This Week's Citation Classic®

von Heijne G. Patterns of amino acids near signal-sequence cleavage sites.
Eur. J. Biochem. 133:17-21, 1983.
[Research Group for Theoretical Biophysics, Department of Theoretical Physics, Royal Institute of Technology, Stockholm, Sweden]

This paper reported a statistical study of the amino-acid sequences of secretory signal peptides. In particular, it demonstrated that only small, uncharged residues are allowed in positions −3 and −1 relative to the site of cleavage between the signal peptide and the mature protein. This observation served as a basis for a scheme that predicts the most likely cleavage site when only the primary sequence of the precursor protein is known. [The *SCI*® indicates that this paper has been cited in over 380 publications.]

Prediction of Cleavage in Secretory Proteins

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As a graduate student in Clas Blomberg's Research Group for Theoretical Biophysics at the Royal Institute of Technology in Stockholm, I had one scientifically very fruitful idea: to brush up my rusty high-school French. A demanding teacher made me subscribe to *La Recherche*, a French popular-science magazine. Flipping through its pages one day, I stumbled across a short piece on protein secretion. It described the classic G. Blobel and B. Dobberstein paper[1] that presented the first full-blown version of the signal hypothesis. A small figure illustrated the main idea: a signal peptide initiating cotranslational protein translocation across the membrane of the endoplasmic reticulum (ER). The hydrophobic signal peptide was shown as somehow squeezing through a likewise hydrophobic membrane, ending up, after cleavage, as a freely soluble peptide in the lumen of the ER.

This didn't make sense to me: a hydrophobic peptide ought to become anchored in the membrane, most likely with its charged amino-terminal end remaining in the cytoplasm. I later found out that this was the essence of the so-called "loop model."[2] Fortunately, I didn't know this at the time, or I would never have been drawn into the field of protein sorting.[3] At any rate, this inspired me to write a paper dealing with the energetics of a polypeptide chain passing through a lipid bilayer.[4]

I then got interested in the primary sequences of secreted proteins, and a study of the then-known signal peptides was a fairly obvious step. Again, I didn't know that this had been done before on smaller collections of sequences,[5] and it turned out that my sample was just the right size for discerning what has later become known as the (−3,−1)-rule for the cleavage site between the signal peptide and the mature protein: only small, uncharged residues are allowed in positions −3 and −1. As it happened, an equally well-cited paper[6] with essentially the same message was published by D. Perlman and H. Halvorson within a few weeks of my paper.

The main reason for the many citations is that genes and cDNAs for secretory proteins represent a large proportion of current DNA-sequencing efforts. The (−3,−1)-rule allows one to make a reasonable prediction of the site of signal peptide cleavage in such proteins. If a few thousand new protein sequences are deduced from their DNA sequences per year, and if, say, 20 percent of these represent secretory proteins, and if a good number of the papers reporting these sequences cite the (−3,−1)-rule, one is bound to end up with quite a few centimeters of *Science Citation Index*® column-space. It is thus simple mass-market effects, rather than profound insight or theoretical sophistication, that marks the success of this *Citation Classic*. As for a moral, I guess that the story underlines the well-documented importance of ignorance and French in all scientific work.

1. **Blobel G & Dobberstein B.** Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* 67:835-51, 1975. (Cited 1,895 times.) [See also: **Blobel G.** Citation Classic. *Current Contents/Life Sciences* 28(11):18, 18 March 1985.]
2. **DiRienzo J M, Nakamura K & Inouye M.** The outer membrane proteins of Gram-negative bacteria: biosynthesis, assembly, and functions. *Annu. Rev. Biochem.* 47:481-532, 1978. (Cited 340 times.)
3. **von Heijne G.** Transcending the impenetrable: how proteins come to terms with membranes. *Biochim. Biophys. Acta* 947:307-33, 1988.
4. **von Heijne G & Blomberg C.** Trans-membrane translocation of proteins: the direct transfer model. *Eur. J. Biochem.* 97:175-81, 1979. (Cited 170 times.)
5. **Austen B M & Ridd D H.** The signal peptide and its role in membrane penetration. *Biochem. Soc. Symposium* 46:235-58, 1981. (Cited 15 times.)
6. **Perlman D & Halvorson H.** A putative signal peptidase recognition site and sequence in eukaryotic and prokaryotic signal peptides. *J. Mol. Biol.* 167:391-409, 1983. (Cited 355 times.)

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