic purposes. PtdIns-3,4,5-P_3 is definitely another important second messenger. It is involved in the binding of proteins to cellular membranes and in the activation of the serine/threonine-protein kinase B [Hemmings, 1997] (see fig. 8).

In addition to Ins-1,4,5-P_3, other inositol polyphosphates such as inositol hexakisphosphate (not shown in fig. 8) have been demonstrated to regulate different cellular systems as well. This compound for example inhibits different serine/threonine-phosphoprotein phosphatases resulting in the activation of voltage-dependent L-type Ca^{2+} channels of the plasma membrane [Larsson et al., 1997].

Figure 8 provides a simplified summary for the formation of the three major second messengers derived from the PtdIns metabolism and for their intracellular targets. In addition to the selected compounds, effects of other phosphoinositides, not included in figure 8, have been delineated. It is thus appropriate to point out that our presentation is deliberately intended as an introductory guideline rather than as an exhaustive compilation.

Phosphorylation-Mediated Signaling

As already referred to with respect to the enzymatic generation of cyclic nucleotides and the intrinsic activity of growth factor receptors, protein phosphorylation is a reversible post-translational modification of great physiological significance (fig. 6, 7). Moreover, one consequence of the enzymatic generation of polyphosphoinositides is the increase of protein phosphorylation (fig. 8). While the reader is familiar with text book examples of the impact of protein kinases on target enzyme activities for example in glycogen metabolism, the far-reaching significance of this type of protein modification touches upon a wider array of biological activities. As already indicated, lectin-induced receptor dimerization (fig. 1B) is a trigger for tyrosine phosphorylation. Thus, growth factor receptors with this intrinsic activity become transiently modified upon lectin binding. Therefore, binding of ligands to these receptors results in receptor transphosphorylation. The phosphorylated sites in the receptor establish new adaptor determinants which enable recognition events with distinct protein modules. Based in these interactions, a series of proteins containing either Src homology 2 domain (SH2 domains) [Margolis, 1992] or phosphotyrosine-binding domains [van der Geer and Pawson, 1995] recognizes the phosphorylated tyrosine residues located in the receptor. Among these are the previously mentioned phospholipase Cγ and phospholipase A_2, and also phosphatidylinositol-3-kinase (PtdIns3K), phosphatidylinositol-4-kinase and phosphatidylinositol-4-phosphate 5-kinase (fig. 6, 8). Other gathered proteins, however, merely have an adaptor function, such as growth factor receptor-bound protein 2 (Grb2), bridging the receptor to target proteins with enzymatic activities such as the GTP exchanger factor Son of sevenless (Sos). In any event, the enrollment of these proteins acts as initiation point for different signaling pathways. The role of specific recognition sequences in the assembly of the different signaling pathways aided by adaptor and scaffold proteins has been recently reviewed [Pawson and Scott, 1997]. It is at this stage pertinent to reinforce the validity of the concept that the separately introduced phosphorylation pathways are intimately intertwined, as exemplified by the mitogenactivated protein kinase (MAPK) pathways shown in figure 9.

The connection between plasma membrane-bound receptors channeling extracellular signals to a given protein kinase cascade pathway is often secured by the above mentioned adaptor proteins coupled to monomeric G proteins of the Ras family [Haubruck and McCormick, 1991; Denhardt, 1996] (fig. 9). The different small monomeric G proteins, among which Ras, Rho and Rab are the most prominent members, not only regulate cell growth, differentiation and apoptosis, but also phagocytosis, vesicular trafficking including endo- and exocytosis, cytoskeletal dynamics, cell attachment and adhesion as well as cellular motility [Haubruck and McCormick, 1991; Denhardt, 1996; Lacal, 1997; Narumiya et al., 1997].

Although these processes at first sight appear to be very difficult to delineate unambiguously in vivo, valuable information is provided by the elegant approach of using selective inhibitors for signal transduction mechanisms to pinpoint relevant mediators within the flow of information. Using this method, protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II were judged to be involved in ganglioside-stimulated phagosome/lysosome fusion, while arachidonic acid generation and leukotriene synthesis appeared to be associated with granule release by NK cells and lectin-mediated neutrophil adhesion [Cifone et al., 1997; Timoshenko et al., 1997, 1998; Yamaguchi et al., 1997]. Interestingly, not only the turn-on, but the turn-off of a signal, namely dephosphorylation of phosphotyrosine residues by a phosphatase, can follow lectin-dependent cell contacts, as evidenced for the invasion of protozoa by the causative agent of the Legionnaires' disease [Venkataraman et al., 1997]. An evident advantage of the inhibitor approach is the possibility of assessing the potentially additive contributions of various pathways to the generation of a distinct parameter change [Timoshenko et al., 1997, 1998]. Further pursuits to unravel intimate details of the involved effectors can thus be focused by singling out functionally neutral reactions on the basis of the lack of any response interference by a target-selected signaling inhibitor, for example a kinase, phosphatase or NO synthase inhibitor.

As already noted, the strategically placed tyrosine phosphate groups in activated growth factor receptors are the initiation points of signal transduction pathways. Thus, the SH2/Src homology 3 domain-containing protein Grb2 binds at the same time to phosphorylated growth factor receptors such as the EGF receptor and the PDGF receptor, and to the nucleotide-exchanger factor Sos and analogous proteins. In some instances, however, the adaptor protein Shc is also involved in the activation process of certain growth factor receptors (see fig. 9). Plasma membrane-bound Ras is thus activated by Sos replacing GDP by GTP in its binding site, basically analogous to the Gα subunit ligand exchange. Thereafter, GTP-Ras targets and activates Raf-1 and other related serine/threonine-protein kinases. These kinases are the first constituents of the MAP kinase cascade and are therefore denoted MAPK kinase kinase (MAPKKK) (fig. 9). However, the activation of some MAPKKK such as MEK kinase/SAPK kinase kinase (MEKK) is mediated by p21-activated kinase (PAK), an upstream protein kinase, that in turn is activated by Rac, a PtdIns3K-activated small G protein that receives the signal for its activity enhancement from Ras [Giancotti, 1997] (see fig. 9). The MAPKKK act on a family of dual-specific threonine/tyrosine-protein kinases such as MAP/ERK kinase and SAPK kinase 4 (MKK4) as substrates for phosphate transfer. Therefore, and as second downstream components of this cascade, they are also denoted MAPK kinase (MAPKK) (fig. 9). These kinases have in turn as

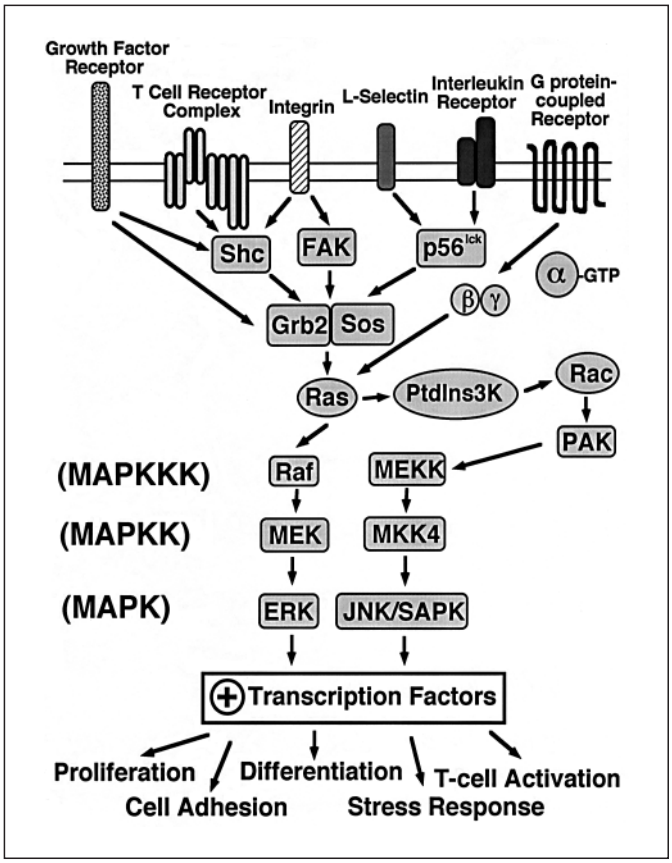

Fig. 9. Multiple systems activate the mitogen-activated protein kinase (MAPK) pathways. Two parallel MAPK pathways (Raf → MEK → ERK, and PAK → MEKK → MKK4 → JNK/SAPK) are represented as collectors of signals derived from growth factor receptors, the T cell receptor complex, integrins, L-selectin, interleukin receptors, and G protein-coupled receptors, which result in transcriptional activation and the subsequent occurrence of specific cellular responses, such as proliferation, differentiation, T cell activation, cell adhesion and stress responses among others. The MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK components of the two pathways are represented at the same level in the scheme. For the identification of the individual components of the different signaling pathways see text. MEK = MAP/ERK kinase; ERK = extracellular signal-regulated kinase; PAK = p21-activated kinase; MEKK = MEK kinase/SAPK kinase kinase; MKK4 = SAPK kinase 4; JNK/SAPK = c-Jun N-terminal kinase/stress-activated protein kinase. The C-type lectin L-selectin, involved in neutrophil activation and adhesion to endothelium, represents a clear example in which the activation of the MAPK pathway occurs upon lectin-glycoligand interaction [see Waddell et al., 1995].

substrates a family of serine/threonine-protein kinases denoted MAPK (fig. 9) such as extracellular signal-regulated kinase and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) among others, which translocate to the nucleus and phosphorylate a number of transcription factors, e.g. Elk-1, CREB and c-Fos, with veritable implications for gene expression [Janknecht et al., 1993; Gille et al., 1995; Okazaki and Sagata, 1995; Xing et al., 1996] (see fig. 9). The importance of nuclear pores as sites to direct the traffic in the transport of proteins and RNA transcripts into and out of the nucleus, as occurring during transcriptional activation, has recently been reviewed [Nigg, 1997].

It is also important to mention that certain components of the signaling cascades such as the adaptor protein Shc help to converge signals derived from different sensor molecules, for example certain growth factor receptors, the T cell receptor complex and integrins, into a common downstream MAPK pathway [Ravichandran et al., 1995; Giancotti, 1997; Meredith and Schwartz, 1997] (see fig. 9). Similarly, L-selectin and interleukin receptors such as the interleukin 2 receptor share $p56^{lck}$ as a common component, converging the pathway at the Grb2/Sos complex to signal via the MAPK cascade [Taniguchi et al., 1995; Brenner et al., 1996] (see fig. 9).

With respect to lectins, it is noteworthy that the increased tyrosine phosphorylation-dependent stimulation of MAPK by cross-linking L-selectin, a C-type lectin, is suggested to contribute to neutrophil activation and adhesion to the endothelium during the inflammatory response [Waddell et al., 1995]. Similarly, the binding of the ligand for another selectin, namely P-selectin glycoprotein ligand-1, leads to MAPKK activation and reveals tyrosine phosphorylation as a biologically important event in neutrophil response to selectin binding [Hidari et al., 1997]. Signal transduction by selectins is the focus of a recent review article, which is recommended as source of further information on this subgroup [Crockett-Torabi, 1998].

As documented in the preceding paragraphs, one well-investigated case of carbohydrate-mediated cell contact formation which serves as a model for elucidation of signaling is the interaction between leukocytes and endothelial cells via selectins [Gabius, 1997a]. For example, L-selectin guides lymphocyte rolling on the endothelial cell layer in concert with E- and P-selectins as a first step to aim at its transmigration across the capillary [for illustration of the orchestrated reactions towards egress from capillaries, see Kaltner and Stierstorfer, 1998]. Binding of cognate sulfatides to L-selectin produces activation of the MAP kinase pathway in neutrophils, as already mentioned [Waddell et al., 1995]. Within these processes in T lymphocytes, the *src*-tyrosine kinase $p56^{lck}$ and the subsequent activation of Rac proteins have a positive impact on the stress-activated protein kinase JNK/SAPK (see fig. 9) [Brenner et al., 1996, 1997]. Apparently, these pathways are also important for the formation of the free radical superoxide anion ($O_2{}^{*-}$) during the activation of leukocytes.

Moreover, relevant regulation of gene expression has been observed in the realm of cell adhesion molecules (see fig. 9). Tyrosine kinase receptors can regulate the expression of a large panel of proteins involved in cell-to-cell adhesion and cellular adhesion to the extracellular matrix, starting already at fertilization [Kinsey, 1998]. These processes are unquestionably of great importance in development and morphogenesis. For example, the overexpression of the EGF receptor upregulates CD44, a cell surface adhesion molecule which increases cell attachment to hyaluronic acid in the extracellular matrix [Zhang et al., 1997]. Furthermore, integrin-mediated cell adhesion to the extracellular matrix conveys cell survival signals preventing the occurrence of programmed cell death.

The signaling pathways activated by integrins are apparently similar to those activated by mitogenic factors [Giancotti, 1997] (see fig. 9). Moreover, the absence of integrin-mediated signaling can induce downregulation of Bcl-2 and the activation interleukin-1β-converting enzyme-like proteases (caspases) which play a role in facilitating biochemical events characteristic for apoptosis [Meredith and Schwartz, 1997]. Integrin-mediated cell adhesion to laminin and fibronectin for example activates the MAPK pathway [Chen et al., 1994; Giancotti, 1997] which is essential for proliferation and differentiation processes (fig. 9). However, it has been proposed that this activation may be independent of Ras, but dependent on the activation of focal adhesion kinase [Chen et al., 1996]. Although all the pathways for integrin signaling are not yet entirely known, the participation of PtdIns3K and Rac as downstream components from Ras that exploit an alternative MAPK cascade formed by PAK, MEKK, MKK4 and JNK/SAPK has been documented [Giancotti, 1997] (fig. 9). Nevertheless, this pathway does not appear to be universally relevant, since not all cell types react identically. Hence, an integrin appears to be capable of exerting the downregulation of the MAPK cascade in platelets [Nadal et al., 1997]. Interestingly, the activation of the Ras/MAPK pathway can also be achieved by G protein-coupled receptors. This appears to be mediated by the dissociated Gβγ dimer released from the activated G protein upon receptor interaction with its ligand [Graves et al., 1995] (fig. 9).

As a summary, figure 9 depicts in a simplified manner the activation of two parallel MAPK pathways and other

kinases by growth factor receptors, the T cell receptor complex, integrins, selectins, interleukin receptors, and G protein-coupled receptors. Like pieces of a puzzle, figures 5–9 should be placed together to get an idea of the potential of the intracellular information traffic which presently is deciphered at a surely incomplete level. Nevertheless, these basic reactions provide a mechanistic framework of cellular events which partake in the translation of receptor-ligand recognition at the molecular level into morphological changes.

As already mentioned, not only the presence, but also the removal of an amino acid-bound phosphate group has crucial consequences. This has been documented by the use of vanadate as an inhibitor of tyrosine-specific phosphoprotein phosphatases. Proteins phosphorylated at tyrosine residues are dephosphorylated by a large group of phosphotyrosine-protein phosphatases, some of which appear to be receptors located in the plasma membrane such as CD45, and others are located intracellularly. Receptor-like phosphatases appear to be involved in ligand-cell and cell-cell interactions. Among the latter function, the participation of these enzymes in cell adhesion and aggregation processes now comes into view [Mauro and Dixon, 1994; Dixon, 1995]. Explicitly implicating an animal lectin, CD45 binds to the B lymphocyte I-type lectin CD22, which recognizes distinct glycoproteins with the sialic acid-α2,6-galactose disaccharide, and to the antigen-receptor/CD3 complex. The interaction of CD22-CD45-CD3 results in the activation of the phosphatidylinositol-specific phospholipase Cγ1. This enzyme forms the already mentioned messengers 1,2-DAG and Ins-1,4,5-P_3. As illustrated in our schemes (see fig. 6, 8), protein kinase C activation by 1,2-DAG and Ca^{2+} mobilization from intracellular stores by Ins-1,4,5-P_3 eventually ensue from this molecular interplay [Sgroi et al., 1995].

Further inspection of phosphatases reveals proteins capable of dephosphorylating phosphotyrosine and phosphothreonine residues, which assumably play a role in the deactivation of the MAPK pathway (see fig. 9) and hence in the regulation of cell cycle among other functions [Mauro and Dixon, 1994; Dixon, 1995].

Involvement of Tyrosine Kinases in Complex Developmental Processes

The readers of this journal, whose expertise lies primarily in the examination of tissue architecture, may wonder how to link these biochemical events to pattern formation. In addition to the obvious consequences of cell proliferation in this context, as documented by the activation of growth factor receptors, it is instructive to give some additional examples of how this is achieved during development and morphogenesis.

The cooperation of different tyrosine kinase receptors to attain the successful formation of complex structures such as the embryonic vascular system is exemplified by pointing to the participation of several endothelium-specific tyrosine kinase receptors in vasculogenesis and angiogenesis. The vascular endothelial growth factor form a family which includes ligands for three of these receptors (Flk-1/KDR, Flt-1, and Flt-4), while two additional members of this family (Tie-1 and Tie-2/Tek) are orphan receptors without known ligand(s) [Merenmies et al., 1997].

Moreover, the role of tyrosine kinases in development is also exemplified by the Eph family of tyrosine kinase receptors and their ligands denoted ephrins [Eph Nomenclature Committee, 1997]. Receptors and ligands are expressed in different cell types, where they play a pivotal role in the segmentation patterns of early embryos. Moreover, these molecules also intervene in the development of the central nervous system. They play a crucial role in axonal guidance and pathfinding by eliciting repulsion signals, as illustrated in figure 10, contributing to the overall regionalization of the different neural structures [Nieto, 1996; Orioli and Klein, 1997; Pasquale, 1997; Sefton and Nieto, 1997]. Other growth factors such as hepatocyte growth factor/scatler factor, brain-derived neurotrophic factor, neutrophin-3, nerve growth factor and fibroblast growth factor have likewise been implicated in axonal guidance in addition to their role as trophic agents [McFarlane and Holt, 1997].

These complex processes also involve endogenous lectins of the nervous system [Zanetta, 1998], whose action by signaling presents an attractive topic for future research. When considering the role of tyrosine kinases in pattern formation and development, their emerging role in fertilization deserves attention [Kinsey, 1998]. Due to the importance of protein-carbohydrate recognition in this process [Gabius, 1987b; Sinowatz et al., 1997, 1998] it is an attractive hypothesis to partially link such events with level alterations of phosphorylated tyrosine moieties.

The preceding paragraphs have led us from the biochemical level of the description of events which initiate signaling cascades to the histochemical level, as these biochemical processes finally yield visible morphological changes. Although many details remain to be elucidated, an intriguing picture is emerging by the delineation of the actual chain of events. This task resembles a molecular puzzle to be solved with diligence and perseverance. Complex

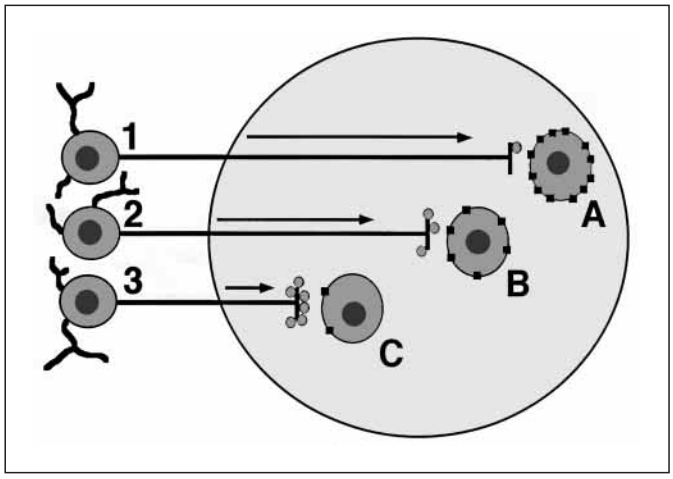

Fig. 10. A mechanism for axonal guidance and migration. A neuron expressing low levels of Eph tyrosine kinase receptors (neuron 1) deeply projects its axon to a reception area (large ovoid) where cells are organized forming a gradient with increasing numbers of exposed ligands (ephrins) in their distal region (right). These axons migrate until they encounter cells which express high levels of this ligand (cell A). Upon the establishment of intercellular interactions, repulsion signals are elicited that prevent further axonal migration. The axonal projection of neurons expressing progressively higher concentrations of Eph receptors (neurons 2 and 3) would have proportionally shorter axonal migration than the neurons expressing a lower number of this receptor (neuron 1). Axonal migration of neurons 2 and 3 would thus be arrested sooner by their interactions with cells expressing progressively smaller numbers of ligands (ephrins) (cells B and C) present on their plasma membranes and located either in the central region (center) or in the proximal region (left) of the reception area (large ovoid) [for a compilation of the role of endogenous lectins in the nervous system, see Zanetta, 1998].

events such as cell proliferation are thus the consequence of defined biochemical processes linked to transmit and often to amplify extracellular signals. Historically, prime attention has been paid to peptide growth factors in this area. As depicted in figure 1B, the initial trigger reaction, among others, can also be the accomplishment of a snug fit between a lectin and its glycoligand.

Mitogenic and Apoptotic Effects of Lectins

During the different morphogenetic and developmental stages of multicellular organisms, cells undergo spatially and temporally exquisitely tuned proliferation, migration, differentiation and apoptotic processes (see fig. 2). In this review we shall concentrate on the description of the effect of exogenous lectins and the implication of endogenous lectins in the two processes by which the total number of cells in an organisms can be modified, that is proliferation and apoptosis. Nevertheless, it is important to emphasize that during development cell proliferation, migration, differentiation and apoptosis are not unrelated events. Conversely, they are intimately connected and balanced to yield the generation of a developmentally normal organism.

To build a bridge between the exemplified studies at individual signaling pathways and the morphological changes in lectin research, it is pertinent to briefly point out that lectins can excel at eliciting immediately visible changes in cellular parameters. Known from immunology laboratories, plant lectins are readily available immune cell mitogens [Borrebaeck and Carlsson, 1989; Villalobo et al., 1997a]. Additionally, these exogenous lectins are able to mimic the action of some growth factors. For example, the popular concanavalin A stimulates the EGF receptor tyrosine kinase [Zeng et al., 1995], the subsequent growth factor-dependent Ca^{2+} mobilization [Wheeler et al., 1987] and the activation of the Na^{+}/H^{+} exchanger [Green and Muallem, 1989]. This cascade of events is readily apparent on figures 6 and 8.

Concerning these activities it should not be necessarily assumed that endogenous lectins will display the same capacity in this respect. The differences in the fine specificities of plant and animal lectin pairs would otherwise be underestimated, as emphasized by the deliberate recommendation to employ animal lectins in histochemical studies instead of exogenous model proteins to increase the physiological relevance of the results [Gabius and Gabius, 1993, 1997]. At any rate, the responses of animal cells to plant lectins paved the way for attempting a delineation of physiological functions of animal lectins. Likewise, cell proliferation is assessed with plant lectins and homologous cells or parasites to define functions within plants [Rüdiger, 1997, 1998]. To document a positive influence on proliferation for an animal lectin, we cite the case of an endogenous mannose receptor with at least a close relationship to the tandem repeat C-type lectin expressed in macrophages. This lectin elicits a mitogenic response in lung smooth muscle cells upon its stimulation with a mannosylated neoglycoprotein [Lew et al., 1994]. Enhancement of proliferative activity is not restricted to this lectin. The endogenous cerebellar soluble lectin CSL is known to induce mitogenesis in Schwann cells [Badache et al., 1995]. A further example of the mitogenic action of lectins is provided by galectin-1, a β-galactoside-specific lectin, which stimulates the proliferation of vascular cells, thus favoring angiogenesis and vascularization [Sanford and Harris-Hooker, 1990]. However, the effects of galectins on cell proliferation cannot be readily predicted, since a rather complex chain of events

underlies this process. As an example, recombinant galectin-1 has been shown to have a dual role in cell proliferation, mediating a mitogenic action by binding to β-galactosides on the cell surface at low concentrations, and inhibiting cell growth in a β-galactoside-independent fashion at elevated concentrations [Adams et al., 1996]. By virtue of ganglioside sialidase-dependent increases in the level of ganglioside GM_1 presentation galectin-1 appears to play a crucial role in triggering cell growth arrest and differentiation in cultured human neuroblastoma cells [Kopitz et al., 1998]. Ligand properties of gangliosides for galectins add a new facet to the already documented array of their functions in cellular physiology [Kopitz, 1997; Hakomori, 1998].

The cellular proliferative program is not only positively affected by lectins. On the contrary, lectins are also capable of diminishing the cell number. Programmed cell death, also called apoptosis, is induced by internal and external cellular signals and is an essential mechanism to remove unwanted and no longer required cells during the normal development of multicellular organisms (see fig. 11). Moreover, apoptosis also plays an important role in the normal immune response, for example during cytotoxic T-lymphocyte-induced apoptosis of target tumor cells. When considering induction of apoptosis as a therapeutic means, concomitant upregulation of the normal mediators for this process in a paracrine fashion may yield an immunologically privileged site in the vicinity of for example tumor cells, which may have unfavorable consequences for the patient [Krammer, 1997].

During apoptosis cells undergo characteristic morphological changes upon activation of a number of cellular processes that could be initiated for example by tumor necrosis factor (TNF) in cells expressing TNF receptors or by Fas ligand in cells expressing CD95 (Fas/APO-1). Interestingly, certain cytokines such as interleukin 6 and interferon-γ, and growth factors such as EGF can also induce apoptosis [Chin et al., 1997]. Moreover, a wide panel of chemicals also display this activity, when the concentration reaches a permissive level. These changes including DNA fragmentation that yields the typical electrophoretic ladder pattern are distinct from those occurring during necrotic cell death. Therefore, no inflammatory response is elicited during apoptosis. The apoptotic cells are eliminated by surrounding cells without the exposure of the intracellular content to the immune system, and the different biochemical events underlying the morphological changes during apoptosis that will be mentioned below have been extensively reviewed [Gerschenson and Rotello, 1992; Chinnaiyan and Dixit, 1996; Zhivotovsky et al., 1996; Chin et al., 1997; Cohen, 1997; Wallach et al., 1997; Enari et al., 1998]. Interestingly, the clearance of apoptotic cell bodies can also be performed by a carbohydrate-mediated process with the participation of endocytic receptors such as the C-type asialoglycoprotein receptor of hepatocytes [Dini et al., 1992].

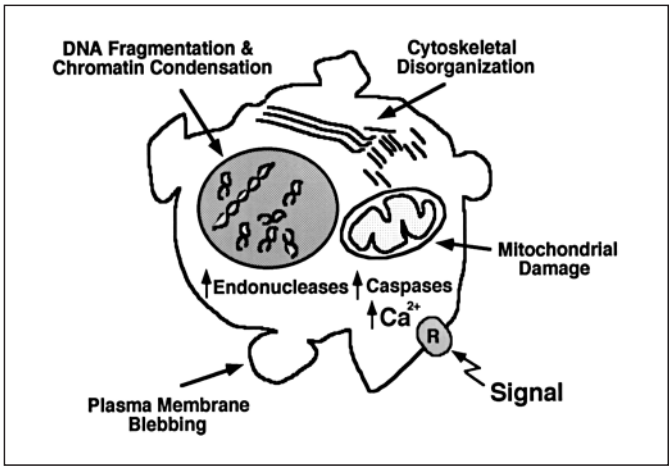

Fig. 11. Major events during apoptotic cell death. External apoptotic signals can deliver their messages by specific plasma membrane receptors (R) such as CD95 (Fas/APO-1) and TNF receptors. This results in the activation of caspases, endonucleases, formation of active oxygen species, inactivation of cytochrome c and increase in the cytosolic concentration of free Ca^{2+}. These processes are followed by irreversible DNA fragmentation, chromatin condensation, plasma membrane blebbing, disruption of cytoskeletal organization and mitochondrial damage. The resulting cellular debris (apoptotic bodies) are eliminated by macrophages employing for example lectin-dependent endocytosis of glycoepitope-bearing fragments [see Dini et al., 1992].

The first event responsible for signaling an apoptotic response appears to be the activation of specific receptors such as CD95 (Fas/APO-1) and TNF receptors. Thereafter, a family of intracellular aspartate-specific cysteinyl proteases, denoted caspases, are activated and play an important role in the overall apoptotic process. Furthermore, activation of a specific caspase-activated endonuclease, which fragments nuclear DNA at the linker regions between the nucleosomes, is readily observed, because DNA degradation results in a characteristic fragmentation pattern with the appearance of segments of DNA of regular length. Moreover, a characteristic blebbing of the plasma membrane and cytoskeletal disruption are seen during apoptosis. Furthermore, mitochondria produce reactive oxygen intermediates in apoptotic cells that lead to lipid peroxidation and cytochrome c inactivation. Both processes contribute to evoke a drop in membrane potential and inhibit oxidative phosphorylation. Figure 11 presents a summary of major events in a cell undergoing apoptosis.

As can be expected, the cell also has means of averting apoptosis. A major component of this system is a membrane-bound protein denoted Bcl-2. Thus, overexpression of this protein prevents the inactivation of cytochrome c, a feature associated with apoptosis as mentioned above. Based on limited sequence similarity to Bcl-2, most importantly the NWGR motif in the BH1 domain, and its capacity to confer resistance to apoptosis, human galectin-3 has been defined as a cell death inhibitor for human leukemic T lymphocytes and breast carcinoma cells [Yang et al., 1996; Akahani et al., 1997].

Induction of apoptosis by different plant lectins has been described in several cell types. For example, the lectin from *Griffonia simplicifolia* I-B_4 and wheat germ agglutinin trigger apoptosis after their receptor-mediated internalization into murine tumor cells expressing binding sites for these lectins, while resistant tumor cells are devoid of these receptors [Kim et al., 1993]. Moreover, ricin, a galactose-specific lectin, induces apoptosis in macrophages nonattached to a surface by a Ca^{2+}-independent mechanism, while preadherence of the macrophage to a surface prevents apoptosis [Khan and Waring, 1993]. However, plants are not the only source of lectins capable of inducing apoptosis. Animal lectins are also active in this context, two members of the galectin family being prominent examples [Goldstone and Lavin, 1991; Perillo et al., 1995; Rabinovich et al., 1997]. A major concern in the interpretation of results derived from in vitro experiments in this area is the use of often rather high concentrations of lectins required to trigger apoptosis. Therefore, only if it is documented that these concentrations can be reached in vivo, physiological relevance can be ascribed to these experimental data.

Concluding Remarks

It certainly is an ideal situation being able to correlate the presence of a lectin or a glycoconjugate with a histochemically visible pattern and to straightforwardly and unerringly draw a scheme of the involved signaling mechanism. However, this is not always the case. This article intended to document established routes of information transfer which are often taken to elicit ensuing cellular responses. They could also be used downstream of the initial recognition event by endogenous and exogenous lectins.

The outlined observations on the signaling events that may be brought into action upon lectin-glycoligand interactions attest the possible physiological relevance of these molecules for triggering such cascades during their effects on development and morphogenesis. Indubitably, our knowledge in this area is still very limited, and the puzzle resembles an unfinished Escher's drawing, in which it is presently difficult to predict the final outcome. The desirable refinement of our knowledge on the individual cellular signaling pathways, their cross talk, and the impact of lectin-ligand recognition on information transfer will put us in an appropriate position to understand the role of lectins in modulating cellular activities. In this sense, we face the difficult, though definitely not insurmountable task of deciphering the glycocode as well as the triggered intracellular transduction pathways on our way to comprehend the physiological situation as a means to devise therapeutic strategies for different pathological processes.

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