23 Genes, 23 Years Later

Commentary

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George Palade's pioneering work in the 1960s had established the secretory pathway and its connection to the biogenesis of organelles as a major theme in eukaryotic cell biology (Palade, 1975). His disciples, including David Sabatini and Gunter Blobel, extended Palade's morphological approaches to the next level of analysis, involving subcellular fractionation and biochemical reconstitution to understand the mechanism of secretory polypeptide synthesis and vectorial translocation (Redman and Sabatini, 1966; Dobberstein and Blobel, 1975). However, as of the end of the 1970s, little progress had been made in identifying proteins required for the organization and execution of the secretory pathway.

Beisson and colleagues developed a genetic approach to the discovery of proteins required for secretion to investigate mucocyst discharge in Paramecium (Beisson et al., 1976). Unfortunately, rudimentary and cumbersome genetic and molecular cloning techniques limited the utility of this system. However, Saccharomyces cerevisiae was known to secrete glycoproteins (Van Rijn et al., 1972), and some intracellular organelles characteristic of a mammalian style secretory system had been visualized (Linnemans et al., 1977). Thus, a genetic approach in yeast, analogous to that used to dissect the cell division cycle (Hartwell, 1974), seemed appropriate to begin to investigate molecular aspects of the secretory pathway.

We assumed that a clever genetic selection procedure would be necessary to identify rare, secretion-selective mutations. Unfortunately, neither of us knew much genetics, but our initial effort suggested that such mutants would not be so rare. In 1979, we published our first report on the isolation of a pleiotropic secretion mutant sec1 (Novick and Schekman, 1979). sec1 mutant cells accumulated secretory proteins and vesicles at the expense of cell wall and plasma membrane expansion. However, the procedure used in the isolation of this mutant, from a relatively small collection of temperature-sensitive growth mutants, was not exactly as we had described in that paper. The seemingly clever idea was to select against cells expressing an inducible plasma membrane permease, the SO_4^{2-} permease, which was known to actively concentrate chromate, a toxic mimic of sulfate. Mutagenized cells were incubated at a restrictive temperature, induced for sulfate permease, and then incubated overnight in the presence of chromate in an effort to kill cells competent in the cell surface

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expression of the permease. sec1 emerged from this selection, but the procedure was abandoned when we found, in a reconstruction experiment, that sec1 mutant cells were actually killed more efficiently than wild-type cells during the prolonged incubation at 37°C in medium containing chromate. Apparently, the secretory block imposed at the restrictive temperature is enough to kill a sec mutant cell even though it does not take up chromate.

Given that sec1 was isolated despite the "antiselection" procedure, we figured that a random screen would be preferable, so a set of ~100 ts isolates was surveyed for the accumulation of an enlarged intracellular pool of two secretory enzymes, invertase and acid phosphatase. This resulted in one more sec mutant, but the procedure was tedious, so we resumed the hunt for an effective selection procedure.

A couple of years earlier, Susan Henry, at Albert Einstein Medical School, had documented a tight coupling between net cell surface growth and inositol metabolism, suggesting that plasma membrane enlargement and phosphoinositide production were inextricably linked. She showed that an auxotrophic strain starved for inositol developed a substantial increase in buoyant density in relation to wild-type or unstarved cells (Henry et al., 1977). Although we found that this condition does not block secretion, the behavior of sec1 seemed similar in respect to the halt in net surface growth at a restrictive temperature.

This idea set the stage for a dramatic experiment, which is depicted in Figure 1 of Novick et al. (1980). A 100-fold excess of wild-type cells was mixed with sec1 mutant cells and the mixture incubated at 37°C for 3 hr, followed by sedimentation on a gradient of Ludox, a commercial floor polish that had been adapted for the purpose of cell separations. Although this density gradient material was not commercially available, we found a local supply at the Protex Wax Manufacturing Co., a business in downtown Oakland that sold Ludox in 55-gallon drums! Fortunately, they parted with a 5 gal. sample, portions of which we cleaned up by passing samples over an activated charcoal column. Subsequently, Percoll, a coated and reagent-grade form of this material, was produced and is now used widely in cell and organelle fractionation. In any case, Ludox gradients resulted in a substantial separation of the wild-type and sec1 mutant cells, and it became clear that the gradient was a viable means of enriching new sec mutants.

Through the repeated application of this approach, and with the skilled genetic advice of our lab technician, Charles Field, we collected nearly 200 mutant isolates that defined 23 complementation groups. Like sec1, all the mutants accumulate enzymatically active invertase and acid phosphatase, in most cases in a pool that is reversibly returned to the secretory pathway when cells are cooled to a permissive temperature. Most of the mutants affect the steady-state level of a membrane organelle, in particular, the endoplasmic reticulum and secretory vesicles.

Some of the mutants had an anomalous effect on

Table 1. The Function of the Original SEC Genes

Gene	Gene Product Function	Transport Stage	Reference
SEC1	SNARE binding protein	Fusion of secretory vesicles to PM	Carr et al., 1999
SEC2	Exchange protein for Sec4p	Delivery of secretory vesicles to PM	Walch-Solimena et al., 1997
SEC3	Component of exocyst complex	Tethering of secretory vesicles to PM	TerBush et al., 1996
SEC4	Rab GTPase	Delivery of secretory vesicles to PM	Salminen and Novick, 1987
SEC5	Component of exocyst complex	Tethering of secretory vesicles to PM	TerBush et al., 1996
SEC6	Component of exocyst complex	Tethering of secretory vesicles to PM	TerBush et al., 1996
SEC7	Exchange protein for Arf	Vesicle budding from Golgi	Sata et al., 1998
SEC8	Component of exocyst complex	Tethering of secretory vesicles to PM	TerBush et al., 1996
SEC9	t-SNARE	Fusion of secretory vesicles to PM	Brennwald et al., 1994
SEC10	Component of exocyst complex	Tethering of secretory vesicles to PM	TerBush et al., 1996
SEC11	Component of signal peptidase	Exit of certain proteins from ER	Böhni et al., 1988
SEC12	Exchange protein for Sar1p	Vesicle budding from ER	Barlowe and Schekman, 1993
SEC13	Component of COP II coat	Vesicle budding from ER	Barlowe et al., 1994
SEC14	PI/PC transfer protein	Vesicle budding from Golgi	Bankaitis et al., 1990
SEC15	Component of exocyst complex	Tethering of secretory vesicles to PM	TerBush et al., 1996
SEC16	Scaffold protein at ER exit site	Vesicle budding from ER	Espenshade et al., 1995
SEC17	SNARE recycling, α-SNAP	Vesicle fusion, multiple stages	Griff et al., 1992
SEC18	SNARE recycling, NSF	Vesicle fusion, multiple stages	Wilson et al., 1989
SEC19	Rab recycling, GDI	Vesicle delivery, multiple stages	Garrett et al., 1994
SEC20	Component of tethering complex	Tethering of Golgi to ER vesicles	Lewis et al., 1997
SEC21	Component of COP I coat	Budding of Golgi to ER vesicles	Hosobuchi et al., 1992
SEC22	v-SNARE	Fusion of ER to Golgi vesicles	Lian et al., 1994
SEC23	Sar1 GTPase activating protein; component of COP II coat	Vesicle budding from ER	Yoshihisa et al., 1993; Barlowe et al., 1994

organelle structure. One in particular, sec7, accumulates a ring- or cup-shaped tubule that we called a Berkeley Body in honor of the odd-shaped denizens of Berkeley's famed Telegraph Avenue, as well as certain members of the faculty. Unlike most of the other sec mutants, sec7 cells did not discharge secretory proteins on return to a permissive temperature; thus, the Berkeley Body could not be considered an authentic intermediate in the secretory pathway. Fortunately, subsequent work showed that growth conditions influence the reversibility of the sec7 mutant. Incubation in low glucose allows cells to accumulate invertase reversibly within structures that more closely resemble Golgi stacks (Novick et al., 1981). Double mutant analysis showed that this structure follows the ER and gives rise to mature secretory vesicles, just as had been established by more traditional cytologic analysis by Palade and colleagues in their pioneering studies on the secretory pathway in pancreatic exocrine cells (Palade, 1975).

Three other organelle anomalies have also been clarified by subsequent work. sec11 mutant cells accumulate secretory proteins, though fairly selectively (Novick and Schekman, 1983), but seem not to exaggerate any organelles. Böhni et al. (1988) showed that SEC11 encodes a subunit of the signal peptidase, and in its absence only a subset of transported proteins accumulate unprocessed within the ER. Apparently, the bulk of transported proteins either lack a cleavable signal or are unimpeded in their transport by the failure to remove the signal peptide.

sec19 mutant cells accumulate a smattering of all the major secretory organelles, suggesting that the SEC19 gene product acts at recurring stages along the pathway such that a partial defect creates multiple rate-limiting delays. A resolution of this curiosity came with the discovery by Salminen and Novick of one of the first of the rab family GTP binding proteins and their role in vesicle targeting (1987). In the course of their action, Sec4p and Ypt1p, two members of the family that target vesicles at different stages in the pathway, must be retrieved from a target membrane in a GDP-bound form and be delivered to a forming vesicle where the GTP-bound form sustains a transport event. This recycling requires a GDP dissociation inhibitor (GDI), and Garrett et al. (1994) showed that SEC19 encodes the yeast version of this protein. sec19 mutants fail to recycle Sec4p and Ypt1p, and probably other rab proteins, causing organelles devoid of these essential targeting molecules to build up.

Finally, sec17, sec18, and sec22 accumulate ER tubules as well as clusters of small vesicles, distinctly different in size from the mature vesicles that accumulate in sec1. A refined morphologic analysis of the sec mutants blocked early in the pathway showed that ER-Golgi genes can be classified in two categories: those required for vesicle formation, and those required for vesicle targeting and fusion (Kaiser and Schekman, 1990). SEC17 and SEC18 were shown to encode the yeast versions of α-SNAP and NSF, proteins required for SNARE recycling (Wilson et al., 1989; Griff et al.,

1992). *SEC22* was shown to encode a SNARE protein required for vesicle fusion (Jiang et al., 1995). Thus, mutant alleles of these genes block vesicle fusion, and the system quickly backs up and leads to ER proliferation.

We now know a great deal about each of the genes first identified in this paper (see Table 1). Of course, in the years since this report, many more genes that display a *sec* phenotype have been reported (e.g., Deshaies and Schekman, 1987; Newman and Ferro-Novick, 1987; Wuestehube et al., 1996). At this point, the level of the analysis has moved well beyond a descriptive phase, and much of the excitement focuses on mechanistic and atomic-level resolution of Sec protein structure and function. We were most fortunate to be in a position to begin this journey, and it has been a privilege to witness the pathway revealed in its molecular detail through the efforts of laboratories around the world.

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