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PRINTS and PRINTS-S shed light on protein ancestry

T. K. Attwood[1,2,*], M. J. Blythe[3], D. R. Flower[3], A. Gaulton[1], J. E. Mabey[1], N. Maudling[1], L. McGregor[4], A. L. Mitchell[1,2], G. Moulton[1], K. Paine[3] and P. Scordis[1]

[1]School of Biological Sciences, The University of Manchester, Manchester M13 9PT, UK, [2]EMBL Outstation, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK, [3]Edward Jenner Institute for Vaccine Research, Compton, Newbury, Berkshire RG20 7NN, UK and [4]INSERM Unit 331, Faculty of Medicine R.T.H. Laënnec, Rue G. Paradin, 69372 Lyon cedex 08, France

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ABSTRACT

The PRINTS database houses a collection of protein fingerprints. These may be used to make family and tentative functional assignments for uncharacterised sequences. The September 2001 release (version 32.0) includes 1600 fingerprints, encoding ~10 000 motifs, covering a range of globular and membrane proteins, modular polypeptides and so on. In addition to its continued steady growth, we report here its use as a source of annotation in the InterPro resource, and the use of its relational cousin, PRINTS-S, to model relationships between families, including those beyond the reach of conventional sequence analysis approaches. The database is accessible for BLAST, fingerprint and text searches at http://www.bioinf.man.ac.uk/dbbrowser/PRINTS/.

INTRODUCTION

Fingerprints are groups of motifs observed in sequence alignments; taken together, the motifs characterise the aligned family and hence provide a specific diagnostic signature. Fingerprints thus derive much of their potency from the biological context afforded by matching multiple motifs; this makes them at once more flexible and more powerful than single-motif approaches. The technique further departs from other pattern-matching methods by readily allowing the creation of discriminators at super-family-, family- and sub-family-specific levels. Such a hierarchical approach has been used, for example, to resolve G protein-coupled receptor (GPCR) super-families into their constituent families and receptor sub-types (1), and to classify a variety of channel proteins, transporters and enzymes.

To date, 1600 fingerprints have been developed, manually annotated and deposited in the PRINTS database (2). Overall, the database is still relatively small, largely because the detailed annotation of entries is extremely time-consuming. However, the extent of manually-crafted annotations sets the database apart from the growing number of automatically derived 'family' resources, for which there is no biological documentation and no result validation, and in which family groupings may change between database releases.

PRINTS was originally built as a single ASCII (text) file. However, with the continued growth of the database, maintenance was becoming inefficient and error-prone. We therefore migrated the resource to the PostgreSQL relational database management system (DBMS) in order to both address the maintenance issues and allow more complex queries of the underlying data (3). Here we describe recent progress and new developments with the database, and highlight the power of relational PRINTS (PRINTS-S) in exploring evolutionary relationships that reach beyond the limits of sequence similarity search tools.

SOURCE DATABASE AND SEARCH TOOLS

PRINTS is released in major and minor versions: minor releases reflect updates, bringing the contents in line with the current version of the source database [a SWISS-PROT/TrEMBL composite (4)]; major releases denote the addition of new material to the resource. Major releases are made quarterly, each release including 50 new annotated families. Eight major releases have been made since the last report.

The two main tools available for searching PRINTS are: (i) a BLAST (5) server, which allows similarity searches against 'sequences' matched in the current version of the database (6); and (ii) the FingerPRINTScan suite (7), which allows sequence searches against 'fingerprints' contained in the current release. FingerPRINTScan, now used within the EDITtoTrEMBL (8) package as part of the EBI's automatic protocol to annotate TrEMBL, is a powerful diagnostic tool, affording greater specificity than the BLAST implementation (6).

A particular strength of PRINTS is that its motifs are stored in the form of un-gapped, local sequence alignments. This allows different implementations to be established with alternative scoring methods. Thus, a Blocks-format version of the resource that exploits Blocks scoring methods is available at the Fred Hutchinson Cancer Research Center (9). In addition, the EMOTIF database at Stanford overlays a permissive regular expression approach over PRINTS' multiply-aligned motifs, offering different levels of stringency from which to infer the significance of matches (10).

*To whom correspondence should be addressed at: School of Biological Sciences, The University of Manchester, Manchester M13 9PT, UK. Tel: +44 161 275 5766; Fax: +44 161 275 5082; Email: attwood@bioinf.man.ac.uk

RHODOPSIN family links:

Identifier	Accession	Views
7TM	PR90007	[Fingerprint] [Relations]
GPCRCLAN	PR90006	[Fingerprint] [Relations]
GPCRRHODOPSN	PR00237	[Fingerprint] [Relations]
OPSIN	PR00238	[Fingerprint] [Relations]
RPERETINALR	PR00667	[Fingerprint] [Relations]
PINOPSIN	PR00666	[Fingerprint] [Relations]
OPSINLTRLEYE	PR00578	[Fingerprint] [Relations]
OPSINRH3RH4	PR00577	[Fingerprint] [Relations]
OPSINRH1RH2	PR00576	[Fingerprint] [Relations]
OPSINREDGRN	PR00575	[Fingerprint] [Relations]
OPSINBLUE	PR00574	[Fingerprint] [Relations]
PEROPSIN	PR01244	[Fingerprint] [Relations]
RHODOPSNTAIL	PR00239	[Fingerprint] [Relations]

Figure 1. The rhodopsin family hierarchy depicted by PRINTS-S. The different levels of the hierarchy are colour-coded as follows: red, children; green, siblings; purple, parents; brown, grandparents (or great-grandparents).

RELATIONAL PRINTS

One of the most important developments of the database has been its migration to a relational DBMS. This 'streamlined' version of the database, PRINTS-S (3), reduces redundancy, maintains consistency and facilitates routine maintenance. It also permits more complex queries, and allows us to support both new display and flat-file formats. PRINTS-S is accessible for interactive use via the Web. The interface allows both strict keyword searching and more powerful queries using a combination of regular expressions and logical operators.

A valuable attribute of PRINTS-S is the ability to model relationships explicitly by defining parent–child and sibling relations within, and implied by, the PRINTS family hierarchy (Fig. 1). This means, for example, that members of a clan (a group of families for which there are indications of an evolutionary relationship, but between which there is no statistically significant similarity in sequence) can nevertheless be linked. Thus, it is possible to transcend relationships evident at the sequence level and gain structural insights from a realm beyond the theoretical limits of conventional sequence analysis tools [this is the so-called 'Midnight Zone', the region of identity where sequence comparisons fail completely to detect structural similarities (11)].

As an illustration, consider the relationships encoded in the database for the rhodopsin-like GPCRs shown in Figure 1. The FingerPRINTScan suite has been modified to exploit these relationships in such a way that when we search the database with a query sequence, all child/sibling/parent/grandparent relations between matched fingerprints are revealed. For example, take the result of searching with the sequence of ovine rhodopsin, shown in Figure 2. For each matched fingerprint (RHODOPSIN, GPCRRHODOPSN and OPSIN are the only matches with significant E-values highlighted in the table), the relationships between them are traced back through the family hierarchy to the most remote putative ancestor. Thus, we see that RHODOPSIN is a child of the OPSIN family, which is a child of GPCRRHODOPSN (the rhodopsin-like GPCR superfamily), whose parent is the GPCR clan (which includes the secretin-like receptors, metabotropic receptors, etc.), which is derived from a putative '7TM' architectural ancestor. Such an 'ancestral perspective' is only possible because PRINTS-S models the biological associations between families within an internal relational structure, allowing a hierarchical representation of connections between database entries, including those outside the realm of sequence similarity searches.

RELATED DATABASE DEVELOPMENTS

Another landmark in the evolution of PRINTS has been the international collaborative project to integrate the database with PROSITE (12), profiles (12), Pfam (13) and ProDom (14) within a unified protein family annotation resource. This initiative, known as InterPro (15), which primarily exploits the detailed family annotations provided by PROSITE and PRINTS, aims both to reduce duplication of effort in the laborious, bottle-necking process of annotation, and to facilitate communication between disparate resources. InterPro provides a convenient one-stop shop for the analysis of newly determined sequences and has already found application in the analysis of the *Drosophila* and human genomes.

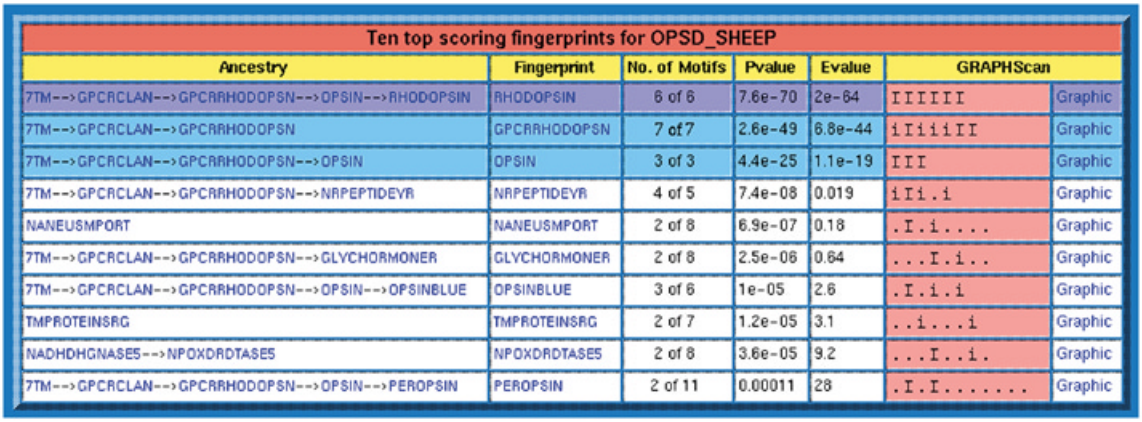
A more recent development is a pilot project to provide an automatic supplement to PRINTS, termed prePRINTS. This exploits an automatic pipeline for sequence alignment, motif detection, iterative database searching and annotation. Interactive versions of parts of the pipeline are also being developed to: (i) allow users to create their own fingerprints for use in conjunction with FingerPRINTScan; and (ii) generate annotation for groups of user-specified sequences. This is PRECIS [Protein Reports Engineered from Concise Information in SWISS-PROT (16); http://bioinf.man.ac.uk/cgi-bin/dbbrowser/precis/precis.cgi]. prePRINTS and its associated tools will ultimately help to increase the family coverage of PRINTS and so improve its effectiveness as a sequence analysis tool.

AVAILABILITY

For local installation, PRINTS flat-files may be retrieved from the anonymous FTP servers at Manchester (ftp://ftp.bioinf.man.ac.uk/pub/prints), HGMP-RC (ftp://ftp.hgmp.mrc.ac.uk/pub/database/prints), EBI (ftp://ftp.ebi.ac.uk/pub/databases), EMBL (ftp://ftp.embl-heidelberg.de) and NCBI (ftp://ncbi.nlm.nih.gov).

CONCLUSIONS

PRINTS is an evolving resource and the new developments help to increase its utility as a tool for protein sequence analysis and genome annotation. In addition, PRINTS-S sheds light on evolutionary relationships between families that were formerly hidden in PRINTS. Together, PRINTS and PRINTS-S are thus complementary tools that facilitate genome annotation, and add greater depth to sequence analyses by offering new ancestral perspectives on protein family relationships.



Ten top scoring fingerprints for OPSD_SHEEP

Ancestry	Fingerprint	No. of Motifs	Pvalue	Evalue	GRAPHScan	
7TM-->GPCRCLAN-->GPCRRHODOPSN-->OPSIN-->RHODOPSIN	RHODOPSIN	6 of 6	7.6e-70	2e-64	IIIIII	Graphic
7TM-->GPCRCLAN-->GPCRRHODOPSN	GPCRRHODOPSN	7 of 7	2.6e-49	6.8e-44	iIiiiII	Graphic
7TM-->GPCRCLAN-->GPCRRHODOPSN-->OPSIN	OPSIN	3 of 3	4.4e-25	1.1e-19	III	Graphic
7TM-->GPCRCLAN-->GPCRRHODOPSN-->NRPEPTIDEVR	NRPEPTIDEVR	4 of 5	7.4e-08	0.019	iIi.i	Graphic
NANEUSMPORT	NANEUSMPORT	2 of 8	6.9e-07	0.18	.I.i....	Graphic
7TM-->GPCRCLAN-->GPCRRHODOPSN-->GLYCHORMONER	GLYCHORMONER	2 of 8	2.5e-06	0.64	...I.i..	Graphic
7TM-->GPCRCLAN-->GPCRRHODOPSN-->OPSIN-->OPSINBLUE	OPSINBLUE	3 of 6	1e-05	2.6	.I.i.i	Graphic
TMPROTEINSRG	TMPROTEINSRG	2 of 7	1.2e-05	3.1	..i...i	Graphic
NADHDHGNASE5-->NPOXDRDTASE5	NPOXDRDTASE5	2 of 8	3.6e-05	9.2	...I..i.	Graphic
7TM-->GPCRCLAN-->GPCRRHODOPSN-->OPSIN-->PEROPSIN	PEROPSIN	2 of 11	0.00011	28	.I.I.....	Graphic

Figure 2. Result of searching PRINTS-S with the sequence of ovine rhodopsin using FingerPRINTScan. The table shows the top 10 matches (significant matches are highlighted, the best is coloured purple), and traces the relationships between each matched fingerprint from its position in the familial hierarchy back to its most distant ancestor. Here, each rhodopsin-like GPCR match can be traced back through its parent super-family, through the ancestral GPCR clan, ultimately to a presumed '7TM' architectural predecessor.

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