Inositol derivatives: evolution and functions

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Abstract | Current research on inositols mainly focuses on *myo*-inositol (Ins) derivatives in eukaryotic cells, and in particular on the many roles of Ins phospholipids and polyphosphorylated Ins derivatives. However, inositols and their derivatives are more versatile than this — they have acquired diverse functions over the course of evolution. Given the central involvement of primordial bacteria and archaea in the emergence of eukaryotes, what is the status of inositol derivatives in these groups of organisms, and how might inositol, inositol lipids and inositol phosphates have become ubiquitous constituents of eukaryotes? And how, later, might the multifarious functions of inositol derivatives have emerged during eukaryote diversification?

Archaea
The third kingdom of life, distinct from Bacteria and Eukarya. Archaea are unicellular and grow in diverse environments that are often physically extreme. Their DNA replication apparatus is more similar to Eukarya than to Bacteria.

Eukarya
The kingdom of life that is characterized by cells that have nuclei and other membrane-bounded organelles. Eukarya include animals, plants, fungi, amoebozoans and numerous phyla of unicellular organisms.

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The inositols are the nine isomeric forms of cyclohexanehexol, a group of small and chemically very stable polar molecules that have versatile properties. *Myo*-inositol (with D-*myo*-inositol abbreviated as Ins) is the most often used form in biology, which also uses at least five of the others (*scyllo*-, *epi*-, *neo*-, D-*chiro*- and *muco*-inositols; see FIG. 1). The synthesis of Ins consumes the central glycolytic metabolite glucose-6-phosphate (Glc6P) and requires two enzymes. First, NAD^+-dependent *myo*-inositol-3-phosphate synthase (MIPS, called Ino1 in yeast) catalyses the cyclization of D-glucose-6-phosphate to D-*myo*-inositol-3-phosphate (Ins3P)[1,2], and then inositol monophosphatase (InsPase) dephosphorylates Ins3P (and other Ins monophosphates) to free Ins[3] (FIG. 2). Limited evidence suggests that the other biological inositols may be made from Ins by simple inversion of the configuration (epimerization) of one or two Ins hydroxyls[4], but further studies are needed. So, Ins and other inositols that are derived from it — as well as all metabolites that contain any of these — are probably made as a result of a minor flux of carbon along a single short side-branch from central energy metabolism.

Ins-containing phospholipids (FIGS 2,3) are plentiful constituents of the membranes of many archaea and of all eukaryotes, and during the past 25 years they have become increasingly central to discussions of eukaryotic cell-control processes (see also the accompanying review in this issue by van Meer, Voelker and Feigenson on general aspects of the lipid composition and organization of cell membranes). Many eukaryotes use inositol-based stress-protective cytosolic solutes, all probably need plasma membrane phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) for endocytosis, exocytosis and sub-plasmalemmal cytoskeleton integrity, and all use PtdIns3P — and probably also PtdIns(3,5)P_2 — to regulate membrane trafficking in secretory and endocytic pathways. Hydrolysis of PtdIns(4,5)P_2 to the second messengers Ins(1,4,5)P_3 and *sn*-1,2-diacylglycerol by phosphoinositidase C (PIC; also known as phosphoinositide-specific phospholipase C) (FIG. 2) is ubiquitous in eukaryotes, and the liberated Ins(1,4,5)P_3 also serves at least sometimes as a precursor of Ins hexakisphosphate ($InsP_6$, also known as phytic acid) and of $InsP_6$-derived diphosphates (commonly termed pyrophosphates (PPs); these include PP-$InsP_5$ (or $InsP_7$) and $(PP)_2$-$InsP_4$ (or $InsP_8$)), the functions of which are starting to be understood. PtdIns(4,5)P_2 is also the substrate for synthesis of the plasma membrane second messenger PtdIns(3,4,5)P_3. Most of the functions of Ins lipids are brought about as a result of their interactions with diverse proteins that interact specifically with particular lipids, as discussed in the accompanying review by Lemmon in this issue.

The importance, distribution and usage of inositol derivatives varies across the three biological kingdoms: Archaea, Bacteria and Eukarya. Ins and its derivatives are made and used by most archaea and are found ubiquitously in eukaryotes, whereas relatively few bacteria make use of them. So, how did the usage of inositol derivatives evolve — and what new insights can recent genomic studies offer into the evolution of the usage of inositols? In this review, I discuss the status of inositol derivatives in the three kingdoms and consider their possible

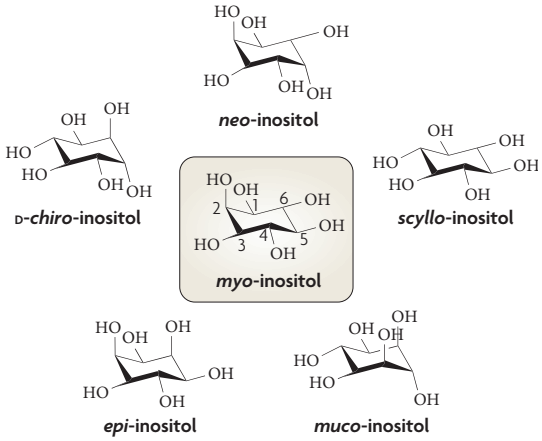

Figure 1 | **The diversity and nomenclature of inositols and their derivatives.** The inositols are the nine isomeric forms of cyclohexanehexol, with the empirical formula $C_6H_{12}O_6$. The inositols constitute a subgroup of a broader class of compounds known as cyclitols — polyhydroxylated and non-reducing compounds in which the core structure is an all-carbon ring of six (or occasionally five or seven) carbons, in which at least three ring carbons have directly attached hydroxyl groups[122]. Biology uses the six inositols depicted (*myo*-, *scyllo*-, *epi*-, D-*chiro*-, *neo*- and *muco*-), plus at least two cyclohexanepentols, or 'monodeoxyinositols' (quercitol and viburnitol, often as monomethyl derivatives), and also some singly unsaturated cyclohexanetetrols (the conduritols). The usual convention, which is followed in this review, is to number the carbons of all biological derivatives of *myo*-inositol (abbreviated as Ins) as shown in the figure. See www.chem.qmul.ac.uk/iupac/cyclitol for further definition and nomenclature, and see REF. 15 for further information.

α-proteobacteria
An ancient bacterial phylogenetic group that includes most phototrophic genera, several genera that metabolize 1-carbon compounds (such as *Methylobacterium*), plant symbionts (such as Rhizobia) and the Rickettsiaceae (including several pathogens). α-proteobacteria are the ancestors of mitochondria.

Cyanobacteria
Also known as Cyanophyta and blue-green algae. An ancient phylogenetic group of aquatic bacteria that obtain their energy through photosynthesis. Cyanobacteria are the ancestors of chloroplasts.

Actinobacteria
Also known as Actinomycetes. This large group of high G + C Gram-positive bacteria includes pathogenic mycobacteria and soil organisms that decompose organic material. Actinobacteria are sources of commercially valuable secondary metabolites, including antibiotics.

Phospholipid headgroup
The hydrophilic headgroup of a membrane phospholipid that is exposed at the membrane surface.

Halophilic archaea
Archaea that grow only, or preferentially, in high-salt environments.

Archaetidyl
A backbone structure in archaeal glycerophospholipid that comprises glycerol-1-phosphate modified with isoprane-based long-chain ethers on carbons 2 and 3.

evolutionary history therein. I then focus on eukaryotic inositol derivatives and how their functions might have diversified during eukaryote diversification.

The evolutionary context

The evolution of the biological roles of inositols can only be considered in the context of a selected model of the origins of extant organisms. According to some models of the origins of the three kingdoms of life, Archaea and Bacteria evolved earliest and in parallel, and the common ancestor of all of the extant Eukarya then emerged from mutual symbiotic relationships between two or more archaeal and bacterial progenitors. An alternative, and now more favoured, view considers Archaea and the deep ancestors of eukaryotes to be sister taxa that are descended from a shared cenancestor[5,6]. All models envisage that mitochondria and chloroplasts are the evolved descendants of ancient symbiotically captured α-proteobacteria and cyanobacteria, respectively[7,8]. (For general discussions of the recognition of the Archaea as a third kingdom of life see REFS 6,9–11.)

We have direct functional information on the structures, metabolism and physiological roles of inositols and inositol compounds from rather few organisms: a few archaea, a few bacteria and some eukaryotes — notably mammals, yeasts and green plants — plus sporadic information from other eukaryotic taxa. Inositol, particularly as a component of membrane lipids (FIG. 3), is widely used by the Archaea and is ubiquitous in the Eukarya. By contrast, few bacteria make Ins and/or Ins phospholipids. Actinobacteria are exceptional in that they have a diverse armoury of inositol derivatives[12,13] that are essential[14]. Taken together, these observations suggest that an archaeal contributor brought the synthesis and utilization of Ins into the first common ancestor of eukaryotes. Then, once eukaryotes had emerged and as they diversified (BOX 1, FIG. 4), functions for inositol derivatives proliferated dramatically.

Inositol distribution in three kingdoms

The usage and distribution of inositol and inositol derivatives in the three domains of life, as discussed below, allows us to propose an evolutionary history for inositol. FIGURE 4 and TABLE 1 summarize the ways in which various major groups of organisms use inositol derivatives. (For further references to inositol-containing compounds that are not discussed in detail in this review see REF. 15.)

Many archaea make and use Ins. MIPS and InsPase, the enzymes that synthesize Ins (FIG. 2), are common in the Archaea of both major clades, the Euryarcheota and Crenarchaeota (FIG. 4; TABLE 1). These organisms use Ins as a major phospholipid headgroup in their membranes[2,3,10]. The fact that Ins-1-phosphate (Ins1P) is a phospholipid headgroup in many archaea[10,16] suggests that the emergence of MIPS and InsPase and the use of Ins as a phospholipid headgroup were both early events in archaeal evolution, predating the divergence of the major archaeal clades. Extremely halophilic archaea, such as *Halobacterium* spp., lack MIPS and Ins lipids (TABLE 1). However, Ins lipids do not form stable bilayers in the near-saturated salt solutions in which these extremely halophilic archaea grow, so it is likely that their ancestors lost the genes that encode MIPS and InsPase as they became redundant during adaptation to life in salt pans and other high-salt environments[17].

In archaeal membrane glycerophospholipids (GPLs) that contain Ins, the Ins is linked to the *sn*-1-linked phosphate of an *sn*-2,3-diradylglycerol-1-phosphoryl (*sn*-2,3-diradylGro1P; also known as archaetidyl) backbone. This is the mirror image of the attachment of Ins to an *sn*-1,2-diradylglycerol-3-phosphoryl (*sn*-1,2-diradylGro3P; also known as phosphatidyl) backbone in eukaryal and bacterial GPL[18] (FIG. 3). Despite the 'reversed' configuration of the archaeal GPL backbone, the inositol headgroup is always linked through the D1-hydroxyl[19].

Many archaeal membranes are standard bilayers of two back-to-back monolayers of archaetidyl GPL, the same arrangement as that adopted by phosphatidyl-containing GPL in the membranes of eukaryotes. Membranes of some archaea include double-headed caldarchaetidyl GPLs that span the bilayer (FIG. 3). When one of these double-headed lipids bears one or more sugar groupings at one end and Ins1P at the other, these headgroups are in the same orientations that glycolipids and phosphoinositides adopt in eukaryote plasma membranes,

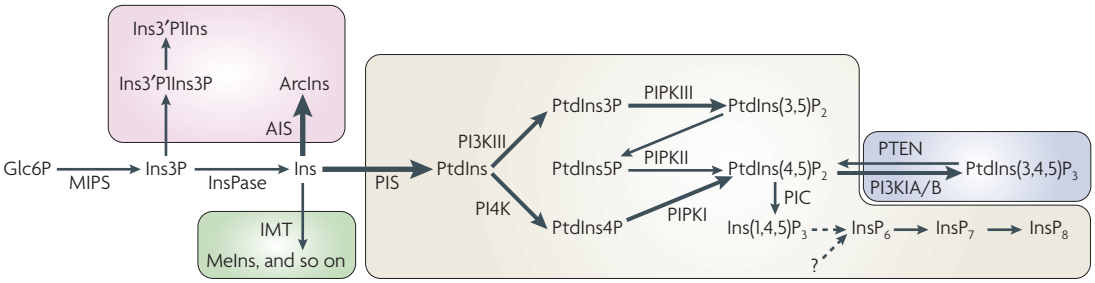

Figure 2 | **Metabolic pathways for the synthesis of inositol derivatives.** The widths of arrows give some indication of the relative rates of the pathways. Pathways that are found in all kingdoms are shown without a background, and other pathways are colour-coded: pink, Archaea; green, green plants; beige, all Eukarya; blue, Metazoa. The question mark acknowledges the existence of multiple routes to Ins hexakisphosphate ($InsP_6$), which are not yet fully understood. The dashed arrows indicate a multistep pathway from Ins(1,4,5)P_3 and other pathways to $InsP_6$. AIS, archaetidylinositol synthase; ArcIns, archaetidylinositol; Glc6P, glucose-6-phosphate; IMT, inositol methyltransferase; InsPase, inositol monophosphatase; MeIns, methylinositol; MIPS, Ins3P synthase; PI3KIA/B, PtdIns(4,5)P_2 3-kinase types A and B; PI3KIII, PtdIns 3-kinase; PI4K, PtdIns 4-kinase; PIC, phosphoinositidase C; PIPKI, PtdIns4P 5-kinase; PIPKII, PtdIns5P 4-kinase; PIPKIII, PtdIns3P 5-kinase; PIS, PtdIns synthase; PtdIns, phosphatidylinositol; PTEN, PtdIns(3,4,5)P_3 3-phosphatase.

with sugar headgroups exposed at the cell surface and Ins1P headgroups facing the cytosol[10].

With regard to the biosynthesis of archaeal GPLs that have an Ins1P headgroup, archaeal CDP:alcohol transferases, known as archaetidylinositol (ArcIns) synthases (AISs), might donate *sn*-2,3-diradylGro1P (archaetidate) — a molecule that is functionally homologous to eukaryotic *sn*-1,2-diradylGro3P (phosphatidate) — to Ins to make the Ins GPL of archaea (FIG. 2; TABLE 1). These putative AISs have not yet been enzymologically validated, so whether they discriminate between the 1- and 3-diradyl-glycerophospho donors remains unknown[10,16]. How eukaryotes evolved only to use bacterial-like *sn*-1,2-diradylGro3P-based lipids (see below), rather than 2,3-diradylGro1P-based lipids like those of their sister taxon Archaea, has been extensively discussed but remains unresolved[10,11].

Some hyperthermophilic archaea also use two Ins phosphodiesters as thermoprotective solutes. These are an InsPIns (two Ins units linked through a single phosphate group) and a glycerophosphoinositol (Gro1P3Ins) — this isomer is different from the Gro3P1Ins of eukaryotes[20,21]. The intracellular concentrations of these archaeal solutes rise dramatically when some hyperthermophilic archaea are heated above their already very high normal growth temperatures. Presumably, the cytoplasmic accumulation of these compounds enhances the thermostability of cell components, particularly proteins. After earlier disagreement, it now seems that the, or at least the major, archaeal InsPIns is Ins-1-phosphoryl-3′-Ins (Ins1P3′Ins; shown as Ins3′P1Ins in FIG. 2 for clearer understanding of the biosynthetic route). Ins1P3′Ins is made by a novel route that involves two molecules of MIPS-synthesized Ins3P (FIG. 2): Ins3P is coupled to CMP to make Ins3P-CMP, Ins3P-CMP donates Ins3P to the 1-hydroxyl of another second Ins3P molecule, and the exposed monoester 3-phosphate is removed by a phosphatase[20].

Relatively few bacteria use Ins. Those bacteria — of various types — that contain Ins derivatives are in the minority. They either synthesize Ins using enzymes that were recruited from archaea by lateral gene transfer sometime in the past[22] or use H^+-driven antiporters to import it from their environment[23]. *Myo*- and *scyllo*-inositol are, however, precursors of several characteristic molecules of the actinobacteria, a large and diverse grouping that consists mainly of environmental organisms, but also includes pathogens that are responsible for tuberculosis, leprosy, diphtheria and other conditions. The Ins-containing components of these complex bacteria include complex mannosylated derivatives of PtdIns, Ins lipid glycans, important antibiotics that include *scyllo*-inositol-derived elements and mycothiol (which is the major protective cytosolic thiol reductant of most actinobacteria) (see REFS 12,15,24). PtdIns synthase (PISs) catalyse the incorporation of Ins into PtdIns (FIG. 2). Comparison of the sequences of actinobacterial and other bacterial PISs (TABLE 1) with the PISs of eukaryotes and with the putative AISs (TABLE 1) of archaea suggests that bacterial PISs might have been recruited from archaea by lateral gene transfer in a similar manner to the bacterial MIPSs and InsPases (R.H.M., unpublished observations).

Not only are Ins derivatives used by rather few bacteria (see above), but they also have few known functions in the mitochondrial or chloroplast descendants of α-proteobacteria or cyanobacteria. Chloroplasts contain only a small amount of PtdIns[26], but for unknown reasons they can sometimes synthesize Ins with a chloroplast-specific MIPS[27]. Mitochondria contain less PtdIns than other eukaryotic organelles and do not make Ins or PtdIns, and have yielded little evidence for specific functions of Ins. However, I have replicated (R.H.M., unpublished observations) the old and still unexplained observation that addition of PtdIns, but not of other major phospholipids, to isolated and 'aged' mitochondria supports their ATP-energized morphological transitions[28].

All eukaryotes use inositols. All eukaryotic cells from both unicellular and multicellular organisms use Ins, always in their membrane phosphoinositides and usually for diverse other processes. The rare cells that lack cytosolic Ins polyphosphates, which include the anucleate

Caldarchaetidyl
A double-headed backbone structure in archaeal glycerophospholipids that comprises two glycerol-1-phosphate groups, each of which is modified with isoprane-based long-chain ethers on carbons 2 and 3.

Hyperthermophilic archaea
Archaea that grow only, or preferentially, at very high temperatures (70–100 °C).

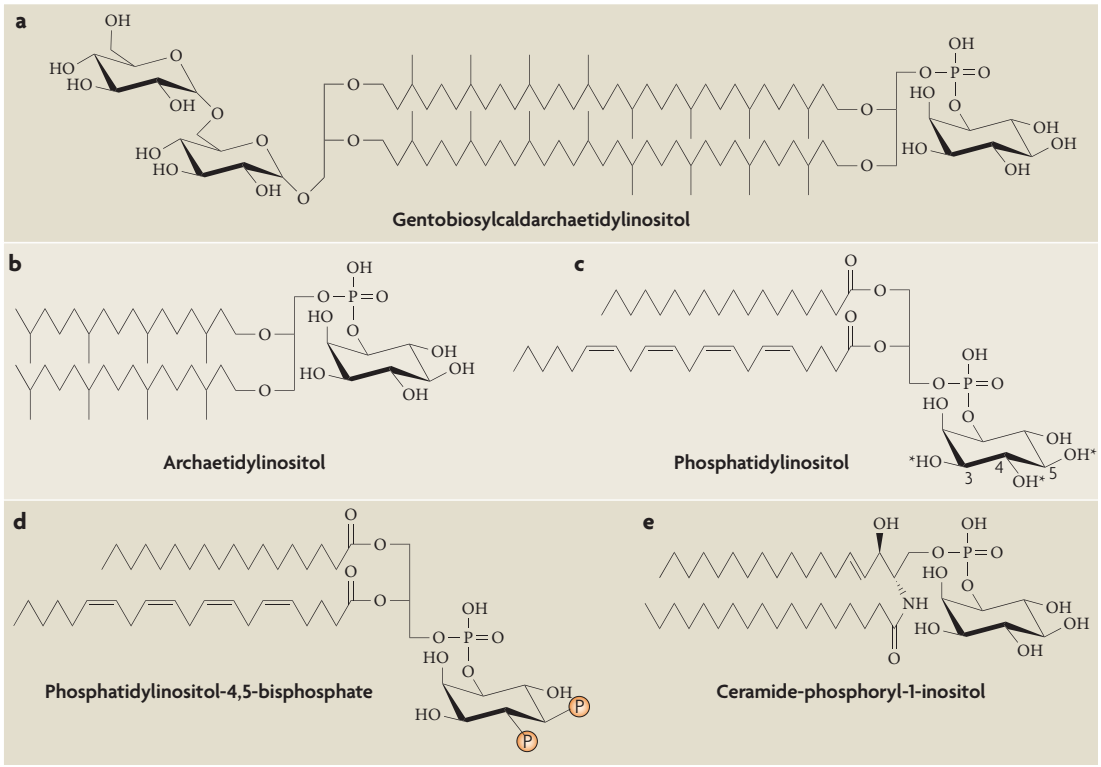

Figure 3 | **Examples of Ins-containing phospholipid structures. a**,**b** | Glycerophosphoinositides of archaea have glycerol backbones in which the Ins1P headgroup is attached to the *sn*-1 hydroxyl group of glycerol, and polyisoprane *O*-ethers are attached to the *sn*-2 and *sn*-3 carbons. The sidechains vary in different archaea — they may include one or more cyclopentane or cyclohexane rings, double bonds or other modifications. **c**,**d** | By contrast, phosphatidylinositol (PtdIns) of eukaryotes and some bacteria has the same Ins1P headgroup, but it is attached to the *sn*-3 hydroxyl group of glycerol. The fatty acyl residues of PtdIns molecules are attached to the *sn*-1 and *sn*-2 hydroxyls through carboxylic ester groups. Many of the PtdIns and other glycerophosphoinositide molecules in the membranes of most mammalian cells carry the depicted pairing of fatty acids (*sn*-1-stearoyl,2-arachidonyl), but in other organisms the fatty acid pairings of PtdIns usually have slightly shorter (16–18 C) chains and fewer double bonds. The 3-,4- and 5-hydroxyl groups of the PtdIns headgroup (marked with asterisks) in structure **c** are the sites of phosphate group attachment in the seven phosphorylated derivatives of PtdIns that are unique to eukaryotes. **e** | Ins-containing sphingolipids, such as ceramide-P-Ins and more complex derivatives thereof, have been studied mainly in fungi, but are found in at least some plants[15].

erythrocytes of mammals, may reflect an evolutionary loss of function. By contrast, the nucleated erythrocytes of birds and reptiles (crocodilians and a turtle), which are animals with an evolutionarily recent common origin, synthesize Ins(1,3,4,5,6)P_5 (REF. 29), as do those of a few fish[30].

Some cells make their own Ins using cytosolic MIPS and InsPase — in mammals, the testis, non-neural cells of the brain and some other tissues do this[31–33] — and other cells use environmentally regulated Na^+- or H^+-linked symporters (usually abbreviated as MIT or SMIT (Na^+-linked) or HMIT (H^+-linked)) to harvest Ins from the environment[23]. Some cells can either harvest available Ins or make their own when none is available[34]: in yeast, this is achieved by tight transcriptional regulation of the synthesis of MIPS by the environmental Ins concentration[34,35]. Some cultured mammalian cells can make their own inositol, but others require an exogenous supply[36,37]. Dietary manipulation of mammals (rats and gerbils) can provoke an inositol deficiency state, especially in lactating females[38]. Ins deficiency causes retention of unsecreted low-density lipoproteins in the liver and/or intestinal mucosa[38]. This fat accumulation is sometimes enhanced by antibiotic treatment, which suggests that animals might obtain Ins from their intestinal flora[39].

The uses to which eukaryotes regularly put Ins and its derivatives are diverse. They are used as 'compatible' cytosolic solutes (see below), often after conversion to a different cyclitol and/or conversion to derivatives such as methyl esters; in the abundant membrane lipid PtdIns; in PtdIns as substrates for synthesizing the seven known polyphosphoinositides (abbreviated as PPIn); in diverse Ins polyphosphates (and pyrophosphates made from these); and in the Ins glycerolipid or sphingolipid cores of the membrane anchors of many externally exposed cell proteins.

How did Ins and GPL with Ins headgroups find their way into early eukaryote progenitors during the evolution of ancestral organisms from which they arose? Given the infrequent usage of Ins by bacteria, and depending on the assumed model of eukaryote origins, the donor of MIPS and InsPase is likely to have been either the hypothetical common ancestor (cenancestor) of Archaea and eukaryotes or the archaeal contributor to the first

eukaryote (see above). Although the first synthesis of Ins and its first use as a major constituent of membrane lipids with an Ins1P headgroup probably occurred in an archaeal–eukaryote cenancestor, the seven phosphorylated derivatives of PtdIns (the polyphosphoinositides; PPIn) and diverse free Ins polyphosphates ($InsP_n$) have only been found in — and are ubiquitous in — eukaryotes. Thus, it is likely that both of these eukaryote-restricted groups of molecules were first made soon after the archaeal and eukaryote lineages diverged. This view is supported by the fact that most of the enzymes that synthesize the various Ins-containing GPLs are found in almost all groups of eukaryotes (TABLE 1). The few eukaryotes that appear to lack some of these enzymes include microsporidians such as *Encephalitozoon cuniculi*, an intracellular fungal pathogen of rabbits that has a genome so reduced that it encodes only ~2,000 proteins[40,41]. As suggested in TABLE 1, these microsporidians retain a PtdIns synthase (which presumably uses host-derived Ins, given the lack of MIPS and InsPase) and the kinases that are needed for synthesis of PtdIns4P, PtdIns(4,5)P_2 and PtdIns3P, but they probably cannot independently synthesize PtdIns(3,5)P_2 or degrade PtdIns(4,5)P_2 by the PIC pathway[15,40].

Evolution of eukaryotic inositol usage

Above I mentioned that most of the direct functional information on the metabolism and physiological roles of inositols and inositol compounds has come from relatively few organisms. However, the multitude of recently sequenced genomes of organisms from all three kingdoms permits prediction of their protein complements, and thus strong inferences as to how these organisms use Ins compounds. The distributions of some of the enzymes and other proteins involved in the metabolism and functions of Ins derivatives in various types of organism are shown in TABLE 1 (see REF. 25 for a more detailed table of the phylogenetic distributions of proteins that are involved in the metabolism and functions of PtdIns(3,5)P_2).

Given the limited number of ways in which archaea and bacteria use inositols, compared with the diversity and near-ubiquity of the Ins derivatives found in eukaryotes, it is likely that many of the Ins derivatives that are widespread in eukaryotes first emerged in very deep ancestors of the extant eukaryotic lineages. Some of the hallmark functions for inositol derivatives seem, however, only to have become established during the later stages of eukaryote diversification (see below; BOX 1). For example, compelling evidence for signalling through receptor-regulated PICs and Ins(1,4,5)P_3 receptor/Ca^{2+} channels is only available for metazoa, and receptor-regulated synthesis of the membrane-integral messenger PtdIns(3,4,5)P_3 probably emerged in a late common ancestor of amoebozoa and metazoa (FIG. 4).

Inositol derivatives as compatible solutes. Many organisms, both archaeal and eukaryotic, make or accumulate various small and polar, but often uncharged, organic solutes at substantial intracellular concentrations, particularly in response to exposure to environmental stresses such as heat, freezing, dehydration or high salinity.

Box 1 | Eukaryote diversification

Views of eukaryote phylogeny have recently been in flux, but phylogenetic analyses using multiply concatenated protein sequences are now generating a degree of consensus. Many of the diverse groups that have historically been loosely grouped as protists (including *Giardia* spp., *Trichomonas* spp., *Plasmodium* spp., cryptosporidia, trypanosomes, *Leishmania* spp. and euglenids) progressively diverged at various stages during early eukaryote diversification, probably between ~2,500 and ~1,500 million years ago (MYA)[116–119]. Plants of various classes diverged somewhat later, ~1,500 MYA.

Metazoa, fungi, and close allies that include choanoflagellates, constitute a eukaryote crown group that is collected into the supergroup Opisthokonta, with amoebozoa slightly more distantly related, and these groupings have diversified since maybe 1,300–1,400 MYA[120,121]. The phylogenetic tree outlined in FIG. 4 particularly emphasizes organisms for which there is information on inositol usage.

The physically benign properties of these molecules allow cells to sustain major changes in their intracellular concentrations without major effects on cell function. Indeed, these compatible solutes, including various polyols, seem somehow to stabilize cell proteins during environmental challenges. This is one of the most widespread, yet least understood, roles of inositol derivatives. Cytoprotection of this type is important in a few archaea (see above), but is most diversified in eukaryotes — various eukaryotes in diverse environments use several different inositols and Ins-derived cyclitols as compatible solutes (for example, synthesis of various methylinositols in plants)[15,42,43] (FIG. 2).

A study that used an unusually salt-tolerant form of MIPS, cloned from a salt-tolerant wild relative of rice, provides a striking example of the potency of this type of cytoprotection. Expressing this salt-tolerant MIPS in *Escherichia coli* or in *Schizosaccharomyces pombe* (neither of which normally makes Ins), or in two plants that are normally salt-sensitive, had dramatic effects. Salt exposure normally inhibits the growth of all of these organisms, but the variant organisms that expressed the salt-tolerant MIPS made substantial amounts of inositol and continued to grow even under normally toxic levels of salt stress[44].

Osmotic regulation of Ins concentrations in the renomedullary cells that line kidney tubules is a prime example of cytoprotection in mammals. These cells are exposed to continuous changes in their osmotic environment (such as the salt concentration in the glomerular filtrate), to which they respond by regulating the intracellular concentrations of solutes such as sorbitol and Ins. The Na^+/Ins co-transporters SMIT1 and SMIT2 support Ins accumulation, and the *SMIT1* gene is transcriptionally regulated in part through an osmotic response element[45]. Inhibition of SMIT activity is sufficiently nephrotoxic to cause acute kidney failure[46]. SMIT1 has similar importance in neuronal function: deletion of the mouse *SMIT1* gene leads to death soon after birth,

Protists
An informal portmanteau term for the many unicellular eukaryotes that are not animals, plants or fungi.

Choanoflagellates
The closest extant relatives of metazoa and fungi. They are free-living (single-cell or colony-forming) eukaryotes that are ubiquitous in aquatic environments, and typically have an ovoid cell body that has a single apical flagellum surrounded by a collar of microvilli.

Microsporidians
Parasitic fungi, some of which are pathogens of metazoa (such as *Encephalitozoon cuniculi*, the genome of which has been sequenced), that have extremely reduced genomes and mitochondrial remnants (mitosomes).

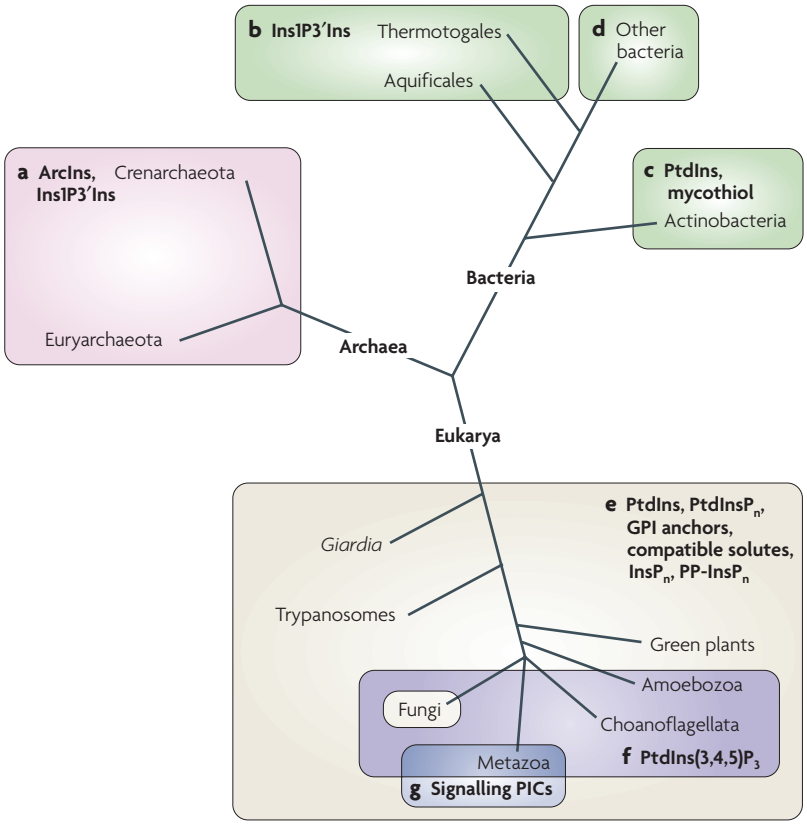

Figure 4 | **Development of the diverse usage of Ins derivatives through evolution.** This phylogenetic tree, which emphasizes those organisms from which there is information on the utilization of inositols, is based on recent attempts at reconstructing the Tree of Life, especially the emerging close relationship between the Metazoa, Amoebozoa and Fungi (see the main text and BOX 1 for references). Panels **a**–**g** emphasize the most central and characteristic aspects of the utilization of inositols in each group of organisms. Other uses of inositols by those groups are listed below in the main text, and in TABLE 1. In addition to phosphatidylinositol (PtdIns) and mycothiol, Actinobacteria also use PtdIns mannosides and PtdIns-anchored lipomannans (panel **c**). A few types of bacteria can synthesize D-*myo*-inositol (Ins) (and occasionally PtdIns), and some use Ins as a carbon source following the hydrolysis of $InsP_6$ by bacterial phytases (panel **d**). Eukarya use $PtdInsP_n$ and other complex Ins derivatives for various cell functions (cytoskeletal, secretory, membrane trafficking and so on) (panel **e**). The use of $PtdIns(3,4,5)P_3$ as a signal appears to be confined to a crown group of eukaryotes that includes Metazoa and Amoebozoa, but may have been lost from Fungi (panel **f**, and see the main text). GPI, glycophosphoinositide; Ins1P3′Ins, Ins-1-phosphoryl-3′-Ins; PIC, phosphoinositidase C (also known as phosphoinositide-specific phospholipase C); $PP\text{-}InsP_n$, Ins pyrophosphates; $PtdIns(3,4,5)P_3$, phosphatidylinositol-3,4,5-trisphosphate. Figure modified with permission from REF. 15 © (2007) The Biochemical Society.

apparently after incomplete nerve development and failure of respiratory control by the brainstem[47,48]. The recent observation that oral administration of *scyllo*-inositol (also referred to as AZD-103) or *epi*-inositol can ameliorate behavioural and other symptoms in mouse models of Alzheimer's disease might be another example of such a compatible solute effect[49–51].

Given that diverse inositol derivatives are involved, diverse organisms are affected and diverse environmental stresses become better tolerated, it seems likely that these molecules provide highly effective cytoprotection and that many variants of this poorly understood process evolved independently at different times.

Glomerular filtrate
The blood plasma filtrate that emerges from the glomerulus of the mammalian kidney, mainly comprising water and low molecular mass solutes (such as ions, sugars and amino acids) that are actively reabsorbed in later segments of the nephron.

The membrane lipid PtdIns. PtdIns biosynthesis involves transfer of an intact phosphatidyl group from CMP-phosphatidate (synonymous with CDP-diacylglycerol) to the D1 position of free inositol, catalysed by PIS, an enzyme that is conserved in all eukaryotes[32,52] (FIG. 2; TABLE 1) and may be descended from an ancestral archaeal AIS (see above).

PtdIns usually makes up 5–30% of the membrane phospholipids of eukaryote cells, and appears to be essential for normal cell function in the cytosolic leaflets of membranes, although at different concentrations in different organelles (see REF. 53). For example, there is more PtdIns in the endoplasmic reticulum, where it is synthesized, than in mitochondria[32]. As with the other abundant membrane phospholipids of eukaryotes (such as phosphatidylcholine and phosphatidylethanolamine), it is still far from clear how and why particular phospholipid species have different abundances and distributions in the different organelles of cells.

PtdIns4P, mainly a PtdIns(4,5)P_2 precursor. The 4-phosphorylation of PtdIns, mainly at the plasma membrane, is the gateway to almost all functions of PtdIns4P and $PtdIns(4,5)P_2$: little $PtdIns(4,5)P_2$ is made via PtdIns5P (FIG. 2). However, PtdIns4P does have specific roles. For example, it is an essential cofactor for trafficking the precursors of complex glycosphingolipids through the Golgi complex before the completed molecules are transported on to various compartments of the endolysosomal system and to the plasma membrane[54]. All eukaryotes have PtdIns 4-kinases (PI4Ks; FIG. 2; TABLE 1) that make PtdIns4P, the main precursor for $PtdIns(4,5)P_2$ (FIG. 2). PI4Ks are of two divergent types, typified in *Saccharomyces cerevisiae* by Lsb6 and by Stt4 and Pik1 (REF. 55). Genetic studies suggest that Stt4 makes the bulk of the PtdIns4P that serves as a precursor for $PtdIns(4,5)P_2$ (REFS 56,57). Stt4/Pik1-type PI4Ks are found in all eukaryotes, whereas Lsb6-type PI4Ks are found only in metazoa and fungi. This suggests that the Stt4/Pik1 type appeared early and has persisted throughout eukaryote evolution, and that the Lsb6 type did not emerge until maybe 1,300 million years ago.

It seems likely that all eukaryotes, even the microsporidian *E. cuniculi*, make $PtdIns(4,5)P_2$ using PtdIns4P 5-kinases (PIPKIs; FIG. 2; TABLE 1) that are homologues of yeast Mss4. $PtdIns(4,5)P_2$ is a low-abundance membrane lipid that has diverse proven functions, for example in signalling, cell motility and exocytosis[58,59] (see the accompanying reviews by Lemmon and by Wymann and Scheiter in this issue). An ability to synthesize $PtdIns(4,5)P_2$ seems, therefore, to be one of those 'eukaryotic signature' functions that was already present in an ancestral eukaryote and has remained a core requirement of the eukaryotic cell[40].

The first major function of $PtdIns(4,5)P_2$ to be established was as the substrate of PIC-mediated signalling in response to cell-surface receptor activation, which produces the second messengers $Ins(1,4,5)P_3$ and *sn*-1,2-diacylglycerol (FIG. 2). PICs come in at least six subtypes, the β, γ, δ, ε, ζ and η families, which are regulated in various ways. For example, PICγs are activated by

Table 1 | **Phylogenetic distributions of inositol derivatives and proteins (or genes encoding putative protein homologues)**

Phylogenetic group (example species)	MIPS	InsPase	PIS and/or AIS	PI4K	PIPKI	PIC	PI3KIII	PIPKIII	PI3KIA/B	GPI anchors*
Animals (metazoa)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Fungi (ascomycetes; *Saccharomyces*, *Schizosaccharomyces* and so on)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Fungi (microsporidia; *Encephalitozoon*, *Antonospora*)	No?	No?	Yes	Yes	Yes	No	Yes	No	No	Yes
Amoebozoa (*Dictyostelium*)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Plants (Viridiplantae; *Chlamydomonas*, *Oryza*, *Arabidopsis*)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Stramenophiles (*Phytophthora*, *Thalassiosira*)	Yes	Yes	Yes	?	Yes	No	Yes	?	Yes	Yes?
Apicomplexans (*Cryptosporidium*, *Toxoplasma*, *Theileria*, *Plasmodium* spp.)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes
Bacteria (actinobacteria; *Mycobacterium*, *Corynebacterium*, *Streptomyces* and so on)	Yes	Yes	Yes	No	No	No	No	No	No	No
Bacteria (Aquificales, Thermotogales; *Aquifex*, *Thermotoga*)	Yes	Yes	?	No	No	No	No	No	No	No
Bacteria (most others)	No	No	No	No	No	No	No	No	No	No
Archaea (other than extreme halophiles)	Yes	Yes	Yes	No	No	No	No	No	No	Yes?
Archaea (extreme halophiles)	No	No	No	No	No	No	No	No	No	No

*For GPI anchors, 'yes' indicates direct evidence of protein or oligosaccharide anchors with core PtdIns or InsP-ceramide, or of enzymes that catalyse their formation. GPI, glycophosphoinositide; InsPase, inositol monophosphatase; MIPS, *myo*-inositol-3-phosphate synthase; PI3KIA/B, types IA and/or IB phosphoinositide 3-kinase (PtdIns(4,5)P_2 3-kinase); PI3KIII, PtdIns 3-kinase; PI4K, PtdIns 4-kinase; PIC, phosphoinositidase C; PIPKI, PtdIns4P-5-kinase (type 1, Stt4/Pik1-like); PIPKIII, PtdIns3P 5-kinase (Fab1-like); PIS/AIS, PtdIns and/or archaetidylinositol synthase (CMP-P-archaeol or CMP-P-diacylglycerol:inositol transferase).

tyrosine kinases[60,61], PICβs by G protein-coupled receptors[60], PICε by small GTP switch proteins (notably Ras)[62], and the sperm-restricted PICζ mainly increases in the cytosolic Ca^{2+} concentration. The ultimate evolutionary origin of eukaryotic PtdIns(4,5)P_2-selective PICs remains an enigma. Relatives of PICδ and PICζ, similar enzymes that differ mainly by the presence or absence of a PH domain, make up the only near-ubiquitous PIC subfamily, so they are likely to be structurally and functionally most similar to the common ancestor of all extant PICs[60]. At least one PICδ/ζ-like protein has been predicted from every eukaryotic genome except those of the microsporidian *E. cuniculi* (see above) and of stramenophile plant pathogens of the genus *Phytophthora*[64] (TABLE 1). Unfortunately, despite much effort, we still do not understand the physiological regulation or the functions of these ubiquitous PICδ/ζ enzymes in most situations. One hint is that the sole PIC in yeast (Plc1 in *S. cerevisiae*) — and maybe also the equivalent enzyme in the unicellular alga *Dunaliella*[65] — can be activated by hypo-osmotic stress[57].

The main biological function of the other PICs — the β, γ, ε and η families, all of which are restricted to metazoa — is to make Ins(1,4,5)P_3 and 1,2-diacylglycerol as second messengers. Their metazoa-restricted distributions essentially match the distribution of Ca^{2+}-mobilizing Ins(1,4,5)P_3 receptors[66,67]. The situation in the amoebozoan *Dictyostelium* is unclear: it lacks these receptor-regulated PICs, but possesses an Ins(1,4,5)P_3 receptor-like protein (IplA) that is somehow involved in the regulation of chemoattractant-stimulated Ca^{2+} entry[68].

But what are the functions of the PICδ/ζ enzymes of eukaryotes that fall outside the metazoan clade, which make Ins(1,4,5)P_3 in cells with genomes that do not encode recognizable Ca^{2+}-mobilizing Ins(1,4,5)P_3 receptors? Two answers seem possible. First, Ins(1,4,5)P_3 still seems to stimulate Ca^{2+} mobilization in at least some of these organisms[69,70], so there might be other types of Ins(1,4,5)P_3-activated Ca^{2+} channels that are yet to be identified. Second, it is now clear that PIC-liberated Ins(1,4,5)P_3 has another function, at least in yeast. Ins(1,4,5)P_3 that is liberated when *S. cerevisiae* Plc1 is activated does not persist in the cell as a Ca^{2+}-mobilizing second messenger. Rather, it is rapidly transformed into more highly phosphorylated inositol species — polyphosphates and pyrophosphates — and these are increasingly being assigned various functions, both in the cytosol and nucleus (see below and FIG. 2)[57,71,72]. A recent study demonstrated that in mammalian cells, a combination of overexpression of the kinases involved in conversion of Ins(1,4,5)P_3 to InsP_6 with persistent activation of PIC can cause marked changes in the relative concentrations of

various inositol polyphosphates[73] — but to what degree this mirrors normal events in stimulated cells remains to be determined.

PtdIns3P and PtdIns(3,5)P_2 in trafficking pathways. PtdIns 3-kinase (PI3KIII), which synthesizes PtdIns3P from PtdIns, and PtdIns3P 5-kinase (PIPKIII), which then synthesizes PtdIns(3,5)P_2, seem to be near-ubiquitous in eukaryotes (FIG. 2; TABLE 1). The roles of PtdIns3P in anterograde traffic through intracellular membrane trafficking pathways and of PtdIns(3,5)P_2 in at least some retrograde pathways[25,74–77] appear to be maintained in all or almost all eukaryotes. A lack of PtdIns3P impedes tubulovesicular membrane flux of secretory and endosomal proteins from the Golgi complex outwards through the endosomal system[74,78], whereas a deficit of PtdIns(3,5)P_2 impedes the return of membrane to the Golgi and is therefore characterized by gross enlargement of endolysosomal structures that cannot recycle arriving membrane[25,77]. Inspection of genomes suggests that a few intracellular pathogenic eukaryotes — such as microsporidians and pathogenic apicomplexans (including the malaria parasites) — lack the ability to synthesize PtdIns(3,5)P_2 and maybe sometimes even PtdIns3P[25]. Can they operate without the core functions that require these lipids? This seems unlikely, so perhaps these organisms have invented subterfuges by which they hijack these lipids from their hosts. Possibly in a similar vein, some pathogenic bacteria can use phosphoinositide-specific enzymes (for example, a phosphoinositide 5-phosphatase (SopB) in *Salmonella*[79] and a PtdIns-specific phospholipase C (PlcA) in *Listeria*[80,81]) as virulence factors with which they subvert cell functions in their metazoan cell hosts.

PtdIns(3,4,5)P_3 as a plasma membrane signal. PtdIns(3,4,5)P_3 is synthesized from PtdIns(4,5)P_2 by type I phosphoinositide 3-kinases (PI3KIs; FIG. 2) in response to the activation of receptor tyrosine kinases (for subclass PI3KIA) or G-protein-coupled receptors (for subclass PI3KIB). Changes in plasma membrane PtdIns(3,4,5)P_3 concentrations then exert control over the survival, growth and numerous other functions of cells in most or all metazoa. These diverse animals include all chordates, the subphylum Ecdysozoa, which includes insects and nematodes, and radially symmetrical cnidarians such as sea anemones[82,83]. The finding of a PI3KI gene in a sea anemone, a member of an early diverged metazoan group, suggests that the ancestral lineage from which all extant metazoa evolved probably already possessed a PI3KI[84]. Moreover, examination of Choanobase reveals that *Monosiga brevicollis*, a member of the choanoflagellate group of organisms that is evolutionarily the closest to metazoa, expresses an mRNA that appears to encode a bona fide PI3KI.

Strikingly, *Dictyostelium discoideum* — an amoebozoan that belongs to a phylogenetic group that is generally considered to have branched from the eukaryotic tree before the divergence of fungi and metazoa (TABLE 1) — is the only organism outside the 'Metazoa plus Choanoflaggellata' grouping to possess (multiple) functional PI3KIs. Inhibitor and genetic studies have shown that the localized changes in the concentration of plasma membrane PtdIns(3,4,5)P_3 that occur when *D. discoideum* responds to chemoattractants are important, although non-essential, contributors to the control of chemotaxis[85–87].

These findings create something of a conundrum. PI3KIs are found in metazoans, in at least one choanoflagellate and also in *D. discoideum* — but there are none in the sequenced fungal genomes, despite the fact that fungi diverged from the shared ancestral lineage later than amoebozoans. It therefore seems likely that PtdIns(3,4,5)P_3-synthesizing PI3KIs first appeared in eukaryotes just before the divergence of the ancestral amoebozoan and metazoan lineages. Enzymes of this type were presumably then lost from an early fungal ancestor before the fungi underwent significant diversification. A further surprise came when it was discovered that *S. pombe* can make small quantities of PtdIns(3,4,5)P_3 of unknown function[88]. In accord with the sequence of events suggested above, however, it turns out that this *S. pombe* PtdIns(3,4,5)P_3 is made by an alternative route that does not require a PI3KI: instead, a type I PIPKI 5-phosphorylates PtdIns(3,4)P_2.

Multipurpose inositol phosphates and pyrophosphates. All eukaryotes probably make *myo*-inositol (poly)phosphates ($InsP_n$s), in which some or all of the Ins hydroxyls can be phosphorylated, and at least half of the 63 possible $InsP_n$s can be found in one organism or another[89–92]. Typically, the most abundant $InsP_n$ molecules are multiple isomers of InsP and $InsP_2$ (probably downstream metabolites of polyphosphates), Ins(1,3,4,5,6)P_5 (and sometimes other $InsP_5$ isomers) and $InsP_6$ (see REF. 91 and the Shears laboratory web site). After nucleotide di- and triphosphates, $InsP_n$ molecules are among the more abundant cytosolic phosphate compounds. For the most part, Ins(1,3,4,5,6)P_5 and $InsP_6$ are stable cell constituents, with turnover times of many hours or even days in mammalian cells[91,93], but they can occasionally exhibit rapid changes in concentration in response to stimuli[94]. Remarkably, $InsP_6$ has recently been found at the heart of the crystallographically determined structures of two unrelated proteins, an auxin receptor and an RNA editing enzyme[95,96].There are several routes for the biosynthesis of $InsP_6$: by sequential phosphorylation directly from free Ins (only so far seen in *D. discoideum*, which harbours millimolar concentrations of $InsP_6$)[97]; from the Ins(1,4,5)P_3 that is released by PIC[92] (FIG. 2); and possibly by other routes. The fact that cells have evolved multiple pathways to synthesize $InsP_6$, presumably by convergent evolution, reinforces the view that $InsP_6$ must be important to eukaryotic cell function — the reasons why will increasingly become clear from future work.

A surprise has been that a significant proportion of the $InsP_5$ and/or $InsP_6$ in many cells is further phosphorylated to form pyrophosphates. The resulting molecules, which display rapid turnover, are correctly termed diphospho-$InsP_5$ ((PP)-$InsP_5$ or $InsP_7$) and bis-diphospho-$InsP_4$ ($(PP)_2$-$InsP_4$ or $InsP_8$). In these molecules, which are probably ubiquitous in eukaryotes, monoester phosphate groups are modified to form diphosphate groups that have free energies of hydrolysis akin to those of the 'high

Apicomplexans
A large group of spore-forming protists that are characterized by a unique organelle (the apical complex) and are parasites of animals. Apicomplexans cause diseases such as malaria and cryptosporidiosis.

Ecdysozoa
One of the major groups of animals, which includes both the arthropods and nematodes. Many members of this group regularly shed their cuticle by ecdysis, hence the name Ecdysozoa.

Cnidarians
An ancient phylum of marine organisms with rotational symmetry, of which the anthozoa (including sea anemones and corals) were probably the earliest to diverge.

energy' diphosphate and triphosphate groups of nucleotides[72,98]. Widely conserved enzymes convert $InsP_6$ to at least two isomeric forms each of $InsP_7$ and of $InsP_8$, so all eukaryotes probably make $InsP_7$ and $InsP_8$ (REFS 99,100). $InsP_7$ and/or $InsP_8$ have already been implicated, usually genetically, in a remarkable range of important cell functions, including mRNA processing[71], control of telomere length and insulin secretion[72,101–103]. Remarkably, the mechanism(s) underlying these functions might include direct phosphorylation of proteins by phosphate transfer from $InsP_7$ or $InsP_8$ (REF. 104). More surprising yet, this phosphorylation converts pre-existing phosphoserine residues in proteins into diphosphoserine residues of as-yet-unknown function[105].

One of the most striking aspects of inositol biology is that inositol polyphosphates, particularly hexakisphosphates, make up much of the organic phosphate content in many soils and submarine and estuarine sediments[106,107]. Plants, especially their seeds, contain substantial amounts of *myo*-inositol hexakisphosphate, so plant remnants will contribute to the soil complement either directly or when these compounds are excreted undigested by herbivores[108]. Surprisingly, the inositol pentakisphosphatases and inositol hexakisphosphates of soils and sediments often contain substantial amounts of the unusual inositol isomers *scyllo*-inositol, *neo*-inositol and D-*chiro*-inositol[106,107] (FIG. 1). Because inositol polyphosphates are so far only known from eukaryotes, much of this abundant environmental phosphate load is presumably made by unidentified soil eukaryotes such as various microorganisms and/or fungal mycelia. So far, the only organisms known to make polyphosphates of inositols other than *myo*-inositol are some amoebae[109].

Glycophosphoinositides as cell-surface anchors. Following the discovery that treatment of cells with PtdIns-specific bacterial phospholipases brings about the release of some cell-surface proteins, detailed studies by numerous laboratories established that most or all eukaryote cells display proteins that are anchored to the membrane through C-terminally attached glycophosphoinositide (GPI) structures[110] on their cell surfaces. Linkage of a GPI anchor to a protein is catalysed by a GPI-GlcNAc transferase complex that, in humans, comprises at least six subunits (PIG-A, PIG-C, PIG-H, PIG-P, GPI1, and DPM2)[111,112]. Recognition of a newly synthesized intracellular protein as a substrate for this complex requires a multicomponent C-terminal recognition motif that can be identified by a dedicated computer algorithm[113]. Sometimes the resulting protein anchors appear to contain either L-*chiro*-inositol or *scyllo*-inositol rather than *myo*-inositol[114].

GPI anchors are apparently ubiquitous elements of eukaryote cells, appearing even before the deep branching of the line leading to *Giardia lamblia*, and they do not occur in bacteria[113,115] (TABLE 1). However, two independent types of evidence hint that this mode of protein attachment might be used by some archaea. First, treatment of the archaeon *Sulfolobus acidocaldarius* with phospholipase C provoked release of inositol-labelled surface proteins[115]. Second, *in silico* sequence analysis has found protein motifs that suggest that GPI-anchored proteins might be present in at least four other archaeal species from both of the major archaeal clades (Euryarchaeota and Crenarcheota)[113].

Eukarya and Archaea are already established as the two kingdoms that use Ins lipids most extensively, and these observations hint that the anchoring of proteins to membranes through structures that include PtdIns or some closely related lipid may have evolved before the divergence of Archaea and Eukarya. If this is correct, the use of PtdIns to anchor proteins to cell surfaces may have been a more ancient innovation than the phosphorylation of PtdIns to the various PPIn.

Conclusions

The above analysis of the distributions and functions of inositol and inositol derivatives through the three kingdoms of life leads to several main conclusions. First, Ins was adopted as a membrane lipid headgroup very early, in cells that predated the divergence of the archaeal and eukaryotic kingdoms. Second, a remarkably wide variety of organisms has exploited the benign physical properties of cytosolic inositols as elements in physiological mechanisms for weathering environmental stresses. Third, all eukaryotes use the phosphorylated derivatives of PtdIns for various roles in the functional integration of their multicompartment cells. And fourth, $PtdIns(4,5)P_2$ only took on the remarkable extra role of being the central player in two major cell signalling pathways — PIC-catalysed $PtdIns(4,5)P_2$ hydrolysis and PI3KI-catalysed $PtdIns(3,4,5)P_3$ synthesis — at around the time at which the ancestors of amoebozoa and metazoa diverged.

This is only an initial foray into the evolution of the complex biology of inositol derivatives, which has until now been studied mainly in eukaryotes, especially in metazoa and fungi. Many of the interesting questions that remain will need to be investigated in less-studied organisms. For example, what are the functional relationships between the enzymes that make ArcIns in archaea and PtdIns in other organisms, and what are the sources and functions of the abundant 'unconventional' inositol polyphosphates that are abundant in soils and sediments?

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