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Commentary

New paradigms in signal transduction

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ABSTRACT

Signal transduction is a dynamic field in which established pathways evolve and new pathways emerge. The purpose of this commentary is to highlight new paradigms of signal transduction that have developed over the past few years. This discussion proposes a third member of the generic models of membrane receptors in addition to the 7-transmembrane pass receptor and the enzyme-linked receptor: the non-enzymatic nucleating receptor. Also discussed are the new paradigms of signal transduction by proteolysis which includes signaling by Notch, signaling through the Hedgehog and Wnt pathways, signaling through histidine phosphorylation, and reactive oxygen species in signal transduction.

1. Introduction

Signal transduction touches every other field of life science because it is the means by which cells communicate with each other. Our own eukaryotic cells must be able to communicate with their neighbors as well as with distant cells, and prokaryotic cells communicate with their environment. Signal transduction pathways are used in all of these cases to convey the messages of ligands into changes in the biological activity of the target cells. Moreover, aberrant signaling through these communication pathways results in many diseases. As a result, signal transduction pathways have been the target of drug development. Many drugs have been designed to exploit the receptors that activate signaling pathways, and the intracellular components of signaling pathways are also the target of drug discovery.

The field of signal transduction continues to be dynamic. Over the past few years new paradigms of signal transduction have been recognized and additional details of known pathways have been discovered. The purpose of this commentary is to review these new paradigms in signal transduction and, when possible, to incorporate them into known models of signaling.

2. Fundamental models of membrane receptors for signal transduction

Two generic models of membrane receptors for water-soluble ligands are broadly accepted. In the model of G-protein coupled receptors, a 7-transmembrane pass receptor protein with a ligand binding domain is coupled with a heterotrimeric G protein and a separate effector enzyme protein [1]. Ligand binding to the extracellular loops of the 7-transmembrane pass receptor results in a conformational change in the receptor protein that serves to activate the heterotrimeric G protein. The Gα-subunit releases GDP and binds to GTP; the GTP-bound Gα-subunit protein activates the effector enzyme which initiates the formation of a second messenger inside the cell. This pathway has been widely studied and well-reviewed [2]. The second fundamental model of membrane receptor is the enzyme linked receptor model [1]. In this

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model, the initial signal transducing unit is composed of a single transmembrane protein that contains a ligand binding domain, a transmembrane region, and an effector enzyme domain at the cytoplasmic face of the membrane. Ligand binding to the extracellular region of the receptor causes a change in the conformation of the protein that is transmitted through the transmembrane region and results in activation of the effector enzyme at the cytoplasmic face of the membrane. In many examples of this model the binding of ligand causes dimerization of two receptor proteins. This pathway has also been thoroughly reviewed [3]. An evaluation of the expansion of signal transduction models indicates that a new category should be added to this group of fundamental models of membrane receptors: the non-enzymatic nucleating receptor.

The non-enzymatic nucleating receptor model is composed of an extracellular ligand binding region and a transmembrane region that reaches the cytoplasmic face of the membrane but which has no effector enzyme activity (Fig. 1). Ligand binding to the extracellular region of this receptor causes a conformational change in the receptor that is transmitted through the membrane spanning region of the protein. The conformational change stimulates nucleation of protein complexes at the cytoplasmic face of the membrane; the ligand-bound receptor serves as a scaffold of activation of proteins that form the signaling complex. The number of receptors known to utilize this model has grown in recent years to the extent that it is now time to include this receptor pattern as a separate fundamental model of membrane receptors; this non-enzymatic nucleating receptor model should take its place beside the G-protein-coupled receptor and the enzyme linked receptor models. A variety of ligands use the non-enzymatic nucleating receptor model. For example, signaling from the extracellular matrix through integrins (Fig. 1A), cadherins, or selectins utilizes a nucleating receptor model [4,5]. The cytokine receptor that activates the Janus (JAK) tyrosine kinase also uses this model [6], as well as the Fas (Fig. 1B) and TNFα receptors [7,8], the T cell receptor [9] and the B cell receptor [10].

Integrin signaling is a good model of the non-enzymatic nucleating receptor model (Fig. 1A). Integrins are composed of αβ heterodimers. Ligands for integrins are extracellular matrix proteins such as fibronectin and collagen. The binding of ligand to the integrin dimer nucleates the formation of a large signaling protein complex at the cytoplasmic face of the membrane that

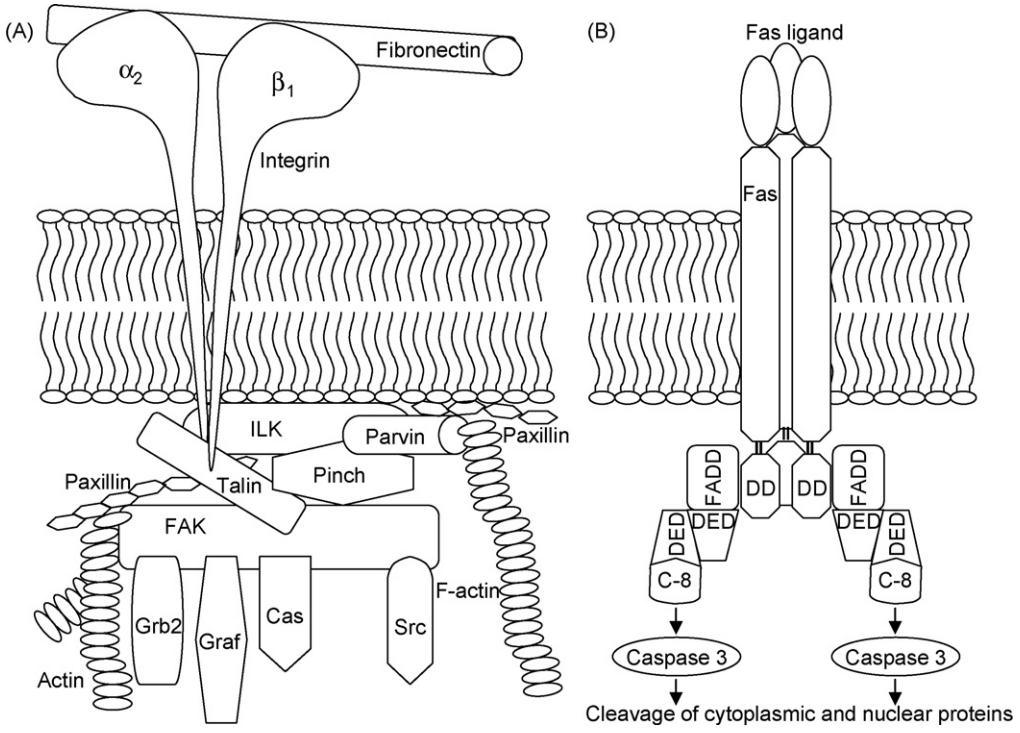

Fig. 1 – Non-enzymatic nucleating receptors. Panel A: signaling through integrins. Pairs of α and β integrins bind to proteins such as fibronectin in the extracellular matrix. This ligand binding causes conformational changes in the integrins that result in the formation of protein complexes at the cytoplasmic face of the membrane that mediate the extracellular signal to the cell. The protein complexes contain protein kinases and cytoplasmic proteins as well as docking proteins such as Grb2, Cas and paxillin. The proteins shown here represent only a small number of those that participate in these protein complexes. ILK: integrin-linked kinase; FAK: focal adhesion kinase; Graf: GTPase regulator associated with FAK; Cas: Crk-associated substrate; Pinch: particularly interesting new cystein-histidine-rich protein. Panel B: the binding of Fas ligand to Fas causes trimerization of the receptor with resultant clustering of the death domains (DD) of the receptor. The adaptor protein FADD (Fas-associated death domain) oligomerizes with the death domains of the receptor. The death effector domain (DED) of FADD then activates the protease, caspase 8, by binding to its death effector domain. Caspase 8 (C-8), in turn, initiates a cascade of proteolytic activity by activating effector caspases such as caspase 3. The end result of this proteolytic cascade is apoptosis.

connects the integrins to the actin cytoskeleton. The formation of the protein complex itself is a signal that the cell is attached to the extracellular matrix. Moreover, many of the proteins within the complex are protein kinases (integrin-linked kinase, focal adhesion kinase, Src) [11,12]. In cells such as lymphocytes that move along a surface, these adhesion complexes form transiently at the point of contact between the cell and the subcellular surface.

Transmembrane receptors that lack an enzymatic domain have often been called ''decoy receptors''. The binding of ligand to these non-enzymatic decoy receptors was thought to sequester the ligand in order to prevent its binding to a receptor with enzymatic activity. It is likely that there are some receptors whose sole purpose is to act as decoys. However, it is also likely that some of the so-called decoy receptors are actually non-enzymatic nucleating receptors whose function was uninterpretable at the time of observation because the mechanism was still unknown. If the receptor experiments were designed to detect enzymatic activity, then the bias would have been to interpret receptors/binding sites without enzymatic activity as inactive decoys rather than seeking to identify an alternate explanation. We now recognize that nucleation of cytoplasmic proteins constitutes such an alternative mechanism of action. Since the non-enzymatic nucleating receptors function through protein–protein interactions and the sequences of protein–protein interaction domains are known, it would be worthwhile to revisit the cytoplasmic regions of decoy receptors with the tools of bioinformatics in a search for such domains to determine whether decoy receptors are capable of forming signal transduction complexes.

3. Signal transduction by proteolysis

Signaling by proteolysis is a new paradigm of signal transduction. The unique feature that sets this mechanism of signal transduction apart from other mechanisms is its irreversibility. The familiar signal transduction pathways that use protein or lipid phosphorylation/dephosphorylation cascades for the passage of signal are readily reversible. The cycle of protein phosphorylation–dephosphorylation–rephosphorylation can be carried out at the same site of a substrate protein or lipid multiple times without damaging or ''using up'' the substrate. In contrast, once a protein is proteolytically cleaved it cannot reenter the cycle of signaling. The cleaved protein carries out its function and is then destroyed and a new protein is required in order to send a new signal through the pathway. It is this absolute lack of reversibility that distinguishes the fundamental difference of signal transduction by proteolysis from other means of signal transduction.

Signal transduction by proteolysis is a separate concept from intracellular proteolysis as a means of destroying damaged, worn out, or misfolded proteins, although some of the same mechanisms are involved in both cellular functions [13,14]. Signaling by proteolysis includes extracellular proteases and intracellular proteases, as well as a process called regulated intramembrane proteolysis.

An example of the involvement of extracellular proteases in signal transduction is the proteinase-activated receptor (PAR) family of proteases. Of these, the best known example is the thrombin signaling system [15]. Thrombin is a protease and its receptor is a 7-transmembrane pass receptor that signals through a heterotrimeric G protein. Instead of binding to its receptor as do conventional ligands, thrombin cleaves a peptide from the amino terminus of its 7-transmembrane pass receptor. The new amino terminus then acts as a tethered ligand which interacts with the extracellular loops of the 7-transmembrane pass receptor to activate the receptor [15].

The most widely recognized intracellular proteases involved in signal transduction are the caspases that are involved in apoptotic signaling [7,16]. Activation of Fas (a non-enzymatic nucleating receptor) by the trimeric Fas ligand leads to trimerization of the receptor (Fig. 1B). The death domains (DD) of the three receptor proteins are brought into juxtaposition with each other. The aggregated death domains of the receptor bind to the death domains of the adaptor protein, Fas-associated death domain (FADD). The death effector domains of FADD then bind to the death effector domains of caspase 8 which activates the proteolytic activity of caspase 8. Caspase 8 is an initiator caspase. It cleaves caspase 3, an effector caspase, to initiate a cascade of proteolytic cleavage reactions catalyzed by caspase 3 and other caspases which ultimately lead to the death of the cell through apoptosis [16].

Regulated intramembrane proteolysis is a process that is shared by signal transduction, the intracellular pathway that recognizes misfolded proteins in the endoplasmic reticulum, and the sterol regulatory element binding protein pathway that regulates genes that control lipid synthesis and uptake [13,14]. One would expect that the transmembrane regions of a transmembrane protein would be protected from proteolysis by the surrounding membrane lipids and by the structure that a protein takes in order to be imbedded in the membrane [14]. In most cases this assumption is correct. However, a subset of proteases is capable of attacking transmembrane proteins within the plane of the membrane. A good example of regulated intramembrane proteolysis in signal transduction is the relatively new Notch signal transduction pathway.

The Notch signaling pathway is important for cell fate determination and pattern formation during embryonic development, and it has been implicated in human genetic diseases and in tumorigenesis [17] (Fig. 2). The known ligands for the Notch pathway are the DSL (for Delta, Serrate, Lag-2) proteins in invertebrates and Delta-like (DLL) and Jagged in vertebrates [17]. The DSL/DLL ligands are transmembrane proteins. As transmembrane proteins, these ligands are fixed and signaling can only take place between two neighboring cells. The intracellular regions of these proteins are not conserved and their functions are unknown. Notch is the receptor for this pathway and it, too, is a transmembrane protein [17] (Fig. 2). Notch does not have enzymatic activity. It is composed of two peptides that are covalently linked; one peptide crosses the membrane and the other is entirely extracellular. Notch is synthesized as a single protein and is cleaved into two peptides during processing; the two peptides remain attached by a disulfide bridge after insertion into the membrane [18].

When the DSL/DLL ligand on one cell binds to the Notch receptor on a neighboring cell, the Notch protein is cleaved at

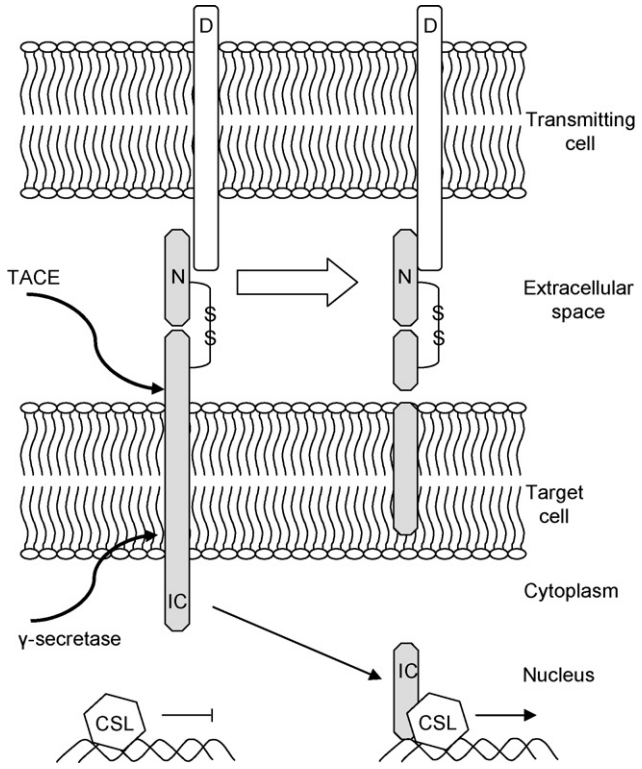

Fig. 2 – Signaling through the Notch pathway. The DLL/DSL ligand (D), a transmembrane protein in the transmitting cell, binds to its receptor, Notch (N), in a neighboring target cell and triggers two proteolytic cleavages. The first cleavage is extracellular and is catalyzed by TNFα converting enzyme (TACE) (top arrow). The second cleavage occurs by a process of regulated intramembrane proteolysis (bottom arrow), catalyzed by γ-secretase, and releases the intracytoplasmic (IC) fragment which translocates to the nucleus where it binds to the transcription factor, CSL. IC is a transcriptional co-activator for CSL; the IC/CSL complex activates DNA transcription.

an extracellular site by TNFα-converting enzyme (TACE). This extracellular cleavage triggers a regulated intramembrane cleavage of the Notch protein by the γ-secretase complex just inside the cytoplasmic face of the membrane [19]. This cleavage releases an intracytoplasmic (IC) peptide that translocates to the nucleus where it binds to a transcription factor, CSL. The IC peptide is a transcriptional co-activator for CSL [17]; the protein complex initiates the transcription of Notch target genes.

Recently the ErbB-4 tyrosine kinase receptor has been reported to undergo a process of regulated intramembrane proteolytic cleavage upon ligand binding by neuregulin that is similar to Notch activation. As with Notch, TACE cleaves the extracellular domain of the ErbB-4 receptor. A regulated intramembrane proteolytic cleavage is then performed by γ-secretase that releases the intracellular domain containing the tyrosine kinase effector enzyme from the receptor and allows it to translocate to the nucleus [14,20,21]. The data suggest that in the nucleus the intracellular domain of ErbB-4 binds to the transcriptional co-activator, YAP65, and activates gene transcription [22], though it may also phosphorylate nuclear proteins. It has been suggested that this process may be a widespread mechanism among receptor tyrosine kinases but the concept remains to be well-established.

For the PAR (thrombin) family pathway and the regulated intramembrane proteolysis signaling (Notch and ErbB-4) pathways, the signals are irreversible. Once the receptors are cleaved they cannot bind a new ligand, they cannot transmit a new signal and they cannot be regenerated. A new protein must be synthesized in order to reestablish the cellular response to ligand. This is very different from the ready reversibility of a phosphorylation cascade in which the same receptor undergoes cycles of regeneration of sensitivity and can transmit a new signal upon binding of a new ligand. Proteolytic signaling through the caspase cascade is even more irrevocable as activation of this pathway results in death of the cell through apoptosis.

Proteolytic cleavage is important to another aspect of signal transduction related to liberation of ligands. We typically think of growth factors as soluble, diffusible proteins. However, many growth factors are synthesized as transmembrane proteins and remain attached to the cell of synthesis as transmembrane proteins [23,24]. In this form some of them may bind to their receptors on immediately neighboring cells. Cleavage of the growth factor from its membrane anchor allows it to diffuse away to act on more distant cells. Similarly, growth factors such as transforming growth factor β (TGFβ) are secreted from the cell and become attached to the extracellular matrix in an inactive latent protein complex [25]. The latent TGFβ can be released from the extracellular matrix by proteolytic cleavage. Growth factors that are transmembrane anchored or bound to the extracellular matrix are in a rapidly releasable storage form. Members of the large family of matrix metalloproteinases are responsible for cleavage of both transmembrane anchored and extracellular matrix bound latent growth factors [26]. The growth factors that have been released from transmembrane or extracellular matrix anchorage by proteolytic cleavage are fully soluble and diffusible and the signaling pathways that they activate are reversible phosphorylation pathways. In contrast to the pathways in which the cleavage step is part of the passage of signal, this proteolytic release of ligand is a step removed from the activation of the signaling pathway. It is still an irreversible step, but the consequences of the irreversibility are different from the proteolytic cleavages that occur in the PAR, regulated intramembrane proteolysis, and caspase pathways.

4. New signaling pathways

4.1. *Hedgehog and Wnt*

Components of the Hedgehog and Wnt pathways were first described two decades ago [27,28] but they have only recently come into their own in the lexicon of signal transduction. Much of what we know of these two pathways comes from studies of development in Drosophila [29]; however their roles in signal transduction in mature/adult cells [30], in vertebrates, and their implications in disease [31,32] are now well-recognized and the interest in the Hedgehog and Wnt

pathways has broadened substantially. The Wnt and Hedgehog pathways (described in Fig. 3) do not fit clearly into the established paradigms of signal transduction.

There are several analogies of these two pathways to known models, but many aspects of their function separate them from the established models of signal transduction. Both the Wnt and Hedgehog pathways utilize a 7-transmembrane pass protein similar to the conventional 7-transmembrane pass receptor [2]. However, in the canonical Wnt and Hedgehog pathways these 7-transmembrane pass proteins do not activate heterotrimeric G proteins [29,31]. In the Wnt pathway, the ligand Wnt binds its 7-transmembrane pass protein in a relatively conventional manner and activates the transmission of signal. However, in the Hedgehog pathway, the 7-transmembrane pass protein does not serve as the receptor. Rather the ligand, Hedgehog, binds to a 12-transmembrane pass protein, Patched. In the absence of Hedgehog, Patched inhibits the 7-transmembrane pass protein in this pathway. In the presence of Hedgehog binding, inhibition of the 7-transmembrane pass protein by Patched is terminated and signal transmission through the pathway is activated. More conventional signaling of Wnt through its 7-transmembrane pass receptor to heterotrimeric G proteins has been reported [36,37], but this is considered a secondary pathway for Wnt signaling.

Another signaling paradigm that is appropriated by the Wnt and Hedgehog pathways is the use of proteolysis in their signaling mechanisms; however, these pathways use proteolysis differently from the mechanisms described above in the section on signaling by proteolysis. In the Wnt and Hedgehog pathways, a component of the signaling apparatus (a transcription factor in the Hedgehog pathway and a transcriptional regulator in the Wnt pathway) is cleaved in the absence of ligand and this proteolytic cleavage must be inhibited in order for signal to be transmitted through the pathway [31,38]. In both of these pathways, the absence of ligand leads to the formation of a cytoplasmic protein complex. Within the protein complex, the transcriptional regulator/transcription factor becomes phosphorylated on multiple sites. Interestingly, both pathways use the ser/thr protein kinases, casein kinase and glycogen synthase kinase 3, to phosphorylate the transcriptional regulator/transcription factor; the Hedgehog pathway uses the cAMP-dependent protein kinase for phosphorylation as well. The multiply phosphorylated protein is then ubiquitinated and thus targeted for proteolytic cleavage. When the Wnt or Hedgehog ligands bind to their respective receptors, the phosphorylation, ubiquitination, and proteolytic cleavage of the transcriptional regulator/transcription factor in the cytoplasmic protein complex is prevented. The intact transcriptional regulator/transcription factor translocates to the nucleus where it modulates the transcription of specific genes. There are two important points to remember about this process. First, proteolysis is used in a different manner in these pathways than those described above. In the Wnt and Hedgehog pathways, it is the inhibition of the proteolytic process rather than activation of proteolysis that conveys the signal into the cell. Second, the proteolytic cleavage in these pathways is irreversible as it is in other signaling pathways that use proteolysis, but the effect of this irreversibility is different. In these pathways, the presence of ligand results in the termination of proteolytic cleavage; in order to pass a signal into the nucleus new protein must be synthesized to carry the signal so there is a delay between the binding of ligand to receptor and the activation of gene transcription [29].

Another interesting analogy between these two pathways that is unique from other signaling ligands involves the ligands themselves. Both Wnt and Hedgehog are secreted palmitoylated proteins [30]. Both also have additional post-translational modifications. Wnt is glycosylated [30] and Hedgehog is modified with a cholesterol moiety [30,39]. In fact, Hedgehog is the only protein known to carry cholesterol as a posttranslational modification [40]. These posttranslational modifications are very unusual in a secreted protein and their functional implications are not clear though speculation abounds [30,39]. They are expected to hold Hedgehog and Wnt in close association with the cell membrane as occurs with lipid posttranslational modification of cytoplasmic proteins; however, it is known that Hedgehog and Wnt both diffuse away from the cell of origin [38].

Thus the Wnt and Hedgehog pathways incorporate aspects of other signaling paradigms in their activation pathways, but the unique features of these pathways suggest that they embody a separate paradigm of their own. Our understanding of these pathways continues to evolve [34,35,41,42] so the categorization of these pathways may change as our understanding increases.

4.2. *Histidine phosphorylation*

The importance of the O-linked phosphorylation of serine, threonine, and tyrosine in the regulation of cell function in both eukaryotic and prokaryotic cells is well-established. The chemical structure of other amino acids such as histidine, aspartate, lysine, and arginine also provides the potential for phosphorylation, but the phosphorylation of alternative amino acids has received less attention than that of the conventional serine, threonine, and tyrosine. The best established case for phosphorylation of alternative amino acids is the N-linked phosphorylation of histidine and the phosphorylation of aspartate in bacteria and plants [43]. The side group of histidine, an imidazole ring, is very different from the hydroxyl groups of serine, threonine, and tyrosine and the phosphorylation of histidine is a very different reaction. The N-linked phosphohistidine bond is a high energy bond, whereas the O-linked phosphate bond of the other three amino acids is not [44].

The signal transduction system for which histidine phosphorylation has been best established is the two component histidine kinase system of bacteria and plants [43]. The two component system uses a phosphotransfer mechanism that takes advantage of the high energy phosphohistidine bond. The two components are the sensor and the response regulator (Fig. 4). The sensor recognizes the appropriate signal for activation and autophosphorylates on histidine. Because the phosphohistidine bond is a high energy bond, the phosphate group can be transferred to a second amino acid. In the simplest case, the phosphate group is transferred from the histidine in the sensor to an aspartate in the response regulator. In more complicated systems the phosphate group may be transferred from histidine to

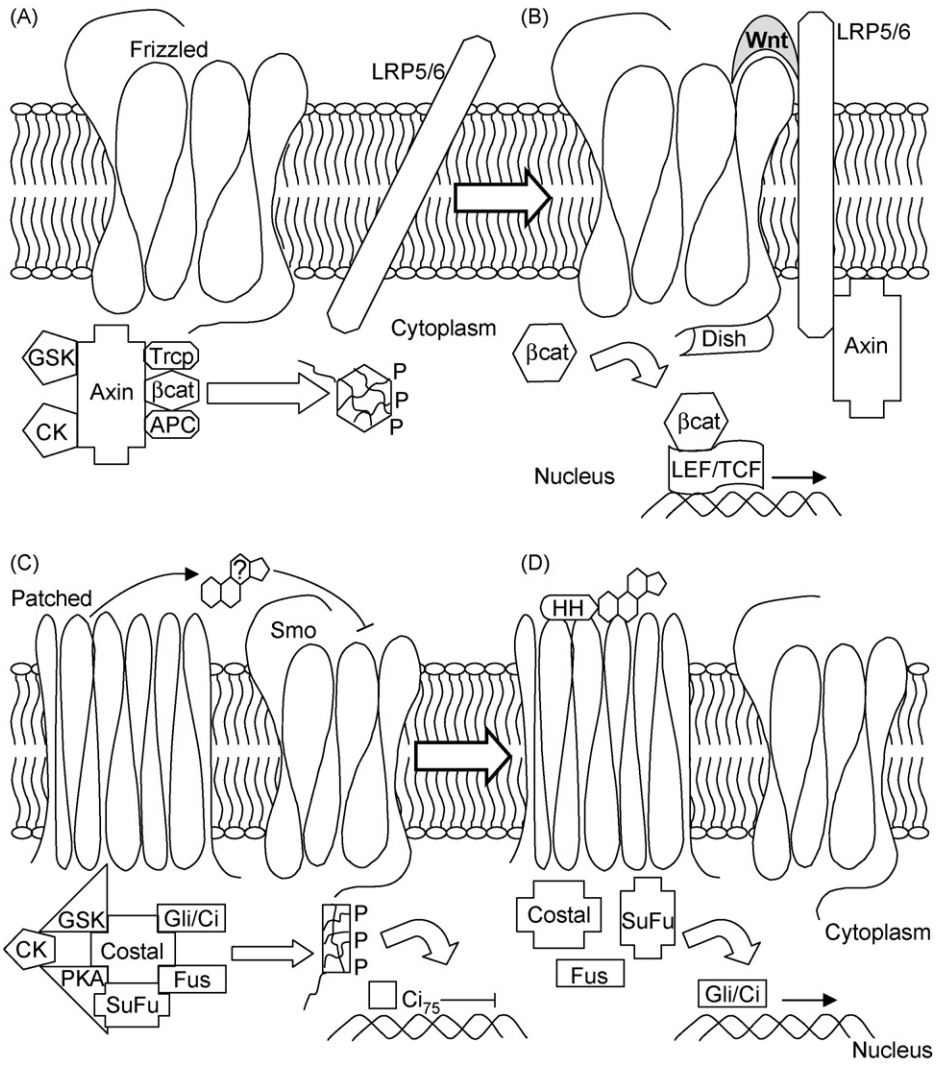

Fig. 3 – Wnt (Panels A and B) and Hedgehog (Panels C and D) signaling pathways. In the absence of the ligand, Wnt (Panel A), a cytoplasmic protein complex forms that contains the scaffolding protein axin, as well as β-catenin (β-cat), adenomatous polyposis coli (APC), and the ubiquitinating enzyme Trcp. β-Catenin is phosphorylated by casein kinase (CK) and glycogen synthase kinase 3 (GSK) while in this complex. Phosphorylation of β-catenin targets it for ubiquitination by Trcp which leads to proteolytic degradation. When Wnt is present it binds to Frizzled, a seven transmembrane pass receptor, and its coreceptor, LDL receptor-like protein 5/6 (LRP5/6). Upon Wnt binding (Panel B) the intracellular domain of LRP5/6 becomes phosphorylated and binds axin. A scaffolding protein, Dishevelled (Dish) binds to the intracellular region of Frizzled. These events result in the dissolution of the protein complex and halt the phosphorylation and ubiquitination of β-catenin so its concentration increases. The increase in β-catenin allows it to translocate to the nucleus where it binds to the transcription factor, LEF/TCF. β-Catenin acts as a transcriptional regulator that activates the transcriptional activity of LEF/TCF. Panel C: in the absence of the Hedgehog ligand, the 12-transmembrane pass receptor, Patched, inhibits the 7-transmembrane protein, Smoothened (Smo). It has been proposed that Patched transports a factor, perhaps a sterol, out of the cell that inhibits Smoothened. In this inhibited state the cytoplasmic components of the pathway form a protein complex composed of the transcription factor, Gli/Ci (Gli in vertebrates, Ci in Drosophila), Fused (Fus), Suppressor of Fused (SuFu), and Costal2. The Gli/Ci protein is phosphorylated by PKA, glycogen synthase kinase 3 (GSK), and casein kinase (CK). The multiply phosphorylated Gli/Ci is targeted for ubiquitination by Slimb and thus targeted for proteolysis. In Drosophila, the Ci protein is cleaved to yield a 75 kDa fragment, Ci_{75}, which translocates to the nucleus where it actively suppresses Hedgehog target genes. In vertebrates there are isoforms of Gli; cleavage products of some Gli isoforms have transcriptional inhibitory activity and others do not. Panel D: when the ligand, Hedgehog (HH), is present it binds to Patched which ceases its inhibition of Smo. Disinhibition of Smo results in disruption of the cytoplasmic protein complex. Full-length Gli/Ci accumulates in the cytoplasm then translocates to the nucleus where it acts as a transcription factor to activate transcription of Hedgehog pathway target genes. Other aspects of these pathways such as secreted antagonists [33], regulation of function by subcellular localization [34], and additional coreceptors [35] are not depicted here.

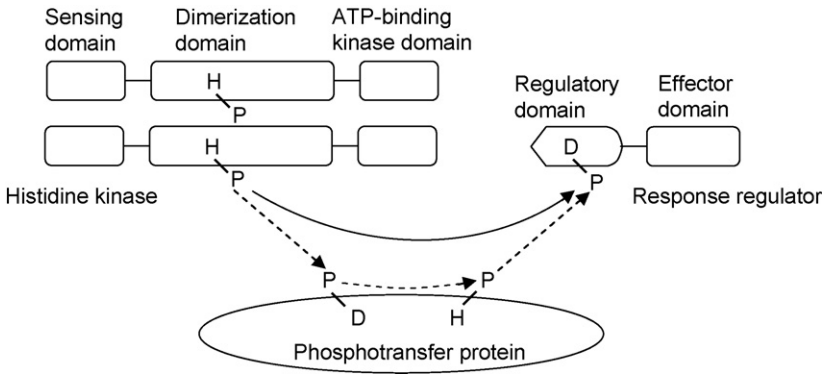

Fig. 4 – Two component histidine kinase signal transduction pathway of bacteria and plants. The histidine kinase is a homodimer, each component of the dimer contains three domains: the sensing domain, the dimerization domain, and the ATP-binding kinase domain. When a stimulus is registered by the sensing domain, the kinase domain is activated and phosphorylates (P) the dimerization domain on a histidine residue (H). In the simplest case, the phosphate group is transferred from the histidine of the histidine kinase to an aspartate (D) in the response regulator (shown by the solid arrow). The phosphate group may also enter a phosphorelay system in which there are many relays of the phosphate group from histidine to aspartate and to histidine to aspartate, and with the histidine and aspartate amino acids located in the same or different proteins. A phosphorelay system is shown with dotted arrows in which the phosphate group is transferred to an aspartate in a phosphotransfer protein, then to a histidine in the phosphotransfer protein, and hence to an aspartate in the response regulator.

aspartate to histidine to aspartate. The histidine and aspartate residues that participate in this phosphorelay system may be within the same protein or may be in separate proteins. The final amino acid acceptor in both the simple systems and the complicated phosphorelay systems is an aspartate in the response regulator of the specific system; the response regulator is activated by phosphorylation on aspartate and initiates action. The functions of response regulators range from bacterial flagellar movement, osmoregulation, and metabolism, to gene transcription [43]. A given bacterium contains many two component histidine kinase systems, each of which carries out a different function. *E. coli*, for example, contains 62 two component histidine kinase systems [43]. The two component histidine kinase systems of bacteria are excellent targets for drug development because vertebrates do not have analogous systems.

Histidine phosphorylation in vertebrate cells is not as well studied nor as well understood as that of the two component histidine kinase systems of bacteria and plants. Phosphohistidine can be identified in vertebrate cells, but the purification, cloning, and understanding of vertebrate histidine kinases and phosphatases is in its infancy. Phosphohistidine in vertebrate cells has the same high energy bond and therefore the same potential for phosphotransfer as that of bacterial and plant two component systems. Such phosphotransfer systems have been proposed for several vertebrate systems, but the evidence is convincing only for pyruvate dehydrogenase kinase and branched-chain α-ketoacid dehydrogenase kinase [45]. Nucleoside diphosphate kinases (NDPK B and Nm23-H1) have been reported to act as histidine protein kinases in addition to their eponymous function of catalyzing the phosphorylation of nucleoside diphosphates to nucleoside triphosphates [45,46]. Vertebrate phosphohistidine phosphatases, necessary to reverse the actions of histidine kinases, have also been identified [46,47]. Examples of vertebrate proteins reported to be phosphorylated on histidine include the β subunit of the heterotrimeric G protein transducin, histone H4, P-selectin, and annexin I [45,46]. The list of histidine protein kinases, phosphohistidine phosphatases, and histidine phosphorylated proteins in vertebrate cells is quite limited. However, if data supporting histidine phosphorylation in vertebrates are scarce, data supporting the phosphorylation of aspartate, lysine, or arginine in vertebrates are practically non-existent [48]. Lysine kinase and arginine kinase have been reported in the literature [48] but the reports are few; aspartate kinase has not been reported. The current data support the concept that phosphorylation of aspartate occurs only through phosphotransfer from phosphohistidine though this may be only because concerted searches have not been carried out. The paucity of information regarding the phosphorylation of alternative amino acids reflects both the limited number of investigators involved in the field as well as the difficulties inherent to the study of phosphohistidine and phosphoaspartate. For example, the partial acid hydrolysis method used for the identification of phosphoamino acids destroys phosphohistidine, thereby preventing its identification in the standard assay [45,46]. It is important to note also that the scarcity of data does not signify that the phosphorylation of alternative amino acids does not occur or that it is not important; rather, the lack of information bespeaks a field that is ripe for development. Thus the phosphorylation of alternative amino acids such as histidine and aspartate describes another new paradigm in signal transduction, especially in regard to vertebrate cells.

4.3. H_2O_2 in signal transduction

Another new paradigm in signal transduction is the involvement of reactive oxygen species, especially hydrogen peroxide

(H_2O_2), as mediators of signal transduction pathways [49,50]. Reactive oxygen species are generally considered deleterious to cells in that they react with and damage proteins, lipids, and DNA, and they are associated with aging, atherosclerosis and cancer [51]. On the other hand, a rapid release of reactive oxygen species is used by neutrophils to destroy phagocytosed bacteria by the ''respiratory burst'' [49]. The use of H_2O_2 as a mediator of signal transduction is another positive biological use of reactive oxygen species [49,50]. Many ligands, including growth factors, interleukins, and vasoactive hormones, have been shown to stimulate the generation of reactive oxygen species in non-phagocytic cells [49]. The most often cited mechanism by which reactive oxygen species modify the actions of proteins is by oxidation of essential cysteine residues [49,50] though other mechanisms have been proposed as well [49]. It has been hypothesized that oxidation of cysteine may be a reversible reaction analogous to reversible phosphorylation [50]. For example, protein tyrosine phosphatases can be reversibly inhibited by oxidization on a conserved cysteine [50]. Many details of signaling through reactive oxygen species remain to be identified but the field shows significant promise. It is somewhat reminiscent of the nitric oxide and carbon monoxide stories in which cells use as signaling molecules factors that are potent poisons at higher concentrations [52,53]. Reactive nitrogen species have also been implicated in signal transduction [54]. The paradigm of reversible cysteine oxidation as a mediator of signal transduction is sure to bring new excitement to the field as details of the mechanism emerge.

5. Challenging the dogma

Signal transduction is a very dynamic field. Established pathways evolve as new details about their function and regulation are identified, and new paradigms emerge as evidence accumulates. In 1985, a paper was published called ''Is there a role for cAMP and adenyl cyclase?'' [55]. This paper questioned ''the validity of the hypothesis that cyclic AMP (cAMP) is the second messenger for cell activation''. It is of note that this paper was published almost 30 years after the first observations of cAMP as an intracellular messenger and at a time that the adenylate cyclase/cAMP pathway was well-described and widely accepted. However, information about other signal transduction pathways was minimal. With the advantage of an additional 20 years of signal transduction research and technological advances, we can address the questions about the cAMP pathway that were raised in this paper. However, the most important point is not that those questions can now be answered; rather, the importance of this paper is that it dared to question an established dogma. Building on that point, the take-home message is that we should continue to ask similar questions about each new signal transduction pathway that is identified or each modification to the established models. Too often new concepts are advanced or links between signaling pathways are made with only a few gene transfection experiments to support the new idea. We must remember to continue to question new ideas until the weight of evidence supports a new concept. Moreover, it is important to be able to recognize which aspects of a pathway are well-established and which require continued effort to fill in the gaps. The Hedgehog, Wnt, Notch, histidine kinase, and reactive oxygen species pathways described in this commentary are good examples of newer pathways for which significant gaps remain in our knowledge.

6. Summary and conclusions

It is part of our nature to seek patterns in science and in art and in our surroundings. The identification of patterns helps us to categorize and understand our world. Signal transduction is an excellent example of a scientific field that serves as a source of ever expanding information that benefits from pattern identification and categorization. This discussion of new paradigms in signal transduction has arisen from efforts to identify patterns in signal transduction pathways, fitting new information into established models, and deriving new paradigms when new information cannot fit previous models. Thus the addition of the paradigm of the non-enzymatic nucleating receptor to the fundamental models of signal transduction was developed from the recognition that a large group of receptors did not fit into the two conventional paradigms of the 7-transmembrane pass receptor/heterotrimeric G protein and the enzyme-linked receptor, but that they did possess a set of fundamental similarities. Likewise, signal transduction by proteolysis encompasses a series of otherwise unrelated functions that share the irreversibility of proteolytic cleavage. The Wnt and Hedgehog pathways share some aspects of signaling with other models, but the sum of their parts is unique. The reversible phosphorylation of alternative amino acids such as histidine and aspartate, and the reversible oxidation of cysteine by H_2O_2, represent new paradigms in signal transduction that use a similar pattern to the conventional reversible phosphorylation of serine, threonine, and tyrosine that is so familiar. By continuing to seek patterns in the function and regulation of signal transduction pathways we will be able to keep abreast of the information in this dynamic and exciting field.

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