A pathway of a hundred genes starts with a single mutant: Isolation of *sec1-1*

Peter J. Novick[1]

Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093

The announcement of the 2013 Nobel Prize for Physiology or Medicine to Randy Schekman, James Rothman, and Thomas Südhof cited a number of papers, including an article published in 1979 in PNAS describing the isolation and characterization of the first yeast secretory mutant, *sec 1–1*. This paper, coauthored with Randy Schekman, was also my own very first publication (1). Subsequent events surrounding the Nobel ceremony helped refresh my memories from 35 years ago.

When I considered joining Randy's laboratory as a first-year graduate student in the Biochemistry Department at the University of California, Berkeley, the Nobel prize had just recently been awarded to George Palade (together with Albert Claude and Christian de Duve) for the description of the secretory pathway. George's Nobel address published in *Science* reviewed the studies in which newly synthesized proteins in pancreatic acinar cells were followed by both cell fractionation and electron microscopy from their site of synthesis on the endoplasmic reticulum, through the stacks of the Golgi apparatus, into secretory granules, and then out into the extracellular environment of the pancreatic lumen (2). Randy gave this now classic review to potential members of his newly formed laboratory with the hope that it would inspire them to try to identify the components of the secretory machinery and to understand the molecular mechanisms by which they direct membrane traffic. Having an interest in biological membranes as well as a natural bent toward genetics, I found Randy's suggestion of a genetic approach to the topic very appealing. The problem was how to identify a secretory mutant.

We used the recent isolation of cell division cycle (*cdc*) mutants in the yeast *Saccharomyces cerevisiae* (3) as a general model for our approach. We started with the assumption that blocking the yeast secretory pathway would be a lethal defect because the yeast cell wall consists in part of secreted glycoproteins, and furthermore, plasma membrane expansion was likely to rely on the incorporation of new membrane from the secretory pathway. Hence, as in the case of the *cdc* mutants, we would be looking for conditionally lethal, temperature-sensitive (*ts*) mutants. Nonetheless, because the secretory compartments in yeast are relatively inconspicuous by electron microscopy, some reports suggested that new surface material was secreted directly through the plasma membrane and that yeast do not have a bona fide secretory pathway analogous to the pathway present in mammalian cells. Thus, we faced both untested assumptions and major uncertainties in our search for secretory mutants.

Being young and naïve, my first efforts were, in retrospect, a bit too elaborate. Having read reports of an inducible sulfate permease activity that would also allow uptake of the toxic compound, chromate (4), I devised what I thought to be a clever enrichment scheme. Mutagenized cells were grown at the permissive temperature in medium containing methionine, repressing the synthesis of sulfate permease. Raising the temperature to the restrictive condition while shifting to medium lacking methionine induced the production of sulfate permease in the cell population. Chromate was then added to the growth medium, and the incubation was continued until viability was reduced by several orders of magnitude. The idea was that secretory-proficient cells would deliver the newly made permease to the cell surface, where it would allow uptake of chromate, leading to cell death, while a secretory mutant would be unable to incorporate the permease into the plasma membrane, thereby avoiding both chromate uptake and cell death.

Hoping for the enrichment of the desired mutants, I then started screening among the survivors for the predicted phenotypes. I first

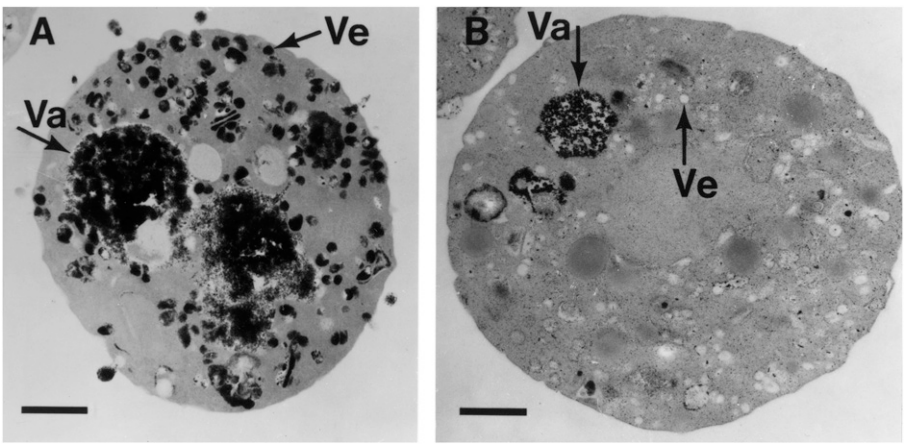

Fig. 1. *sec1-1* cells accumulate a secretory enzyme within vesicles. Thin section electron micrograph of *sec1-1* mutant cells that had been shifted to the restrictive temperature for 2 h before the cell walls were removed, and they were fixed and stained for acid phosphatase activity. (*A*) A strain that synthesizes a secreted form of acid phosphatase. (*B*) A strain that does not synthesize this form of the enzyme. The 100-nm vesicles (labeled Ve) that accumulate in the cytoplasm of *sec1-1* cells as a result of the block in exocytosis are stained only in *A*, indicating that they contain the acid phosphatase activity that is normally exported to the cell wall. The vacuoles (labeled Va), which represent the yeast equivalent to the lysosomes of animal cells, are stained in both panels because of the presence of a different phosphatase activity that is made by both strains. The bar is 0.5 μm. Reproduced from ref. 1.

Author contributions: P.J.N. wrote the paper.

The author declares no conflict of interest.

This article is part of the special series of PNAS 100th Anniversary articles to commemorate exceptional research published in PNAS over the last century. See the companion article, "Secretion and cell-surface growth are blocked in a temperature-sensitive mutant of *Saccharomyces cerevisiae*" on page 1858 in issue 4 of volume 76, and see Inner Workings on page 9021.

[1]E-mail: pnovick@ucsd.edu.

screened for *ts* growth by replica plating colonies at permissive and restrictive temperatures and then tested the *ts* strains for their ability to secrete two different cell wall enzymes, invertase and acid phosphatase, at the restrictive temperature. I also tested for the ability of the mutant strains to incorporate radiolabeled leucine into newly synthesized protein. From a pool of 87 *ts* colonies, two mutants were found that matched our expectations. At the restrictive temperature, they were impaired in the export of the two cell wall enzymes yet continued to synthesize new proteins. The question then became—where were the missing secretory proteins? An independent assay of the internal and external pools of these enzymes demonstrated that in one of the mutants, *sec1-1,* the missing cell wall enzymes had accumulated in an internal pool. Furthermore, shifting the cells back to the permissive temperature in the presence of the protein synthesis inhibitor cycloheximide proved that the internal pool constituted a functional intermediate on the export pathway.*

It was around this time that the biochemistry graduate students at Berkeley hosted George Palade for a seminar, and I was able to describe my work to him at a potluck dinner during his visit. He not only complimented my attempt at moussaka, he was also very encouraging regarding my project, but suggested that I really needed to examine the mutant by thin section electron microscopy. With the help of the departmental electron microscopist, Alice Taylor, we were able to visualize an incredible transformation. Following incubation of *sec1-1* cells at the restrictive temperature the cells became literally packed with membrane-bounded vesicles. Staining for acid phosphatase activity established that the intracellular pool of acid phosphatase resides within the accumulated vesicles (Fig. 1). At this point, it became very clear that, just as in the pancreas, yeast relies on vesicular carriers to bring secreted proteins from their site of synthesis to the cell surface.

The challenge then became to identify more of the components of the secretory machinery by isolating a large number of secretory mutants. This prompted me to reexamine my enrichment scheme. Having a bona fide secretory mutant at hand, I was able to test the efficiency of the enrichment procedure through a reconstruction experiment. The results were not encouraging; although *sec1-1* cells fail to incorporate sulfate permease into the plasma membrane at the restrictive temperature, they actually die faster than WT cells as a result of the effects of the secretory block. In other words, *sec1-1* was isolated despite the enrichment and not because of it. Another *sec* mutant was isolated by direct screening without any enrichment step; however, the inefficiency of this approach led us to search for some phenotype that would allow enrichment of *sec* mutants before they die from the secretory block itself. Randy suggested exploiting cell density based on the logic that if cell surface growth was blocked, then cell volume couldn't expand, whereas cell mass continued to increase as a result of ongoing metabolism. An increase in cell mass without a corresponding increase in volume would lead to an increase in density. This proved to be remarkably effective. Following a shift to the restrictive temperature, *sec1-1* cells became far denser than the densest WT cells, allowing a clean separation on a density gradient. Incorporation of a density enrichment step in place of the chromate selection led to the isolation of more than 200 secretory mutants that together defined 23 different *sec* genes encoding components that act at all stages of the secretory pathway, from the endoplasmic reticulum, through the Golgi apparatus, and to the cell surface (5).

[George Palade] not only complimented my attempt at moussaka, he was also very encouraging regarding my project.

Over the last 35 years, this mutant collection has proven to be a treasure trove, yielding examples of each of the key elements of the vesicular traffic machinery, including coat proteins responsible for vesicle formation, tethers involved in the initial recognition of the target membrane by the vesicle, soluble N-ethylmaleimide sensitive factor attachment protein receptors (SNAREs) that drive the fusion of the vesicle and target membranes, SNARE interactors, such as the Sec1 protein, that regulate assembly of the SNARE complex and couple complex assembly to efficient fusion, and GTPases and their exchange proteins that coordinate the many different components of the machinery at each stage of transport (6). Mammalian homologs have been identified for all of the *SEC* genes, and the evolutionary conservation of the core eukaryotic secretory machinery has been definitively proven. The initial collection of *sec* genes has been expanded severalfold by a variety of genetic screens and by reverse genetics following biochemical analysis. Additional membrane traffic pathways have also been dissected by novel genetic approaches. In vitro assays that reconstitute specific steps of vesicular traffic in cell free extracts and in totally defined systems have allowed detailed analysis of the molecular mechanisms of vesicle budding and membrane fusion.

Although there are certainly still important unanswered questions regarding the detailed mechanisms of vesicle budding and membrane fusion, my interest lies in understanding how all of the different events along the entire secretory pathway are coordinated. GTPases of the Rab and Arf families work through multiple effector proteins to coordinate the biochemical events within a given stage of transport (7). Furthermore, various molecular mechanisms serve to link GTPases at different stages of transport into regulatory circuits that help to coordinate adjacent stages of transport. These circuits act in concert with spatially and temporally regulated changes in lipid content to control the functional identity of the membrane as it flows along a pathway. Our understanding of these regulatory mechanisms is still in its adolescence, with many key lessons to be uncovered. Perhaps another 35 years will be needed.

*A page from my lab notebook, circa 1978, shows a graph of the key experimental results demonstrating that secretion is first blocked in the temperature sensitive *sec1-1* mutant and then reversed following a shift back to the permissive temperature. See Inner Workings on page 9021.

1 Novick P, Schekman R (1979) Secretion and cell-surface growth are blocked in a temperature-sensitive mutant of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 76(4): 1858–1862.

2 Palade G (1975) Intracellular aspects of the process of protein synthesis. *Science* 189(4206):867.

3 Hereford LM, Hartwell LH (1974) Sequential gene function in the initiation of *Saccharomyces cerevisiae* DNA synthesis. *J Mol Biol* 84(3):445–461.

4 Breton A, Surdin-Kerjan Y (1977) Sulfate uptake in *Saccharomyces cerevisiae*: Biochemical and genetic study. *J Bacteriol* 132(1):224–232.

5 Novick P, Field C, Schekman R (1980) Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell* 21(1):205–215.

6 Schekman R, Novick P (2004) 23 genes, 23 years later. *Cell* 116(2 Suppl):S13–S15.

7 Mizuno-Yamasaki E, Rivera-Molina F, Novick P (2012) GTPase networks in membrane traffic. *Annu Rev Biochem* 81:637–659.