Macromolecules such as proteins, lipids, and carbohydrates often have complex structures that underpin their cellular functions. The sugar alcohol myo-inositol is a notable exception—its simple six-carbon structure is rather unremarkable (Fig. 1) but is used in countless cellular processes in all domains of life (1).

For instance, inositol provides the structural backbone of phosphatidylinositol, an essential building block of important signaling molecules such as inositol polyphosphates. Phosphatidylinositol is one of the most abundant membrane phospholipids in eukaryotes, vital for glycosylphosphatidylinositol-anchored proteins, and a component of some sphingolipids (2, 3).

“The fact of the matter is that inositol is absolutely essential [in cells],” says Susan Henry, Professor of Molecular Biology and Genetics at Cornell University (Fig. 2).

Henry has studied inositol metabolism in the yeast Saccharomyces cerevisiae since the 1970s. “I focused on the phospholipids and the metabolites that regulate their formation,” she says. “Inositol turned out to be the strongest regulatory metabolite of these pathways.”

*S. cerevisiae* was Henry’s organism of choice almost immediately from the start of her long career, she says, because it is easier to study inositol and phospholipids in this species than in more complex eukaryotes.

Yeast species have been a workhorse for scientists since the dawn of modern research. Their widespread use in fermentations led to the coinage of the term enzyme (Greek for *in* yeast) (4). *S. cerevisiae* grows rapidly in culture and does so as single cells, a boon for investigating eukaryotic biochemistry under controlled conditions. The species can be stably maintained in the haploid state, and its genes can be easily manipulated.

“Yeast is almost like the Escherichia coli of the eukaryotic world. [It helped us] to figure out exactly where the metabolic components are coming from,” says Henry.

The fully sequenced *S. cerevisiae* genome did not become available until 1996 (5), so earlier studies of the genetics and biochemistry even of simple organisms such as yeast required skilled detective work to find all the players involved in a molecular pathway.

While at Albert Einstein College of Medicine in the mid-1970s, Henry and Ph.D. student Michael Culbertson used the mutagenic agent ethyl methanesulfonate to generate a series of more than 50 *S. cerevisiae* mutants defective in inositol biosynthesis (6). This mutant strain collection provided a resource to launch investigations into the genes involved in inositol and phospholipid metabolism.

In a 1981 JBC paper, Henry and co-author Thomas Donahue reported the first purification and characterization of yeast myo-inositol-1-phosphate synthase (7). This enzyme, called Ino1, is an intramolecular lyase and isomerase that catalyzes the cyclization of glucose 6-phosphate, a reaction that yields inositol 1-phosphate, an immediate precursor to free inositol.

The two scientists also mapped its gene to a locus (called *INO1*) in the yeast genome and developed antibodies for specific detection of Ino1, laying the groundwork for more detailed biochemical and genetic studies.

In three JBC papers published in the late 1980s and early 1990s and recognized as Classics here (8–10), Henry and colleagues at Albert Einstein College and at Carnegie Mellon University reported the sequence and genetic analysis of the *INO1* gene, along with its regulation by a transcriptional repressor and two transcriptional activators.

In the first paper (8), Henry and Margaret Dean-Johnson sequenced the cloned *INO1* gene, including its 5’ and 3’ regulatory regions, and also determined the amino acid sequence of the purified protein. This analysis uncovered an ORF as a prime candidate for encoding the entire enzyme.

When they disrupted the predicted *INO1* ORF in yeast cells, the researchers found that the cells did not express any protein detectable by the antibodies for Ino1 and that the cells grew only when supplied with inositol from the growth medium.

The findings showed that the *INO1* gene encodes myo-inositol-1-phosphate synthase in yeast, representing a major advance because it made available the full-length nucleotide and amino acid sequences of this central phospholipid enzyme in eukaryotes (11).

In keeping with earlier findings of Henry’s laboratory that expression of Ino1 is transcriptionally regulated (12), the Classics paper also uncovered several conserved short DNA motifs in the 5’ promoter region of the *INO1* gene that were likely binding sites of transcriptional regulators (8).

Henry therefore next set her sights on deciphering the regulation of *INO1* expression by inositol and another phospholipid precursor, choline. Using different *INO1* promoter constructs fused to the *E. coli lacZ* gene to measure the promoters’ activities, her team pinpointed the main transcriptional start site, a

Figure 1. myo-Inositol is a sugar alcohol, signaling compound, and ubiquitous precursor to phospholipids and many other important structural and signaling metabolites.
TATA box, and several transcription factor-binding sites in the INO1 promoter (13).

The team also found a region that appeared to be bound by a transcriptional repressor, Opi1 (named after the overproduction of inositol phenotype of yeast strains lacking this repressor), which they had previously identified (14).

In the second Classics paper (9), Henry and colleagues mapped the OPI1 gene in the yeast genome, cloned and sequenced it, and identified key features of the predicted Opi1 protein sequence, including a leucine repeat and polyglutamine stretches also present in other regulatory proteins.

The paper defined a major regulatory mechanism that controls INO1 expression. It also provided critical momentum for work by Henry’s group that later identified a cis-acting regulatory DNA element, the inositol-sensitive upstream activation sequence (UASINO). This sequence is present in the promoters of genes responsible for the synthesis of phospholipids and also the lipid triacylglycerol (14).

The third Classics paper capped off the series by further elucidating the regulatory circuit that controls phospholipid biosynthesis in yeast (10). Henry and colleagues demonstrated that the yeast proteins Ino2 and Ino4 form a heterodimeric complex that binds and activates the INO1 promoter. They also delineated the binding sites of the Ino2-Ino4 complex on this promoter and showed that both proteins contain a basic helix-loop-helix motif characteristic of transcriptional regulators.

The paper was the first to describe a basic helix-loop-helix heterodimeric transcription factor in yeast and represented a milestone in the then budding field of research into the regulation of phospholipid synthesis in eukaryotes.

Looking back, Henry says that the mentorship by two of her early advisors, Seymour Fogel and Alec Keith, helped lay the foundation for her career. Besides sharing their expertise in genetics and biochemistry, they also gave Henry critical material support to get her work off the ground.

“I was really lucky that they were not the kind of people who wanted me to [work exclusively] on their hot project,” she says.

“They were willing to let me come into the laboratory and use their materials to do the things that I wanted to do.”

Moreover, although Henry was working with yeast, she could secure funding through agencies that typically support mainly medical research, she says. “I did not have any trouble getting support from the National Institutes of Health, because of the connection with lipid metabolism in other eukaryotic organisms.”

This investment paid off well, she notes. “Many of the genes that I worked on were homologous to those in other eukaryotes, providing a ladder for other people to find the [corresponding] genes in other organisms.”

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