Microbiology Comment provides a platform for readers of **Microbiology** to communicate their personal observations and opinions in a more informal way than through the submission of papers.

Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others. In other instances we may have a useful piece of methodology that we would like to share.

The Editors hope that readers will take full advantage of this section and use it to raise matters that hitherto have been confined to a limited audience.

Jon Saunders
Editor-in-Chief

Yeast genome and the inositol trisphosphate kinase controversy

Second messengers derived from inositol phospholipids are important regulators of various cellular processes, such as cell growth, fertilization, sensory perception and neuronal responses (1). In particular, inositol 1,4,5-trisphosphate (InsP_3) plays a key role in controlling many complex cellular events by generating internal calcium signals (3). In animal cells, InsP_3 is phosphorylated to inositol 1,3,4,5-tetrakisphosphate by a specific kinase (InsP_3 3-kinase) that occupies a central position in regulating the availability of these two calcium-mobilizing second messengers (3). A similar InsP_3 3-kinase activity has also been found in a unicellular alga, *Chlamydomonas eugametos*, but not in pea roots (2). Although the intracellular localization and regulation of InsP_3 3-kinase are poorly understood, certain properties of this enzyme suggest that it provides an important branch point in the phosphoinositide signalling cascade. For example, InsP_3 3-kinase is stimulated by calcium/calmodulin and is a target of protein kinase C and cAMP-dependent protein kinase A (3, 4). The significance of the phosphoinositide

signalling cascade prompted some authors to elucidate the corresponding pathway in the budding yeast *Saccharomyces cerevisiae*, often referred to as 'a model eukaryote'. As a result, Estevez *et al.* (4) reported that InsP_3 metabolism in yeast is unusual and does not involve InsP_3 3-kinase. Instead, yeast appears to possess InsP_3 6-kinase activity that generates inositol 1,4,5,6- P_4 from InsP_3 and not the expected inositol 1,3,4,5- P_4 . The yeast InsP_3 6-kinase is not regulated by calcium/calmodulin and is not recognized by antibodies to the rat-brain InsP_3 3-kinase. Moreover, these authors also failed to detect in yeast extracts another important activity in InsP_3 metabolism, namely InsP_3 phosphomonoesterase (4). The conclusion of this work has been recently challenged by Robinson *et al.* (7), who reported that InsP_3 is undetectable in yeast extracts due to the presence of both InsP_3 3-kinase and phosphomonoesterase activity. Evidently, more work, in particular on the molecular level, needs to be done to resolve this controversy. However, before such data become available, we were curious to determine whether the InsP_3 3-kinase coding sequence is present in the recently released sequence of the entire *S. cerevisiae* genome.

Our current study indicates that InsP₃ 3-kinase is highly conserved between vertebrates and invertebrates and the level of amino acid identity between the mammalian enzyme and that in insect species is in the range of 60–70% (R. Maleszka and others, unpublished). Although none of the non-animal genes encoding InsP₃ 3-kinase has been sequenced to date, the fact that this activity exists in a green alga implies that InsP₃ 3-kinase is not a metazoan invention and most likely evolved before the *Animalia/Planta* division. Thus, we hypothesized that a yeast homologue of InsP₃ 3-kinase, if available, should be easily detected by searching the complete, translated genome of *S. cerevisiae* with the sequence of the animal protein. We have performed BLAST and FASTA searches using as a probe either the entire protein sequence of InsP₃ 3-kinase (SWISS-PROT accession no. P17105), or shorter, conserved motifs against the 6-frame translation of the entire yeast genome (Stanford Genomic Resources, <http://genome-www.stanford.edu/Saccharomyces/>). Interestingly, regardless of

the search mode, we have been unable to find an open reading frame that would indicate the presence of InsP₃ 3-kinase domain(s) in the yeast genome. Only a few, short motifs have been picked up by this approach. These are low scoring sequences that most likely bear no phylogenetic relationship to InsP₃ 3-kinase. One possible exception may be a motif in a putative yeast protein, YD9335.03c (PIR accession no. S63423), also found in a rabbit protein that stimulates phosphate transport (GenBank accession no. U74297). The consensus pattern for this motif is: LxDLxxxxxxPCxLxKMGxR. Conceivably, this pattern represents a signature of an ancient module that has been used to build InsP₃ 3-kinase and other proteins.

Our analysis adds more weight to the notion that yeast in fact does not possess InsP₃ 3-kinase analogous to that found in animal species and explains the lack of cross-reactivity with InsP₃ 3-kinase antibodies in yeast extracts (4). Since the InsP₃ 3-kinase reaction is the only known pathway to produce inositol 1,3,4,5-P₄ (3), the apparent lack of the corresponding gene in yeast, and possibly in plants (2), has important implications for the evolution of inositol phosphate kinases and mechanisms of calcium signalling. Clearly, in different lineages, the phosphoinositide signalling cascade involves distinct critical precursors that may reflect specific signalling

► GUIDELINES

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

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requirements of divergent cells and tissues.

Gene loss in yeast is not unusual and a number of both metabolic and regulatory functions are known to be missing in *S. cerevisiae*; e.g. yeast does not possess initial steps in D-xylose catabolism (6) and the regulatory kinase Raf (5). Future analysis of the yeast genome will reveal the extent of this phenomenon and provide us with a better understanding of how alternative metabolic pathways and regulatory networks evolved. Genes involved in these alternative and/or unique pathways are likely to account for a considerable proportion of over 30% of yeast

genes that have no known homologues in other lineages.

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