OPINION PAPER

Bioinformatics as a critical prerequisite to transcriptome and proteome studies

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Abstract

Large-scale genomic studies rely strongly on annotations available in databases to design experimental supports such as arrays or to explain results in term of biological meaning. Most of this information originates from bioinformatic predictions. Their accuracy as well as their relevance to existing biological data are critical in avoiding the misinterpretation of experimental results.

Key words: Bioinformatic predictions, biological data, database, genomic studies, interpretation of results.

Introduction

The increasing number of sequences that are available in databases, a hundred times higher than ten years ago (http://www.ncbi.nlm.nih.gov/Genbank/genbankstats.html), makes the accuracy of sequence annotation a great challenge. By contrast with global analyses of transcriptional activity that aim to scan the genome for potential transcription units (Choudhary et al., 2001; Yamada et al., 2003), transcriptome and proteome studies require the structure and function of genes to be determined precisely. Transcriptome studies need arrays designed to follow the expression of specific collections of genes that must be relevant to the biological question addressed. Proteomic approaches rely on the identification of proteins performed using mass spectrometry either from peptide sequencing or from peptide mass fingerprinting.


This paper will provide some examples of misleading annotations with regard to putative protein function that may cause mistakes either in array design or in data interpretation. Examples will be taken mainly from A. thaliana and from published papers or databases such as Uniprot, NCBI, TAIR, TIGR, and MIPS.

Proteins rich in particular amino acids

Cell wall structural proteins provide interesting examples of poor quality annotation because their sequences are rich in particular amino acids. Three classes of structural proteins have been clearly defined: extensins characterized by the presence of numerous Ser-Pro$_n$ ($n \geq 3$) motifs separated by Tyr-, Lys-, His, and Val-rich regions (Kielszewski and Lamport, 1994); Hydroxyproline/Proline-Rich proteins (H/PRPs) characterized by a high content in Pro and Pro-Pro-X-Y-Lys motifs, where X, Y=Val, Tyr, His, or Glu (Showalter, 1993); and Glycine-Rich proteins (GRPs) characterized by a high content in Gly (up to 70%) organized in repeats of the (Gly-X) motif, where X=Gly,

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Proteins containing several functional domains may be a problem when results of sequence comparison or functional domain search are not carefully interpreted. A first example is the protein encoded by At3g22060. It is presently annotated as a receptor protein kinase related in the NCBI, TAIR, and TIGR databases because it contains a PFAM profile named Domain of Unknown Function DUF 26 (PF01657) usually associated with the protein kinase domain PFAM (PF00069) not present in this protein. The protein has therefore no predictable function at the moment. A second example is that of proteins belonging to a family of curculin-like (mannose-binding) lectins (At1g78850, At1g78860, and At1g16900). It is mentioned in the NCBI, TAIR, TIGR, and MIPS databases that they show low similarity to a Ser/Thr protein kinase of Zea mays (GI: 2598067). This similarity does exist with the curculin-like (mannose-binding) lectin domain (PF01453), but not with the protein kinase domain (PF00069) of the Z. mays protein absent in many members of the lectin family. The same is true for At1g53070 that is annotated as a receptor protein kinase related. The encoded protein has a legume lectin beta domain (PF00139) and no protein kinase domain. The annotation of genes At1g78850 and At1g53070 misled the authors of a proteomic study discussing the presence of putative protein kinases in cell wall preparations (Chivasa et al., 2002; Ndomba et al., 2003). They actually found putative lectins with completely different biological functions.

Conclusion

All the above-mentioned misleading annotations originate from the misinterpretation of sequence comparisons or domain searches. A careful and critical bioinformatic analysis of DNA and/or protein sequences therefore appears to be an absolute requirement before starting a transcriptome analysis or discussing the results from a proteomic analysis. The importance of comparing results obtained with different bioinformatic softwares has been clearly shown in the Aramemnon database which was especially designed to collect integral membrane proteins (http://aramemnon.botanik.uni-koeln.de/) (Schwacke et al., 2003). It integrates data from 11 trans-membrane predictions and 8 signal peptide predictions and illustrates the type of discrepancies that may be observed between the results. Moreover, the relevance of bioinformatic predictions to biological data should be checked whenever possible to prevent mistakes. Biological data have been proved to be essential for the improvement of the quality of genome annotations, as recently shown by the systematic sequencing of full-length cDNAs (Haas et al., 2002), the use of oligonucleotide tiling arrays (Yamada et al., 2003), and proteomics (Choudhary et al., 2001; Borderies et al., 2003).

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References


