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Role of phosphoinositides and inositol phosphates in the regulation of mycelial branching

G. D. ROBSON

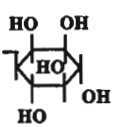
Introduction

All cells have evolved regulatory mechanisms which allow them to respond to external signals and are essential for cell multiplication and survival. In mammalian cells, an array of signal transduction cascades have been described that respond to growth factors and hormones (Mooibroek & Wang, 1988; Su & Karin, 1996). More recently, highly homologous signalling cascades have been reported in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, which are involved in a variety of cellular processes including mating, hyper- and hypo-osmotic sensing, invasive filamentous growth and cell wall integrity (Nishida & Gotoh, 1993; Roberts & Fink, 1994; Waskiewicz & Cooper, 1995; Su & Karin, 1996; Cahil, Janknecht & Nordheim, 1996). The presence of such highly conserved signal transduction pathways suggests that these signal cascades may first have evolved in eukaryotic microbes and have been conserved and adapted during eukaryotic evolution (Janssens, 1987; Kincaid, 1991; Csaba, 1994; Gadd, 1995; Rasmussen *et al.*, 1996).

One of the most highly studied signal transduction pathways in mammalian cells is the phosphoinositide cycle which has been the centre of intense research since the first report that inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$), acts as a second messenger, mobilizing Ca^{2+} from intracellular stores in response to a variety of growth factors, hormones and other ligands (for reviews see Berridge & Irvine, 1984; Nishizuka, 1984; Divecha & Irvine, 1995). In this chapter, current understanding of the structure and function of phosphoinositide signalling in filamentous fungi is reviewed.

Phosphoinositides

The phosphoinositides are a group of minor acidic phospholipids of which phosphatidylinositol (PtdIns) is the principal member, constituting 3–10% of the total phospholipid present in the membrane (Fig. 7.1). PtdIns is synthesized by the membrane-associated phosphatidylinositol synthase from cytidine 5′-phosphate diacylglycerol (CDP-diacylglycerol) and inositol. PtdIns synthase is encoded by a single gene PIS, which when

Phospholipid	% range	Headgroup (X)
Phosphatidylcholine	**38 - 65**	**$-CH_2CH_2N(CH_3)_3$**
Phosphatidylethanolamine	**19 - 33**	**$-CH_2CH_2NH_2$**
Phosphatidylserine	**5 - 15**	**$-CH_2CH(COOH)NH_2$**
Phosphatidylglycerol	**0.4 - 2**	**$CH_2CH(OH)CH_2OH$**
Phosphatidylinositol	**3 - 10**	

Phospholipid structure

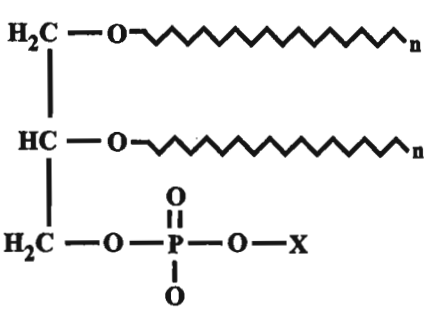


Fig. 7.1. Structure and composition of major phospholipids in eukaryotic membranes.

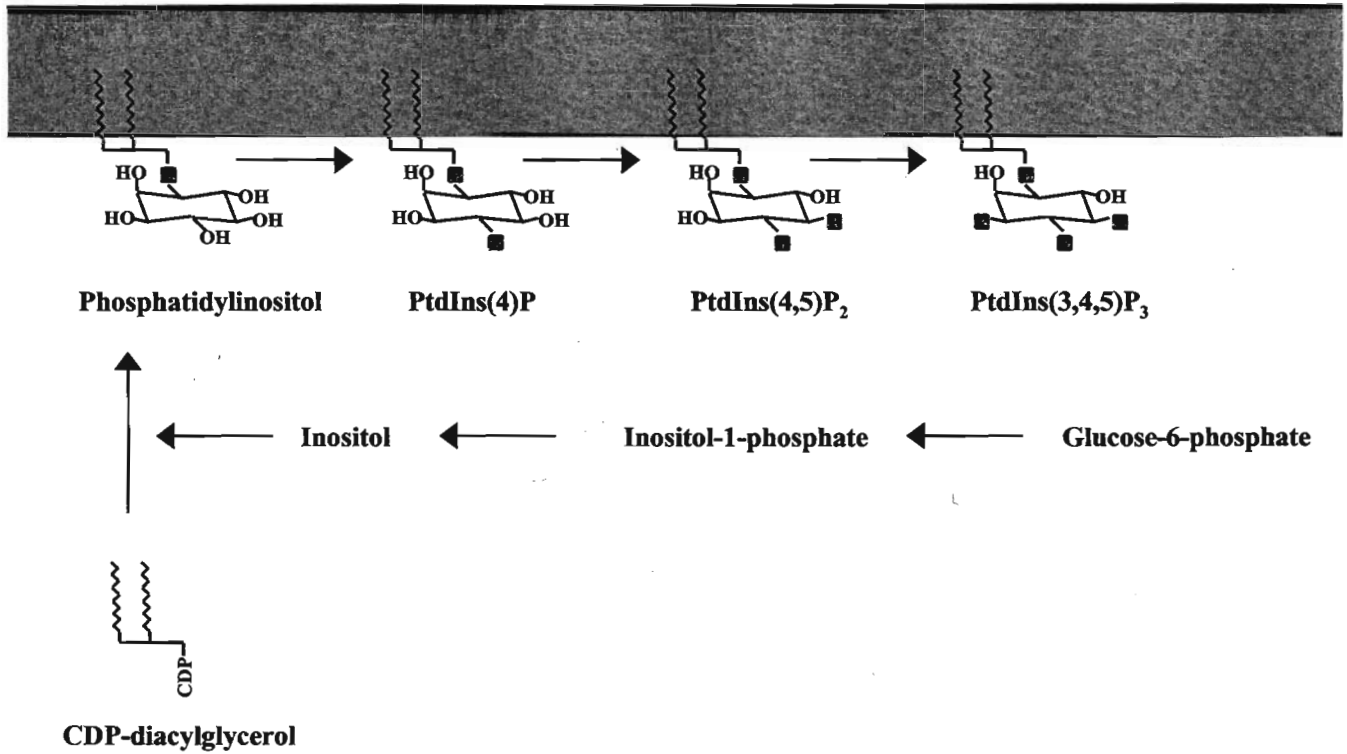


Fig. 7.2. Schematic representation of phosphoinositide biosynthesis in mammalian cells.

disrupted confers a lethal phenotye in *S. cerevisiae* (Nikawa, Kodaki & Yamashita, 1987) and represents the first committed step in the synthesis of these phospholipds. Although there appears to be no direct regulation of this gene, exogenous inositol has been shown to increase phosphatidylinositol synthesis rapidly, apparently by diverting more of the CDP-diacylglycerol precursor from phosphatidylserine biosynthesis (Kelley *et al.*, 1988). Inositol is synthesized by a two-step process from glucose-6-phosphate. The first step, catalysed by inositol-1-phosphate synthase, converts glucose-6-phosphate to inositol-1-phosphate, which is then dephosphorylated by inositol-1-phosphatase to form free inositol (Fig. 7.2). Synthesis of inositol from glucose-6-phosphate appears to be highly conserved in most eukaryotes and has been reported in a number of fungi including *S. cerevisiae* and *Neurospora crassa* (Donahue & Henry, 1981; Zsindely *et al.*, 1983).

PtdIns is itself phosphorylated by PtdIns 4′-kinase forming phosphatidylinositol-4-phosphate (PtdIns(4)P). The PtdIns 4′-kinases are a family of kinases functionally conserved in both mammalian and yeast cells (Wong, Meyer & Cantly, 1997). In *S. cerevisiae*, two genes have been cloned with 4′-kinase activity. The first, *PIK1*, is essential and appears to be predominantly associated with the nucleus whilst disruption of the second gene, *STT4*, leads to slow growth and a phenotype similar to that found in protein kinase C mutants (Flanagan *et al.*, 1993; Garcia-Bustos *et al.*, 1994; Yoshida *et al.*, 1994*a*,*b*). The discovery of two 4′-kinases with different functions and subcellular locations suggests a further level of control in phosphoinositide signalling with discrete compartmentalization of kinases with different cellular functions. PtdIns(4)P is further phosphorylated at the 5′ position yielding phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) in both mammalian and yeast cells and is the substrate for phosphoinositide-specific phospholipase C (Boronenkov & Anderson, 1995). In *S. cerevisiae*, disruption of Fab1p, which encodes the 5′-kinase, leads to vacuolar and growth defects, suggesting a role for PtdIns(4,5)P_2 in the regulation of vacuolar homeostasis (Yamamoto *et al.*, 1995).

In addition to its role in second messenger generation, PtdIns(4,5)P_2 has also been shown to regulate the assembly of the actin cytoskeleton by binding to a number of actin-binding proteins such as profilin in both mammalian and yeast cells. Profilin possess both PtdIns(4,5)P_2- and actin-binding domains, which are located close together so that binding of one excludes binding of the other (Goldschmidt-Clermont *et al.*, 1990;

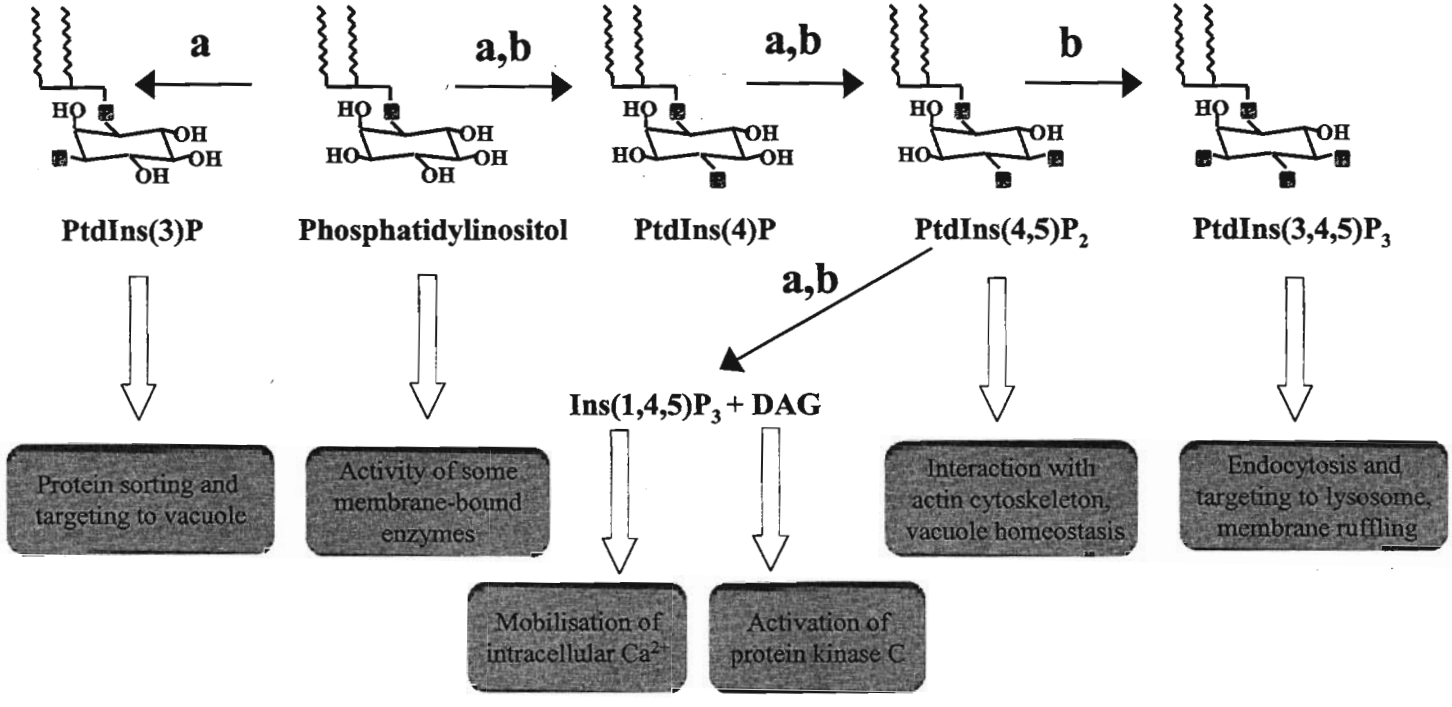


Fig. 7.3. Principal functions of the phosphoinositide cascade in eukaryotic cells; a, occurs in fungi; b, occurs in mammalian cells.

Metzler *et al.*, 1994; Sohn & Goldschmidt-Clermont, 1994). In *S. cerevisiae*, profilin has been found both in the cytosol and associated with the plasma membrane. Moreover, depletion of PtdIns(4,5)P_2 by inositol deprivation of an inositol auxotroph and by glucose starvation, leads to the mobilization of part of the membrane-associated profilin. This mobilization response could be reversed when PtdIns(4,5)P_2 levels were restored to normal (Ostrander, Gorman & Carman, 1995). More recently PtdIns 3′-kinases have been described in both mammalian and yeast cells and represent a further class of kinases with distinctive regulatory roles. In *S. cerevisiae* and *S. pombe*, PtdIns 3′-kinase is encoded by *VSPS34* and disruption of the gene leads to mis-targeting of hydrolases to the vacuole and abnormal vacuole development. PtdIns 3′-kinase differs from the mammalian homologue *P110/P85* in being insensitive to the inhibitor wortmannin and in having a strict substrate specificity for PtdIns leading to the formation of phosphatidylinositol-3-phosphate (PtdIns(3)P) (Auger *et al.*, 1989; Herman & Emr, 1990; Otsu *et al.*, 1991; Schu *et al.*, 1993; Stack & Emr, 1994; Kimura *et al.*, 1995; Takegawa, DeWald & Emr, 1995). In mammalian cells, PtdIns 3′ kinase is wortmannin sensitive and is capable of phosphorylating PtdIns, PtdIns(4)P and PtdIns(4,5)P_2, although *in vivo* PtdIns(4,5)P_2 appears to be the major substrate leading to the formation of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) (Stephens *et al.*, 1994; Woscholski *et al.*, 1994; Brown *et al.*, 1995). Despite these differences, mammalian PtdIns 3′-kinase appears functionally similar to yeast VSPS34, where it is involved in protein targeting to the lysosome, which plays a similar function in mammalian cells to the yeast vacuole. To date, there are no reports of any PtdIns(4,5)P_2-specific 3′-kinases in yeast. Thus, the yeast and mammalian PtdIns 3′-kinases appear to play similar functions in protein targeting, with PtdIns(3,4,5)P_3 fulfilling a similar role in mammalian cells to PtdIns(3)P in yeast cells (Fig. 7.3).

Phosphoinositide-specific phospholipase C

Hydrolysis of PtdIns(4,5)P_2 by phosphoinositide-specific phospholipase C leads to the generation of Ins(1,4,5)P_3 and diacylglycerol, which both possess second messenger functions. A rise in intracellular Ins(1,4,5)P_3 leads to the specific release of intracellular Ca^{2+} from internal pools whilst diacylglycerol acts within the membrane to stimulate protein kinase C (Berridge & Irvine, 1994; Divicha & Irvine, 1995). In mammalian cells, phosphoinositide-specific phospholipase C

represents a family of enzymes which are coupled to either G-protein or tyrosine kinase receptors and are stimulated by a range of agonists, leading to rapid hydrolysis of PtdIns(4,5)P_2 and rise in intracellular Ins(1,4,5)P_3 (Boyer, Hepler & Harden, 1989) (Fig. 7.4). In mammalian cells, Ins(1,4,5)P_3 triggers the release of Ca^{2+} from the endoplasmic reticulum, leading to a transient rise in intracellular Ca^{2+}. The increase in Ca^{2+} above the tightly regulated physiological levels in the cytoplasm activates an array of Ca^{2+}-dependent proteins, includ-

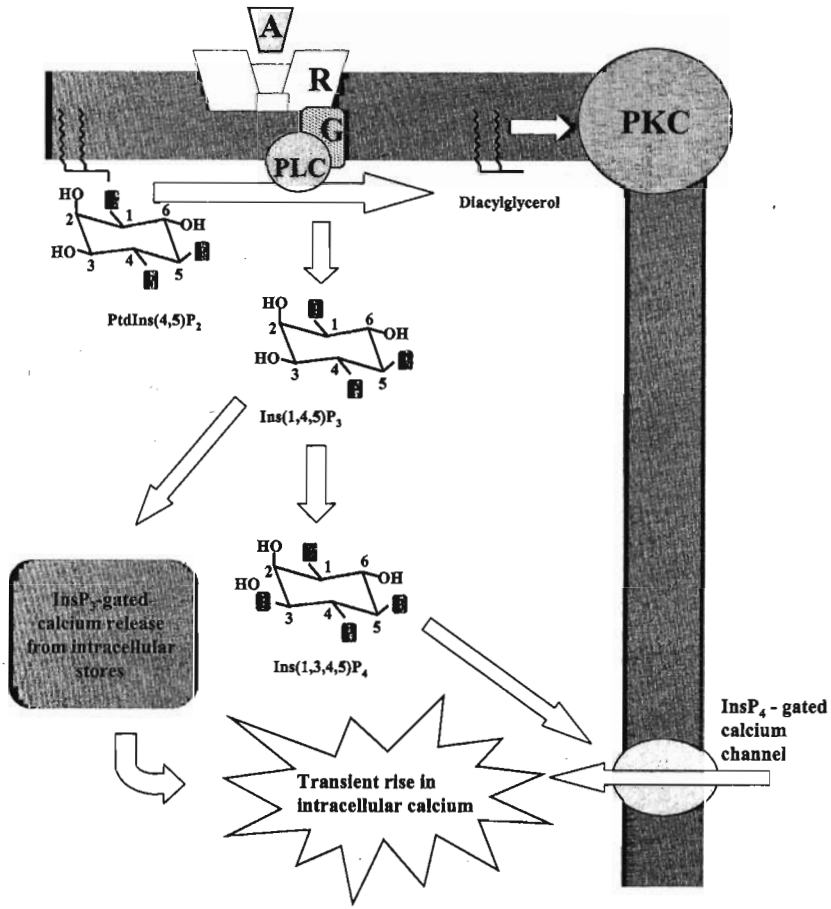


Fig. 7.4. Schematic representation of agonist stimulated inositol trisphosphate generation in mammalian cells. A, agonist; R, receptor; G, G-protein; PLC, phosphatidylinositol-specific phospholipase C; PKC, protein kinase C.

ing the ubiquitous Ca^{2+}-binding protein calmodulin (CAM), which in turn can activate a number of calmodulin-dependent enzymes including CAM-dependent protein kinases and phosphatases (Heinzmann & Hunziker, 1991; Lu & Means, 1993). Ins(1,4,5)P_3 has been shown to release Ca^{2+} specifically from the vacuoles of *S. cerevisiae*, *Candida albicans* and *N. crassa* (Cornelius, Gebauer & Techel, 1989; Schultz *et al.*, 1990; Belde *et al.*, 1993; Calvert & Sanders, 1995), which appear to act as the major intracellular Ca^{2+} store in fungi (Cornelius & Nakashima, 1987; Miller, Vogg & Sanders, 1990) and a phosphatidylinositol-specific phospholipase homologue (PLC-1) has been cloned from *S. cerevisiae*, *S. pombe* (Flick & Thorner, 1993; Yoko-o *et al.*, 1993, 1995; Andoh *et al.*, 1995) and more recently identified in a number of filamentous fungi (Jung *et al.*, 1997). Thus, a functional Ins(1,4,5)P_3 signal transduction pathway appears to exist in fungi although *in vivo* evidence of Ins(1,4,5)P_3-stimulated Ca^{2+} release is lacking and it remains unclear what factors are involved in stimulating phosphatidylinositol-specific phospholipase C activity. In *S. cerevisiae* and *S. pombe*, disruption of PLC-1 leads to a number of phenotypes depending on the strain and the medium the organism is grown in but generally include heat sensitivity and an inability to adapt to osmotic stress, suggesting that PLC-1 may be involved in relaying general stress signals across the membrane (Flick & Thorner, 1993; Payne & Fitzgerald-Hayes, 1993; Yoko-o *et al.*, 1993, 1995; Andoh *et al.*, 1995; Fankhauser *et al.*, 1995). In addition, there have been contradictory reports for a stimulation of phosphoinositide turnover and Ins(1,4,5)P_3 formation on exposure of starved cells to glucose and nitrogen, which would imply a role in nutrient sensing (Schomerus & Kuntzel, 1992; Brandao *et al.*, 1994; Prior, Robson & Trinci, 1994; Giugliano, Dennery & Rana, 1995) although a consensus view has yet to be reached.

Phosphoinositides and inositol phosphates in fungi

In filamentous fungi, all the major components of the phosphoinositide signalling pathway have been identified, including PtdIns, PtdInsP and PtdInsP$_2$ (Hanson, 1991; Robson *et al.*, 1991*a*; Prior *et al.*, 1993). Using HPLC to separate deacylated lipids from *N. crassa*, PtdInsP$_2$ was identified as PtdIns(4,5)P_2 whilst PtdInsP has been resolved and found to be composed of both PtdIns(4)P and PtdIns(3)P with levels of PTdIns(4)P being similar to PtdIns(3)P (Lakin-Thomas, 1993; Prior *et al.*, 1993).

Thus *N. crassa* more closely resembles *S. cerevisiae* and *S. pombe* in containing significant levels of both PtdInsP isomers (Hawkins, Stephens & Piggott, 1993; Stuart *et al.*, 1995) unlike mammalian cells, which contain predominantly only PtdIns(4)P and suggests that both a PtdIns 3′ and a PtdIns 4′ kinases are present. The levels of PtdIns(4,5)P_2 present in fungi as a percentage of the total phosphoinositides present is much lower than reported for mammalian cells, typically between 0.1 to 1% compared with 3.0 to 9.3% respectively. Moreover, the profile of inositol phosphates present in filamentous fungi appears complex. In *N. crassa*, evidence from HPLC separations suggests the presence of multiple isomers of InsP_2, InsP_3 and InsP_4 at similar levels, making their detection, identification and function difficult to establish (Lakin-Thomas, 1993; Prior *et al.*, 1993).

Metabolism of Ins(1,4,5)P_3 is an important control point in the Ins(1,4,5)P_3-mediated Ca^{2+}-release mechanism, serving to reduce Ins(1,4,5)P_3 levels and hence terminate the signal. Metabolism occurs by two mechanisms, phosphorylation by a specific kinase to InsP_4 and by dephosphorylation to InsP_2. InsP_2 is itself further dephosphorylated to InsP and ultimately inositol, which can then return to the phosphoinositide cycle by incorporation back to PtdIns. In mammalian cells, Ins(1,4,5)P_3 is dephosphorylated principally by a 5′-phosphomonoesterase and phosphorylated by a specific 3′-kinase leading to the formation of Ins(1,3,4,5)P_4 (Shears, 1992). The formation of Ins(1,3,4,5)P_4 itself acts as a further signal molecule, stimulating the opening of Ca^{2+} channels in the plasma membrane, prolonging the increase in intracellular Ca^{2+} (Irvine, 1992). In *S. cerevisiae*, no phosphomonoesterase activity was detected in either soluble or microsomal fractions and the phosphorylation by a soluble kinase found to produce Ins(1,4,5,6)P_4, indicating a 6′-kinase rather than a 3′-kinase described in mammalian cells (Estevez *et al.*, 1994). In *N. crassa*, dephosphorylation of Ins(1,4,5)P_3 was associated principally with the microsomal fraction and appeared to occur through the concerted action of 1′, 4′ and 5′-phosphomonoesterase activities. Ins(1,4,5)P_3 metabolism in the cytoplasm appeared to occur largely by phosphorylation by a 3′-kinase leading to the formation of Ins(1,3,4,5)P_4 identical with that found in mammalian cells, although a minor unidentified InsP_4 was also present suggesting *N. crassa* may contain two InsP 3′-kinases (Hosking, Trinci & Robson, 1997). In *C. albicans*, both an Ins(1,4,5,)P_3 kinase and Ins(1,4,5)$_3$ phosphatase activities were demonstrated *in vitro* although isomers formed were not identified (Gadd & Foster, 1997).

Studies with inositol-requiring mutants

The first indications that phosphoinositides may play an important role in hyphal morphology and branching were made by Beadle (1944) who noted that inositol-requiring strains of *N. crassa*, when grown on sub-optimal concentrations of inositol, produced small, compact mycelial pellets in liquid cultures rather than the normal spreading mycelial mat formed by the wild-type strain. This alteration in the mycelial branching pattern was later correlated to a reduction in the content of inositol-containing phospholipids (Fuller & Tatum, 1956; Shatkin & Tatum, 1961). Inositol starvation was found to lead to a rapid drop in viability and a deterioration of cellular membranes in *N. crassa* and was attributed to the uncoupling of membrane synthesis and other cellular components leading to unbalanced growth and so-called 'inositol-less death' (Shatkin & Tatum, 1961; Munkres, 1976). Similar observations were also made for inositol auxotrophs of *S. cerevisiae*, where inositol starvation was also associated with a rapid decrease in cell viability and degeneration of membranes (Atkinson, Kolat & Henry, 1977; Henry *et al.*, 1977). In addition, inositol deprivation was also associated with changes in the cell wall composition of both *S. cerevisiae* and *N. crassa*. In *N. crassa*, the glucosamine content of the wall was reduced, indicating that chitin synthase activity was lower whilst in *S. cerevisiae*, inositol starvation was associated with a decrease in both the level of mannan and glucan in the wall. The decrease in the levels of mannan was correlated to a reduction in the activity of UDP-*N*-acetylglucosamine:dolichol phosphate *N*-acetylglucosamine-1-phosphate transferase (GlcNAc-1-P), a membrane-associated enzyme which catalyses the first step in N-linked mannan synthesis (Hanson & Lester, 1982). GlcNAc-1-P transferase activity could be restored *in vitro* by the addition of PtdIns. Hence, at least for mannan synthesis, a decrease is due to a dependency of GlcNAc-1-P transferase on PtdIns for maximal activity. One of the problems associated with using inositol-requiring strains of *N. crassa* to investigate the relationship between phosphoinositides and morphology is that inositol deprivation leads to a reduction in all classes of phosphoinositides in the membrane, due to the reduction in the synthesis of PtdIns (Table 7.1). As well as acting as the precursor for PtdInsP and PtdInsP$_2$, PtdIns, which comprises 80–95% of the total phosphoinositides in the membrane, is also required for the maximal activity of a number of membrane-bound enzymes. In addition, PtdIns is also the precursor for a further class of inositol-containing lipids, the sphingolipids, which themselves play an

Table 7.1. *Effect of inositol deprivation on the phosphoinositide composition of an inositol-requiring strain of* Neurospora crassa. *Data from Lakin-Thomas (1993)*

	Phosphoinositide content (pmol/g dry weight)	
Phosphoinositide	Inositol-sufficient	Inositol-deficient
PtdIns	2500	240
PtdIns(3)P	29.5	7.3
PtdIns(4)P	29.5	5.0
PtdIns(4,5)P_2	24.3	6.9

essential role in cell viability and are also depleted during inositol deprivation (Hanson & Brody, 1978; Dickson *et al.*, 1990; Patton *et al.*, 1992; Nagiec *et al.*, 1997). Thus, morphological changes associated with inositol deprivation may be due to factors other than interference with phosphoinositide turnover.

Studies with phosphoinositide turnover inhibitors

Studies with inhibitors of phosphoinositide synthesis have also been correlated to changes in colony morphology and hyphal branching. Validamycin A, an aminoglycosidic antibiotic used in the control of *Rhizoctonia solani*, which causes sheath blight in rice plants, was found to induce profuse branching and a reduction in hyphal extension in *R. solani* and *Rhizoctonia cerealis* (Robson, Kuhn & Trinci, 1988). This alteration in the spatial pattern of the colony was correlated to a reduction in the total phosphoinositide content of the membrane, which was only slightly reversed by the addition of exogenous inositol (Robson, Kuhn & Trinci, 1989). Validamycin was reported to lead to an alteration in the wall composition of *R. solani*, suggesting that the observed changes in hyphal extension and branching may be due to an alteration in wall assembly at the hyphal tip. Hanson (1991) examined the effects of lithium chloride on the morphology and phosphoinositide composition of *N. crassa*. Studies on a range of mammalian cells have shown that hydrolysis of inositol phosphates by inositol monophosphatases is highly sensitive to lithium and that exposure leads to a reduction in phosphoinositide synthesis (Drummond, 1987; Nahorski & Potter, 1989). When grown in the presence of LiCl up to a concentration of 4 mM, the morphology of *N. crassa* changed from a spreading mat to compact pellets, similar to that

found during inositol deprivation of inositol-requiring strains of *N. crassa*. This dose-dependent change in hyphal morphology was associated with a decrease in the levels of PtdIns, PtdInsP and $PtdInsP_2$. However, as with the inositol-requiring strains, the primary effects are due to a reduction in PtdIns synthesis and therefore not necessarily due to inhibition of the phosphoinositide signalling pathway.

More recently, we investigated the effect of specific phosphoinositide turnover inhibitors on the growth and morphology of *N. crassa*. Both lysocellin and piericidin B1 *N*-oxide were found to reduce hyphal extension and induce hyphal branching without affecting the overall specific growth rate (Hosking, Robson & Trinci, 1995). An inositol analogue (2*S*, 3*R*, 5*R*-3-azido-2-benzoyloxy-5-hydroxycyclohexanone), which would reduce the levels of all the phosphoinositides in the membrane, also had similar morphological effects (Table 7.2). On further investigation, lysocellin was found to reduce the levels of PtdInsP and $PtdInsP_2$ by about 70% and 90% respectively and to cause a significant increase in the levels of PtdIns *in vivo* (Table 7.3) suggesting that lysocellin specifically blocks conversion of PtdIns to PtdInsP. This was confirmed *in vitro* on isolated membrane fractions from *N. crassa*, where potent inhibition of PtdIns 4′-kinase activity was observed. Thus, in *N. crassa*, lysocellin potently blocks at least PtdIns (4)P synthesis and consequently PtdIns(4,5)P_2 formation. When young mycelia grown on Cellophane overlaid plates in the absence of lysocellin were transferred to plates containing lysocellin, hyphal extension was reduced and profuse branching was initiated throughout the whole mycelium, first appearing about 1 h after transfer (Fig. 7.5). Thus, interference in phosphoinositide signalling by lysocellin leads to a profound effect on hyphal growth and morphology. When the effect of exposure to lysocellin of growing hyphae was investigated, it was found that lysocellin caused a rapid and complete cessation of hyphal extension within 30 s. This was followed by a recovery after 4–7 min to a rate which was only about 30% of the rate prior to treatment (Fig. 7.6). The rapid reduction in hyphal extension rate indicates that phosphoinositide turnover must be very rapid in growing hyphae and that the rate of hyphal extension is in some way dependent on maintaining levels of PtdInsP and/or $PtdInsP_2$. If PtdIns(4,5)P_2 plays an important role in regulating the actin cytoskeleton in filamentous fungi, the reduction in hyphal extension and the induction of profuse branching observed throughout the mycelium on lysocellin treatment may result from a reorganization of the actin cytoskeleton following

Table 7.2. *Effect of phosphoinositide turnover inhibitors on the growth and morphology of* Neurospora crassa. *Data from Hosking, Robson & Trinci (1995)*

Concentration of inhibitor	Hyphal growth unit length (μm)	Specific growth rate (h^{-1})
Control	1443 ± 141	0.34 ± 0.07
8 μM lysocellin	993 ± 141	0.30 ± 0.05
112 μM piericidin	513 ± 48	0.33 ± 0.05
7.6 μM inositol analogue	578 ± 42	0.33 ± 0.08

Table 7.3. *Effect of lysocellin on the percentage phosphoinositide composition of* Neurospora crassa. N. crassa *was grown in the presence or absence of 1.6 μM lysocellin in a medium containing [^{3}H]-inositol and extracted phospholipids deacylated and separated by HPLC against authentic standards. Data from Hosking, Robson & Trinci (1995)*

Phosphoinositide	Absence of lysocellin	Presence of lysocellin
PtdIns	87.6 ± 0.4	96.9 ± 0.1
PtdInsP	5.2 ± 1.6	1.5 ± 0.1
$PtdInsP_2$	0.48 ± 0.1	0.05 ± 0.02

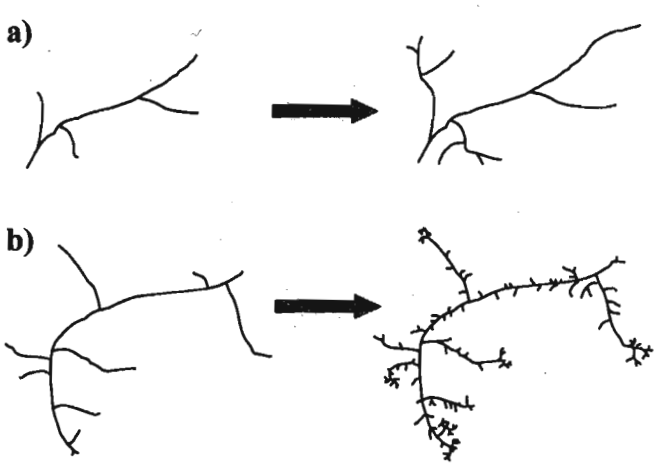


Fig. 7.5. Effect of transferring young germlings of *N. crassa* from agar medium to agar medium lacking (a) or containing (b) 4 μM lysocellin. The effect of the transfer on hyphal morphology is shown after 3 h. (Based on the data of Hosking, Robson & Trinci, 1995.)

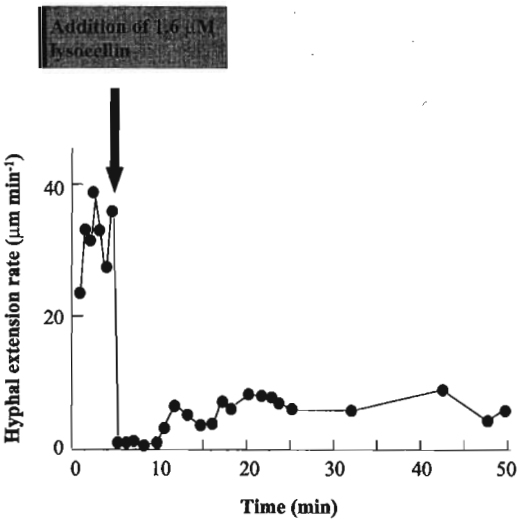


Fig. 7.6. Effect on the extension rate of a single growing hypha of *N. crassa* to exposure to 1.6 μM lysocellin. Hyphae were grown in a cellophane sandwich overlaying agar medium and lysocellin added at the time indicated. (Based on the data of Hosking, Robson and Trinci, 1995.)

the reduction in PtdIns(4,5)P_2 in the membrane. Actin is associated with sites of polarized growth and is important in both polarized hyphal extension and branch initiation (Heath, 1990). Alternatively, the reduction of PtdIns(4,5)P_2 in the membrane by lysocellin treatment would also lead to a reduction in Ins(1,4,5)P_3 and DAG levels, which may result in lower levels of intracellular Ca^{2+} and protein kinase C activity. A protein kinase C homologue has been cloned from a number of fungi including *S. cerevisiae*, *S. pombe*, *C. albicans*, *Aspergillus niger*, *Trichoderma reesei* and *N. crassa* (Antonsson *et al.*, 1994; Morawetz *et al.*, 1996; Paravicini *et al*, 1996). Disruption of the PKC locus in *S. cerevisiae* and *S. pombe* leads to an osmotically sensitive phenotype due to an increase in β-1,3-glucanase activity in the cell wal (Levin *et al.*, 1994; Paravicini *et al.*, 1996). In filamentous fungi, disruption of PKC has yet to be reported. A reduction in the levels of Ins(1,4,5)P_3 may result in a reduction in intracellular Ca^{2+}, which could also affect mycelial morphology. Growth of *F. graminearum* and *N. crassa* in a Ca^{2+}-depleted medium resulted in a more highly branched phenotype and abnormal hyphal development (Schmid & Harold, 1988; Robson, Wiebe & Trinci, 1991*b*) whilst the highly branched phenotypes of two

mutants of *N. crassa*, *frost* and *spray*, could be reversed by high levels of extracellular Ca^{2+} (Dicker & Turian, 1990). However, if changes in mycelial morphology associated with a reduction in PtdIns(4,5)P_2 levels were due to a reduction, in either Ins(1,4,5)P_3 or DAG, it would imply that phosphoinositide-specific phospholipase C actively hydrolyses PtdIns(4,5)P_2 during normal hyphal growth, rather than in response to an outside stimulus.

To date, it is clear that phosphoinositide turnover is intimately linked with hyphal extension and branch induction although the exact nature of the relationship remains unclear. As more homologues of this signal cascade are identified in filamentous fungi, the nature and function of phosphoinositide signalling and its evolution from primitive to higher eukaryotes will be revealed.

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The fungal colony

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