The signal hypothesis, that specific sequences at the N terminus of newly synthesized proteins direct their translocation across cell membranes, was first proposed in 1971 (1), with experimental support for the eukaryotic endoplasmic reticulum in 1975 (2). A spate of papers in the late 1970s explored the implications of this hypothesis for protein translocation in different cell types and across different biological membranes. For bacteria, one of the most important developments was a paper by Bassford et al. published in the Journal of Bacteriology in 1979 (3). This paper was one of a series of studies in which the authors used gene fusions to probe the factors controlling protein export across the plasma membrane and into the periplasm of Escherichia coli.

Bassford et al. made elegant use of the enzyme β-galactosidase, a native inhabitant of the E. coli cytoplasm, as their reporter, fusing the lacZ gene that encodes the enzyme to portions of the malE gene encoding maltose-binding protein (MBP) (3). MBP is a native inhabitant of the E. coli periplasm, exported from the cytoplasm by the system now known as the Sec translocase (4) but then uncharacterized. The aim was to determine which parts of malE are required to successfully direct β-galactosidase to the periplasm, where its enzymatic activity could be detected in periplasmic extracts. Unexpectedly, the authors found that constructs containing substantial N-terminal portions of MBP were not translocated to the periplasm but instead remained associated with the plasma membrane. Furthermore, strong expression of these constructs was lethal, most likely because the constructs clogged up the limited number of translocation sites, thus preventing the export of essential periplasmic proteins (3, 5). The incomplete translocation of recombiant proteins and the limited capacity of the translocation and secretion apparatus are among the key limitations to the use of bacterial recombiant protein secretion for biotechnology (6). The factors that prevent or permit full LacZ translocation to the periplasm remain under investigation to this day (7).

A crucial immediate development from Bassford et al. (3) was the identification of multiple factors required for the operation of the Sec translocation system. The fact that strong, maltose–induced expression of the fusion proteins was lethal allowed a genetic screen for maltose resistance, with selection of mutants deficient in the interaction of the fusion protein with the translocation apparatus. This principle led to the isolation and characterization of the first mutants in the MBP signal sequence (8), leading to understanding of the features of the N-terminal signal peptide that are required for recognition by the translocation apparatus. Then in 1981, Oliver and Beckwith (9) used one of the fusion strains as the starting point for a genetic screen for components of the translocation apparatus itself. They reasoned that mutants defective in components of translocation apparatus would leave fully folded MBP-LacZ fusions in the cytoplasm, resulting in enhanced β-galactosidase activity which would allow a positive screen for lactose metabolism (9). This strategy led to the identification of genes for two cytoplasmic members of the translocation apparatus. These were first secA (9, 10) and then secB (11), genes encoding, respectively, the translocation ATPase and the chaperone that prevents premature folding of the preprotein (4). Later the same approach led to the identification of SecDF (12, 13), a membrane protein complex that is another member of the supporting cast for the Sec translocase (4). Thus, the pioneering study of Bassford et al. (3) was a starting point for characterization of the bacterial Sec translocation system, which is crucial for the construction and maintenance of the cell envelope and is now understood in impressive structural and functional detail (14).

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Address correspondence to c.mullineaux@qmul.ac.uk.
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