TEST OF SIGNAL PEPTIDE PREDICTION ON TRUNCATED SEQUENCES

We quantify the effect of N-terminal sequence truncation on predictive accuracy, showing the problems arising from signal peptide predictions based on partial ORFs. Nielsen *et al.*(1) state that sig nal peptide prediction on EST sequences when the start codon may not be present, and on sequences with N-terminal TM domains, can result in false positive signal peptide predictions. We developed a test case for this, truncating several known nonsecreted proteins, and known secreted with and without internal TM domains. Our results confirm the Nielson *et al.*statement.

METHODS

Evaluation of Signal Peptide Prediction on Truncated Sequences

To evaluate the effect N-terminal signal peptide truncation has on signal peptide prediction, a data set was developed. Three 125-residue subsequences were created for each of twenty-four full-length, known secreted, protein sequences and twenty-four full-length, known non-secreted protein sequences. One started at the N-terminus, one ten residues in from the N-terminus, and one 20 residues in from the N-terminus.

To evaluate how TM domains near the N-terminus affect signal peptide prediction, a second data set was developed, based on twelve frizzled protein sequences. Frizzled proteins were chosen because they contain both an N-terminal signal peptide and internal TM domains (2). For each protein sequence, an exhaustive set of subsequences 125-residues long were created, starting at offset 50 a.a. from the N-terminus, and continuing in steps of 50 a.a.

TargetP was used to analyze both data sets as above. The TM domain prediction software, TMHMM, was used to locate TM domains in the frizzled proteins. The truncated frizzled protein sequences were also analyzed using the secreted protein identification methods listed below. The 24 protein sequences known to be secreted, 24 known not to be secreted, and 12 frizzled protein sequences are appended to this document.

RESULTS

Effect of N-terminal truncation on prediction

The impact of N-terminal truncation of protein sequences on *ab initio* prediction was tested. Twenty-four known secreted protein sequences were correctly predicted to possess signal peptides by TargetP. When the first 10 N-terminal residues of the 24 sequences were truncated, signal peptides were predicted for only 13 sequences, and the predicted cleavage site remained constant for all but one of the 13 sequences.When the first 20 N-terminal residues were truncated, signal peptides were predicted for only 8% of the proteins; sequences with complete N-termini are required for accurate signal sequence prediction. Twenty-four protein sequences known not to be secreted were predicted to not possess signal peptides by TargetP regardless of N-terminal truncation.

The influence of N-terminal truncation at or near a TM domain on *ab initio* prediction of protein sequences was also studied. Twelve protein sequences possessing an N-terminal signal peptide and multiple TM domains were divided into 127 subsequences, 125 residues in length as described in the methods. Correct signal peptide predictions were obtained for all 12 true N-terminal subsequences. The remaining 115 subsequences,

with N-terminal-truncations, produced 47 incorrect signal peptide predictions and 7 incorrect mitochondrial targeting peptide predictions. In 46 of the 47 incorrect signal peptide predictions, at least one TM domain was predicted to exist in the first 50 residues of the subsequence; N-terminal truncation near a TM domain increases incorrect prediction of signal peptides. When these 127 subsequences were analyzed using our methods for EST analysis, only the 12 subsequences containing the actual signal peptide were selected as putative secreted proteins.

REFERENCES

1. Nielsen, H., Brunak, S., von Heijne, G. (1999) "Machine learning approaches for the prediction of signal peptides and other protein sorting signals" *Protein Engineering*, **12**: 3-9.

2. Adler,P.N., Vinson,C., Park,W.J., Conover,S. and Klein,L. (1990) Molecular structure of frizzled, a Drosophila tissue polarity gene. *Genetics*, **126**, 401-416.

TEST CASE SEQUENCES (fasta format)

A. Known secreted protein sequences, first 125 residues
B. Known nonsecreted protein sequences, first 125 residues
C. Frizzled protein sequences

A. Known secreted sequences

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>FP_ZEBRAFISH_FRIZZLED_10_4
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>FP_ZEBRAFISH_FRIZZLED_10_6
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>FP_FZ_8_PROTEIN_11

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