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PERSPECTIVE

Pathway databases and tools for their exploitation: benefits, current limitations and challenges

Anna Bauer-Mehren, Laura I Furlong* and Ferran Sanz

Research Unit on Biomedical Informatics (GRIB), IMIM-Hospital del Mar, Universitat Pompeu Fabra, Barcelona Biomedical Research Park, Dr Aiguader 88, Barcelona, Spain
* Corresponding author. Research Unit on Biomedical Informatics, Universitat Pompeu Fabra, IMIM-Hospital del Mar, PRBB, Dr. Aiguader 88, 08003 Barcelona, Spain. Tel.: +34 9331 60521; Fax: +34 9331 60550; E-mail: lfurlong@imim.es

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In past years, comprehensive representations of cell signalling pathways have been developed by manual curation from literature, which requires huge effort and would benefit from information stored in databases and from automatic retrieval and integration methods. Once a reconstruction of the network of interactions is achieved, analysis of its structural features and its dynamic behaviour can take place. Mathematical modelling techniques are used to simulate the complex behaviour of cell signalling networks, which ultimately sheds light on the mechanisms leading to complex diseases or helps in the identification of drug targets. A variety of databases containing information on cell signalling pathways have been developed in conjunction with methodologies to access and analyse the data. In principle, the scenario is prepared to make the most of this information for the analysis of the dynamics of signalling pathways. However, are the knowledge repositories of signalling pathways ready to realize the systems biology promise? In this article we aim to initiate this discussion and to provide some insights on this issue.
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Introduction

The past decades of research have led to a better understanding of the processes involved in cell signalling. Cell signalling refers to the biochemical processes using which cells respond to cues in their internal or external environment (Alberts *et al*, 2007). With the advent of high throughput experimentation, the identification and characterization of the molecular components involved in cell signalling became possible in a systematic way. In addition, the discovery of the connections between each of these components promoted the reconstruction of the chain of reactions, which subsequently gives rise to a signalling pathway. Ultimately, our ability to interpret the function and regulation of cell signalling pathways is crucial for understanding the ways in which cells respond to external cues and how they communicate with each other.

In this regard, the systematic collection of pathway information in the form of pathway databases and the application of mathematical analysis for pathway modelling are crucial. Several databases containing information on cell signalling pathways have been developed in conjunction with methodologies to access and analyse the data (Suderman and Hallett, 2007). Furthermore, mathematical modelling emerged as a solution to study the complex behaviour of networks (Alves *et al*, 2006; Fisher and Henzinger, 2007; Karlebach and Shamir, 2008). The models, so far obtained, allow formulating hypothesis that can be tested in the laboratory. Iterative cycles of prediction and experimental verification have resulted in the refinement of our knowledge of cell signalling, and have shed light on different aspects of cell signalling at a systems level (regulatory aspects, such as feedback control circuits or architectural features, such as modularity).

Furthermore, signalling cascades are not isolated units within the cell, but form part of a mesh of interconnected networks through which the signal elicited by an environmental cue can traverse (Yaffe, 2008). Ultimately, each cell is exposed to a variety of signalling cues, and the specificity of the response will be determined by the signalling mechanisms that are activated by the cue (Alberts *et al*, 2007). Recent research highlights the importance of the, so called, crosstalks between pathways, such as the recently published connections between signalling through the purinergic receptors and the Ca^{2+} sensing (Chaumont *et al*, 2008); the link between extracellular glycocalyx structure and nitric oxide signalling pathway (Tarbell and Ebong, 2008); the interactions between insulin and epidermal growth factor signalling (Borisov *et al*, 2009) and the crosstalk between phosphoinositide 3 kinase and Ras/extracellular signal-regulated kinase signalling pathways (Wang *et al*, 2009).

An important goal of this research is to achieve a reconstruction of the network of interactions that gives rise to a signalling pathway in a biologically consistent and meaningful manner that in turn allows the mathematical analysis of the emerging properties of the network. In this regard, comprehensive maps of signalling pathways have been developed by manual curation from literature (Oda *et al*, 2005; Oda and Kitano, 2006; Calzone *et al*, 2008). Building such

reference maps requires huge effort and would benefit from information stored in databases and from automatic retrieval and integration methods. Once a reconstruction of the network of interactions is achieved, analysis of the structural features of the network and its dynamic behaviour can take place. A commonly seen architecture of signalling pathways is called 'bow-tie', in which many input and output signals are handled by a common layer constituted by a small number of conserved components. This network architecture provides robustness and flexibility to a variety of external cues due to the redundancy of reactions that are part of the input and output layers (Kitano, 2007a). Robustness refers to the ability of an organism to compensate the effects of perturbations to maintain the organism's functions (Kitano, 2007b). Such perturbations can be changes in the availability of nutrients as well as the presence of mutagens or toxins. Moreover, systems can be subjected to functional disruptions when facing perturbations for which they are not optimized, thus showing points of fragility of the biological system (Kitano, 2007b). For instance, an undesired effect of a drug can be caused by the unwanted interaction of the drug with molecules that represent points of fragility of the physiological system (Kitano, 2007a). In contrast, drugs can be completely ineffective when the robustness of the system compensates their action. It has been suggested that crosstalks between signalling pathways contribute to the robustness of cells against perturbations (Kitano, 2007a). In addition, the points of fragility of the system are sometimes exploited by pathogens causing diseases, or represent processes that are usually found to malfunction in particular diseases, such as cancer. Diseases that arise from dysfunction in cell signalling are usually not attributed to a single gene but to the failure of emerging control mechanisms in the network. It has been reported that the loss of negative feedback loops characterizes solid tumours (Amit *et al*, 2007). These diseases are difficult to diagnose and treat unless accurate understanding of the underlying principles regulating the system is in place. Thus, the interpretation of the global properties of signalling pathways has important implications for the elucidation of the mechanisms that lead to complex diseases, and also for the identification of drug targets.

At present, there are several repositories of information on cell signalling pathways that cover a wide range of signal transduction mechanisms and include high quality data in terms of annotation and cross references to biological databases. In principle, the scenario is prepared to make use of the information for the analysis of the behaviour of the signalling pathways. Thus, are the knowledge repositories on signalling pathways ready to realize the systems biology promise? In this article, we aim to initiate this discussion and to provide some insights on this issue.

First, we present an analytical overview of current pathway databases (see Pathway databases). In section 'Case study: EGFR signalling', we present the results of an evaluation exercise conducted to determine the accuracy and completeness of current pathway databases in front of an expert-curated pathway used as 'gold standard'. Moreover, we propose a strategy for the use of pathway data from public databases for network modelling (Box 1; Table I). Finally, in the section 'Conclusions and perspectives' we discuss the strengths and limitations of the current pathway databases and their usefulness in practical biological problems and applications.

Pathway databases

Pathway databases serve as repositories of current knowledge on cell signalling. They present pathways in a graphical format comparable to the representation in text books, as well as in standard formats allowing exchange between different software platforms and further processing by network analysis, visualization and modelling tools. At present, there exist a vast variety of databases containing biochemical reactions, such as signalling pathways or protein–protein interactions. The Pathguide resource serves as a good overview of current pathway databases (Bader *et al*, 2006). More than 200 pathway repositories are listed, from which over 60 are specialized on reactions in human. However, only half of them provide pathways and reactions in computer-readable formats needed for automatic retrieval and processing, and even less support standard formats, such as Biological Pathway Exchange (BioPAX) (http://www.biopax.org) and Systems Biology Markup Language (SBML) (Hucka *et al*, 2003).

To obtain a complete view of the biological process of interest, combination of information from diverse reactions and pathways is often needed. A recent publication (Adriaens *et al*, 2008), describes a workflow developed for gathering and curating all information on a pathway to obtain a broad and correct representation. However, the described process heavily relies on manual intervention. Consequently, there is a need for the automation of both the pathway retrieval process and the integration of different data sources. This section is devoted to the description of main pathway databases: Reactome, Kyoto Encyclopedia of Genes and Genomes (KEGG), WikiPathways, Nature Pathway Interaction Database (PID) and Pathway Commons. Table II lists all pathway databases and protein–protein interaction resources that are mentioned in this section.

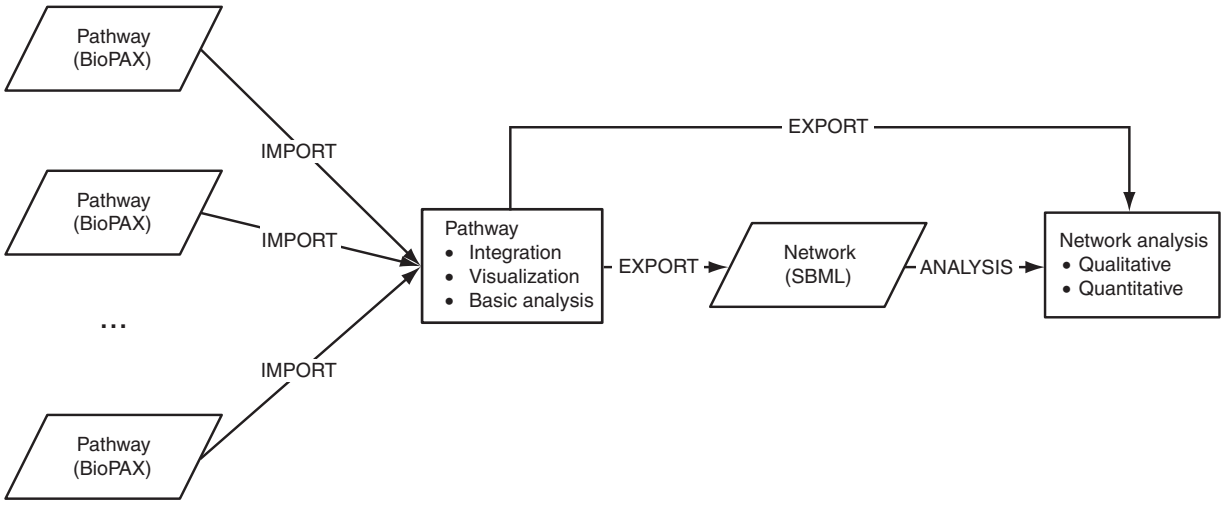
Reactome

Reactome is currently one of the most complete and best-curated pathway databases. It covers reactions for any type of biological process and organizes them in a hierarchal manner. In this hierarchy, the lower level corresponds to single reactions, whereas the upper level represents the pathway as a whole.

Reactome was first developed as an open source database for pathways and interactions in human. Equivalent reactions for other species are inferred from the human data (Vastrik *et al*, 2007), providing coverage to 22 non-human species, including mouse, rat, chicken, puffer fish, worm, fly, yeast, and *Escherichia coli*. Furthermore, other Reactome projects exist focusing on single species, such as the *Arabidopsis* Reactome (http://www.arabidopsisreactome.org).

All pathway and reaction data in Reactome are extracted from biomedical experiments and literature. For this purpose, PhD-level biologists are invited to work together with the Reactome curators and editors on the curation of data on selected biological processes. Once the first outline of the

Box 1 Use of data from public pathway databases for modelling purposes



Box 1 Most public, available pathway databases offer their data in BioPAX format, which was developed for detailed pathway representation and as data exchange format. For storing and sharing of computational models of biological networks, SBML has emerged as standard and is supported by most modelling software. BioPAX and SBML, the two main standards for the representation of biological networks, have been discussed in detail by others (Stromback and Lambrix, 2005; Stromback *et al*, 2006). In Table I, we briefly list the most important features of the SBML and BioPAX standards. A scenario in which pathway data were directly used for network modelling is proposed here. One or more pathways represented in BioPAX format are automatically retrieved from different databases and imported into a pathway visualization and analysis tool. Then, integration of the different pathways can take place to obtain a comprehensive and biologically meaningful representation of the network. In addition, annotations can be added if required or structural analysis of the network can be carried out. The resulting network, which integrates the original pathways retrieved from the databases, is exported to SBML format and subjected to modelling. If a quantitative approach is chosen, additional information, such as rate constants are required to start the modelling process. In this process, conversion between the two formats is required to achieve inter-operability between pathway and model representations. Some solutions are already available. The BioModels (http://www.ebi.ac.uk/biomodels-main/) database, which contains a variety of curated models in SBML format, offers conversion to BioPAX format. The opposite conversion, from BioPAX to SBML, would open the possibility of modelling the pathways stored in public databases. However, the inter-conversion between BioPAX and SBML is not trivial as both formats where developed for different purposes. BioPAX, for instance, does not offer the possibility to store quantitative information needed for kinetic modelling, whereas SBML does not represent relationships between nodes that are not needed for modelling and that are present in BioPAX. Examples of approaches for the conversion from BioPAX to SBML are BiNoM (Zinovyev *et al*, 2008), which is available as Cytoscape plugin, and SyBil, which is part of the model environment for quantitative modelling VCell (Evelo, 2009). Although compatibility of different pathway and network model exchange formats is still not completely achieved, the efforts made towards this goal represent significant contributions to pathway retrieval, integration and subsequent modelling.

biological process is created and annotated, it is inspected by peer reviewers and potential inconsistencies and errors are fixed. Every two years the data are reviewed to keep it updated (Joshi-Tope *et al*, 2005; Matthews *et al*, 2009). Moreover, cross references to different databases, such as UniProt (The UniProt Consortium, 2008), Ensembl (http://www.ensembl.org/index.html), NCBI (http://www.ncbi.nlm.nih.gov), Gene Ontology (GO) (Ashburner *et al*, 2000), Entrez Gene (Maglott *et al*, 2007), UCSC Genome Browser (http://genome.ucsc.edu), HapMap (http://www.hapmap.org), PubMed, as well as to other pathway databases, such as KEGG (Kanehisa and Goto, 2000) are provided.

Pathways are presented as chains of chemical reactions and the same data model is used to describe reactions for any biological process, such as transcription, catalysis or binding (Matthews *et al*, 2007). Altogether, this represents a coherent view of pathway knowledge. The data model is based on classes, such as physical entity or event. Physical entities comprise proteins, DNA, RNA, small molecules but also complexes of single entities. Proteins, RNA and DNA, for which the sequence is known, are linked to the appropriate databases. Chemical entities such as small molecules are linked to ChEBI (http://www.ebi.ac.uk/chebi/init.do). An event can be either a *ReactionLikeEvent*, which represents reactions that convert an input into an output, or a *PathwayLikeEvent*, grouping together several related events. Each class possesses properties, such as information on the type of interaction (e.g. inhibition or activation). Reactome explicitly considers the different states an entity can show in a reaction. The phosphorylated and the unphosphorylated version of a protein are, for example, represented as separate entities. In addition, generalization is allowed. This means that if two different entities have exactly the same function in a reaction, such as isoenzymes, the reaction is only described once and the functional equivalent entities belong to the same *defined set*. Another interesting element of the Reactome data model is the use of *candidate sets*, which act as placeholders for all possible entities in a reaction, in case the exact entity involved in the reaction is not yet known.

Reactome can either be directly browsed or queried by text search using, for instance, UniProt accession numbers. In addition, some tools for advanced queries are provided. The *PathFinder* tool allows connecting an input to an output molecule or event by constructing the shortest path between both. The *SkyPainter* tool can be used to identify events or pathways that are statistically over-represented for a list of genes or proteins. Moreover, Reactome data can be combined

Table I Comparison between SBML and BioPAX

	SBML	BioPAX
Representation format	XML (Extensible Markup Language)	OWL (Web Ontology Language), XML
Main purpose	Representation of computational models of biological networks	Pathway description with all details on reactions, components, information on cellular location etc.
Entities and reactions	Based on species and reactions (Hucka *et al*., 2003): Species (proteins, small molecules etc.) Reactions (how species interact) Compartment (in which interactions take place)	Basic ontology based on three classes (http://www.biopax.org/): Pathway (set of interactions) Physical entity with subclasses, such as RNA, DNA, protein, complex and small molecules Interaction with subclasses, such as conversion having biochemicalReaction as subclass, etc.
Number of pathways represented	One model per SBML file	Several pathways per BioPAX file possible (each object has its own RDF id and is hence uniquely identifiable)
Reaction kinetics	Allows representation of kinetics, including parameters for reaction rates, initial concentrations etc.	No kinetics as BioPAX is not meant for modelling but pathway representation
Levels	Built in levels with different versions. Each level adds new features, such as the incorporation of controlled vocabularies. At the time of writing, the most stable version is SBML Level 2	BioPAX Level 1: representation of chemical reactions involved in metabolism BioPAX Level 2: adds molecular interactions and protein post-translational modifications BioPAX Level 3: any kind of biological reaction, including regulation of gene expression (BioPAX L3 is at the time of writing still in release process) The BioPAX project roadmap envisages two additional levels capturing interactions at the cellular level. (http://www.biopax.org/Docs/BioPAX_Roadmap.html)
Pathway database support	Reactome KEGG	Reactome KEGG (only BioPAX Level 1) PID PathwayCommons
Model database support	BioModels	BioModels (conversion from SBML to BioPAX possible)
Library for reading/writing	libSBML(Bornstein *et al*, 2008)	Paxtools (http://www.biopax.org/paxtools/)
Software support	Standard modelling software, such as CellDesigner or Copasi (Hoops *et al*, 2006) Network visualization software, such as Cytoscape	Network visualization software, such as Cytoscape or VisANT

with other databases such as UniProt, by using the Reactome *BioMart* (http://www.biomart.org) tool.

In addition to browsing pathways through the Reactome web interface, it is possible to download the data for local visualization and analysis using other tools. Different formats are provided for pathway download, including SBML Level 2, and BioPAX Level 2 and Level 3 (for some reactions only), as well as graphical formats. Pathway files, for instance, in BioPAX format can be directly opened in Cytoscape (Shannon *et al*, 2003), a software for the visualization and analysis of networks. Moreover, data can be programmatically accessed through a SOAP web service.

KEGG

KEGG is not only a database for pathways but consists of 19 highly interconnected databases, containing genomic, chemical and phenotypic information (Kanehisa and Goto, 2000; Kanehisa *et al*, 2008). Here we concentrate on the database storing biological pathways. KEGG categorizes its pathways into metabolic processes, genetic information processing, environmental information processing, including signalling pathways, cellular processes, information on human diseases and drug development. However, the best-organized and most complete information can be found for metabolic pathways. KEGG is not organism specific but covers a wide range of organisms, including human. The pathways are manually curated by experts using literature. In addition to the interconnection of all databases underlying KEGG, links to external databases, such as NCBI Entrez Gene, OMIM, UniProt and GO are provided. Pathways can either be browsed or queried by free text search. The user can search for gene names, chemical compounds or whole pathways. A tutorial on how to browse pathways in KEGG and an overview of the multiple representation formats is available (Aoki-Kinoshita & Minoru Kanehisa, 2007).

Each pathway stored in KEGG can be downloaded in its own XML format named KGML, which is supported by VisANT, a software tool for pathway visualization (Hu *et al*, 2008b) and indirectly by Cytoscape using scripting plugins. In addition, metabolic pathways are available in BioPAX Level 1, which was especially designed for metabolic reactions, as well as in SBML. For converting KEGG metabolic pathways to SBML, a tool called KEGG2SBML (http://sbml.org/Software/KEGG2SBML) was developed.

KEGG data can also be accessed using the KEGG API or KEGG FTP. Moreover, for making use of the KEGG resources, several applications exist. KegArray, for example, allows the analysis of microarray data in the context of KEGG pathways.

Table II Online pathway and protein–protein interaction (PPI) databases

Pathway/PPI database	Web link	Standard exchange formats for download	Web service API
Reactome	http://www.reactome.org	BioPAX Level 2 BioPAX Level 3 (only some reactions) SBML Level 2	SOAP web service API Detailed user manual available, example client in Java
KEGG	http://www.genome.jp/kegg/pathway.html	KGML (default format)	SOAP web service API
		BioPAX Level 1 (only metabolic reactions)	Example client in Java, Ruby, Perl
		SBML (using converter) GPML (using converter)	Direct import into Cytoscape
WikiPathways	http://www.wikipathways.org	GPML (default format) Converters to standards, such as SBML and BioPAX are in progress	SOAP web service API Example clients in Java, Perl, Python, R
NCI/Nature Pathway Interaction Database (PID)	http://pid.nci.nih.gov	PID XML (default format)	Access through Pathway Commons
		BioPAX Level 2	
BioCarta	http://www.biocarta.com	BioPAX Level 2 through NCI/Nature Pathway Interaction Database (PID)	
Pathway commons	http://www.pathwaycommons.org	BioPAX Level 2 (default format for pathways) PSI-MI (default format for protein–protein interactions)	HTTP URL-based XML web service through cPath Direct import into Cytoscape
Cancer cell map	http://cancer.cellmap.org	BioPAX Level 2	HTTP URL-based XML web service via cPath
HumanCyc	http://humancyc.org	BioPAX Level 2 BioPAX Level 3	Access through Pathway Commons and Pathway Tools (Karp *et al*, 2002)
IntAct	www.ebi.ac.uk/intact/	PSI-MI	Access through Pathway Commons
HPRD	http://www.hprd.org	PSI-MI	Access through Pathway Commons
MINT	http://mint.bio.uniroma2.it/mint/	PSI-MI	Access through Pathway Commons

WikiPathways

A recently developed resource for pathway information that strongly differs from other pathway repositories is WikiPathways. WikiPathways is an open source project based, like Wikipedia, on the MediaWiki software (Pico *et al*, 2008). It serves as an open and collaborative platform for creation, edition and curation of biological pathways in different species.

WikiPathways aims to achieve a public commitment to pathway storage and curation by keeping pathway creation and curation processes simple. Although the curation process of the previously described databases is subjected to experts, any user with an account on WikiPathways can create new pathways, and edit already existing ones.

The pathway entities are linked to reference databases, based on the criteria provided by the editor. Hence, the identifiers depend on the chosen reference database and can therefore differ between pathways and even within a single pathway.

Pathways in WikiPathways can be browsed by species and categories, for example, *Metabolic Process*. They can also be searched using gene, protein or pathway name or any free text query. In addition, pathways can be programmatically accessed through a web service (http://www.wikipathways.org/index.php/Help:WikiPathways_Webservice).

For pathway data exchange, WikiPathways does not use standard formats like BioPAX or SBML, but offers a much simpler representation called GenMAPP Pathway Markup Language (GPML) that is compatible with visualization and analysis tools, such as Cytoscape, GenMAPP (Salomonis *et al*, 2007) and PathVisio (van Iersel *et al*, 2008). The use of GPML is in agreement with the community annotation nature of the project, as it offers a simple pathway representation and several functionalities for building network diagrams. However, inter-operability with other pathway databases is impeded, and substantial efforts towards combining WikiPathways with the other pathway repositories will be required. In this regard, some approaches with the objective of conversion between GPML and standard pathway exchange formats, such as SBML and BioPAX, are under development (Evelo, 2009). In addition, KEGG pathways in KGML format are also available in GPML format ready for download (http://www.pathvisio.org/Download#Step_3) or can be converted into GPML (http://www.bigcat.unimaas.nl/tracprojects/pathvisio/wiki/KeggConverter).

The exponential growth of biological data poses a challenge to the high-quality annotation and curation of databases. In this scenario, the use of wikis for community curation of biological data have emerged in the past years with the goal of increasing quality of data annotation by combining knowledge from multiple experts (Giles, 2007; Waldrop, 2008; Hu *et al*, 2008a). However, their success will strongly depend on the commitment of the community and WikiPathways authors claim that the initiative represents an experiment, in which the 'community curation' approach is being tested (Pico *et al*, 2008). Thus, WikiPathways can be seen as a complementary

and enhancing source of information for the major pathway databases, like Reactome or KEGG.

In contrast to the aforementioned databases, the systems described below combine diverse pathway repositories, and can be seen as first attempts towards the integration of pathway information from various sources.

Nature pathway interaction database

PID contains data on cell signalling in humans (Schaefer *et al*, 2009). PID combines three different sources: the NCI-curated pathways that are obtained from peer reviewed literature, as well as pathways imported from Reactome and BioCarta. Similar to Reactome, PID structures pathways hierarchically into pathways and their sub-pathways that are called sub-networks in PID.

The PID data model is based on molecular interactions in which input biomolecules are transformed into output biomolecules. Each process can be promoted or inhibited by regulators. Biomolecules are proteins, RNA, complexes or small molecules. DNA is not a part of the PID data model and only output RNA and regulator are represented in transcriptional processes. Each protein is cross-referenced to UniProt, RNA to Entrez Gene, small molecules to the Chemical Abstracts Service (CAS) registry number and complexes are annotated using GO terms. Different states of biomolecules, such as 'active/inactive' or 'phosphorylated' are part of the annotations of the biomolecule. Cellular location, biological processes and molecular function of the entities are cross-linked to GO. Moreover, interactions are annotated with the supporting literature or other evidence, such as *inferred from array experiment* (Schaefer *et al*, 2009).

Pathways can be browsed and queried using gene or protein identifiers, such as Entrez Gene identifier, UniProt accession numbers or HUGO gene symbols, as well as biological process terms from GO, among others. The system returns available results from each of the three sources. Moreover, PID offers advanced queries. The *connected molecules search* option allows finding a possible path between two or more molecules. In the *batch query*, the user can upload lists of gene or protein identifiers and obtain a list of pathways ranked by the probability of including the entities of the query list. Using this application, pathways over-represented in a set of genes, for example, derived from microarray expression experiments, can be obtained.

PID provides different pathway representation formats, including BioPAX Level 2 and a PID proprietary XML format. Data from Reactome are directly imported using the BioPAX Level 2 format and is regularly updated. As not all entities or events stored in Reactome can be presented in BioPAX Level 2, some information is lost during the import. However, this might be avoided once BioPAX Level 3 is released. The BioCarta data are manually assigned to the PID data model, as BioCarta only offers a graphical cartoon view of the pathways and does not provide computer readable download format.

Pathway commons

Pathway Commons is a compilation of the public pathway databases Reactome, PID and Cancer Cell Map as well as protein–protein interaction databases, such as HPRD (Mishra *et al*, 2006), HumanCyc, IntAct (Kerrien *et al*, 2007a) and MINT (Zanzoni *et al*, 2002). Herein, the pathway hierarchies of Reactome and PID are conserved.

Pathway Commons serves as an access point for a collection of public databases and provides technology for integrating pathway information. Pathway creation, extension and curation remain the duty of the source pathway databases. As a consequence, entries in Pathway Commons are cross-linked to their source database, and links to external databases rely on the source database.

A regular search is provided and a filter can be set for restricting the results to source and organism. Furthermore, Pathway Commons provides a web service API for an automatic access of the data. In addition, cPath, a Java open-source software for aggregating, storing and querying pathway data, is offered. One of its key features is the identifier mapping system. It handles mapping tables of equivalent entities, such as the UniProt and the RefSeq accession number of proteins. These tables can in principle be used to integrate data from diverse sources that use different identifiers. Moreover, the system can straightforwardly be extended including self-created mapping tables. PSI-MI (Kerrien *et al*, 2007b) and currently BioPAX Level 2 exchange format are supported. Furthermore, the complete Pathway Commons database can be automatically accessed using the Pathway Commons plugin in Cytoscape.

The systems presented above allow the access to a wide range of data on biological pathways. However, there is overlap in the information offered by different databases. In contrast, for specific pathways some databases offer more accurate and complete information than others. Hence, the user might have difficulties in choosing the right database and in dealing with redundancies and inconsistencies among the pathways. The integration initiatives exemplified by PID and Pathway Commons are attempts to solve these problems. However, the intended integration is not trivial as the data are fragmented and stored in databases that may differ in the representation of the biochemical reactions, as well as in the coverage and accuracy of annotations. In addition, often data are not provided in interchangeable formats hampering the automatic integration.

Case study: EGFR signalling

The epidermal growth factor receptor (EGFR) signalling cascade is one of the best-studied and most important signalling pathways in mammals. It regulates cell growth, survival, proliferation and differentiation. Recently, a detailed and comprehensive map of the EGFR signalling pathway has been reported (Oda *et al*, 2005). As the map was built manually by experts using the literature, it can be seen as a reference representation of the pathway. Other reference maps of important signalling pathways have been reported previously (Oda and Kitano, 2006; Calzone *et al*, 2008; Herrgard *et al*, 2008), providing the scientific community with comprehensive maps that can be used for modelling, which in turn will shed light on important aspects of cell signalling. However, these initiatives constitute huge efforts and, as judged by the

limited number of already available maps, there is a lag between the amount of data available in public databases and the availability of such references map. Hence, we argue that public pathway databases could be used to build such reference maps of signalling pathways. Most pathway databases are also developed by experts in the field and constitute repositories of high-quality data, with the additional advantage of being already represented in machine readable formats that could, in principle, be easily and automatically retrieved, analysed and fed into modelling software tools.

We selected the EGFR pathway (Oda *et al*, 2005), hereafter referred to as *EGFR map*, as a 'gold standard' to evaluate the completeness and accuracy of public pathway databases in the representation of the reactions that are part of the EGFR signalling (Figure 1). We based our selection on the following reasons: (i) signalling through EGFR has been studied for more than 40 years and a lot of information about the reactions is already available (Citri and Yarden, 2006); (ii) it has been carefully curated by experts; (iii) it constitutes an excellent example of crosstalk between different signalling events, thus allowing evaluation of the coverage of crosstalks in the public databases and the ability of network analysis tools to retrieve and combine networks in a meaningful manner; (iv) the study of signalling through EGFR has important implications for understanding several cancer types and the development of new therapeutic strategies. Several computational models have been reported on different aspects of EGFR signalling (Kholodenko *et al*, 1999; Schoeberl *et al*, 2002; Hornberg *et al*, 2005; Birtwistle *et al*, 2007; Borisov *et al*, 2009; Li *et al*, 2009). However, it is worth mentioning that, to the best of our knowledge, no model for the whole *EGFR map* has been reported till now.

In the following paragraphs and in Figures 1–3, we use the same notation of entities as in the SBML file of the *EGFR map* (Oda *et al*, 2005). The *EGFR map* is based on more than 240 publications and contains several crosstalks between the EGFR downstream signalling and other signalling pathways. In the *EGFR map*, depicted in Figure 1, entities are clustered according to their cellular location and function. The functional units comprise receptor endocytosis, recycling and degradation, small GTPase signalling, MAPK cascade, PIP signalling, cell cycle, Ca^{2+} signalling and GPCR-mediated transactivation. Seven phenotypic outcomes of EGFR signalling are depicted: ErbB endocytosis, ErbB degradation, apoptosis, actin reorganization, cell cycle, gene transcription and mitogenesis/tumourigenesis.

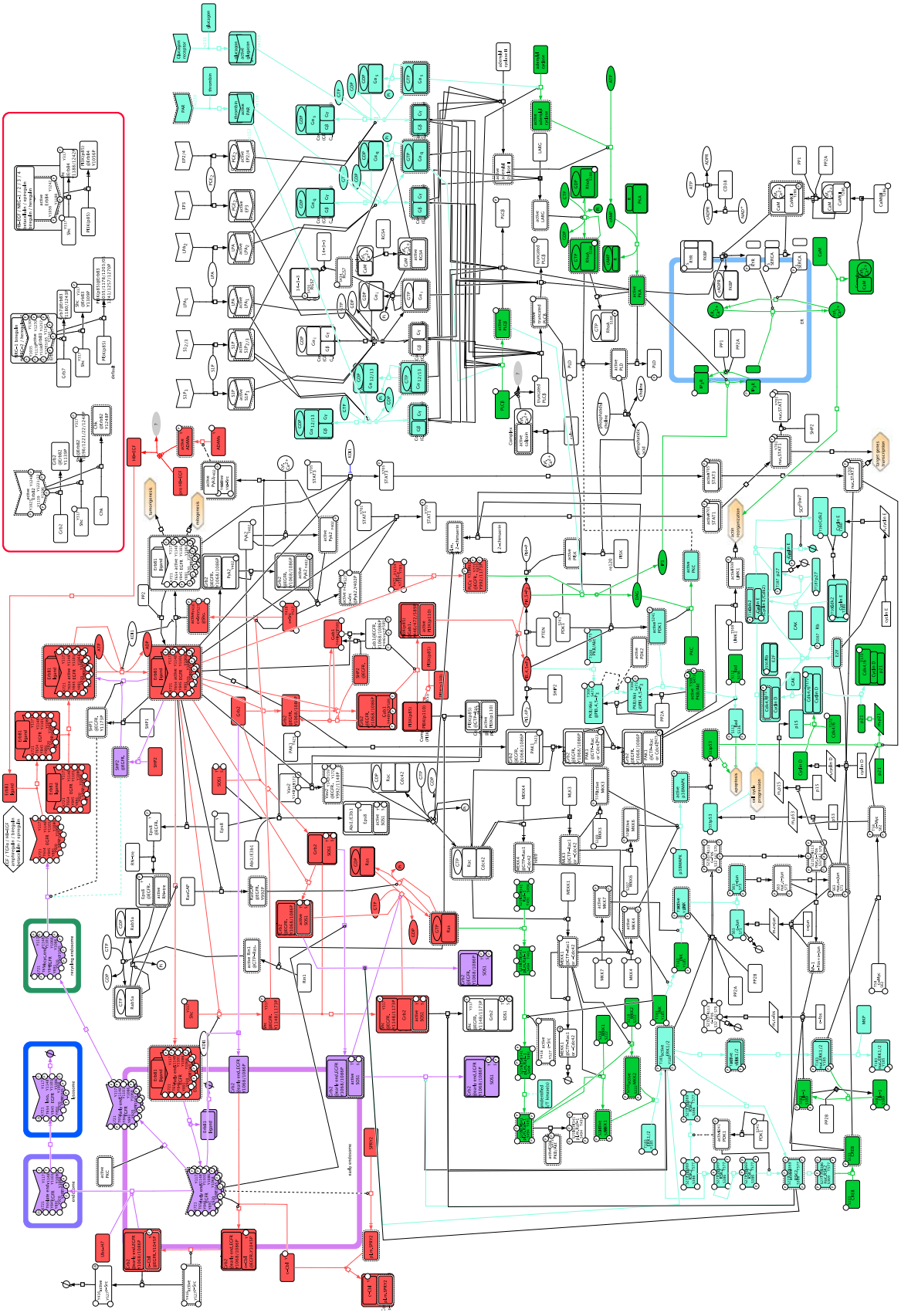
To address the completeness and accuracy of pathway information available in public databases and its automatic retrieval, we tried to recover the complete *EGFR map*. For this purpose we queried Reactome version 26 with the term 'EGFR' and its UniProt identifier 'P00533' and downloaded and visualized the retrieved pathways. Reactome was chosen as it is currently the most detailed pathway repository, and utilizes a data model that accommodates different types of biochemical reactions. For visualization, we chose Cytoscape because of its user-friendly visualization capabilities and its network analysis tools. To map the entities found in Reactome to those in the *EGFR map*, a mapping through standard identifiers was carried out. We compared the original *EGFR map* with the EGFR pathway recovered from Reactome (in BioPAX format) and coloured entities and reactions according to their representation in both resources (see Figure 1). Red entities are found to be identical in the EGFR pathway in Reactome and the *EGFR map*. Purple connotes entities that could be recovered from Reactome but that are differently represented (for instance, if a single protein instead of a complex is described to take part in a reaction).

Only a small proportion of the original EGFR reactions could be recovered from Reactome, and most of them are directly related to signals coming from downstream EGFR signalling. Most of the reactions related to other signalling cascades are not connected with EGFR signalling in Reactome and could therefore not be recovered. Regarding the associated phenotypes, only two were found in Reactome: EGFR endocytosis and EGFR degradation. However, both mechanisms are described in a slightly different manner, in some cases even with more details than in the original *EGFR map*. In a second step, we tried to extend the *EGFR map* by querying Reactome with key entities found in the *EGFR map* to complete signalling cascades, such as the GPCR signalling or the MAPK cascade that are missing in the EGFR pathway in Reactome. All pathways that were added are listed in Table III. We used additional colours to depict the entities that were recovered in this extension process. Green was used for entities found in Reactome and the *EGFR map*, and turquoise was used for entities that differed in their representation in both sources (the coloured *EGFR map* is available in XML and pdf formats as Supplementary information). By this extension, we were able to recover four of the five missing phenotypes: actin reorganization, apoptosis, cell cycle and the transcription of target genes. However, some reactions were still missing in the information recovered from Reactome and in some cases gaps or contradictions appear impeding an automatic integration (Figure 2). In this example, reactions in which ERK1 and ERK2 participate are first separately described and later the representation switches towards a combined ERK1/2 entity.

Regarding the reactions that give rise to regulatory loops in the *EGFR map*, only some of them could be recovered from Reactome. For instance, although the reaction that involves cleavage of pro-HB-EGF by ADAMs is described, its regulation by Pyk2 and c-Src is not included and therefore this positive feedback loop is not coloured in the *EGFR map*. In total, three of the six negative feedback loops were detected: inhibition of EGFR by SHP1, downregulation of EGFR and phosphorylation of SOS1 by ERK1, which leads to SOS1 inhibition.

Although most of the crosstalks between signalling cascades in the EGFR signalling could be established by the extension process, a significant number were not found because the entities that link the different cascades are missing in Reactome. For example, the important crosstalk of the Ca^{2+} and the EGFR signalling by the effect of Ca^{2+} on Pyk2 activity could not be recovered, as Pyk2 is not present in Reactome. Moreover, it is worth mentioning that details about some of the reactions differ between the *EGFR map* and the data found in Reactome. In part, this can be explained by the fact the former is based on literature curated in 2005, and version 26 of Reactome was released in October, 2008.

The extension process was achieved by searching the database with entities representing the main signalling cascades that are known to be connected with the EGFR



signalling, followed by manual identification of the reactions that connect the pathways. In principle, the process of finding the connections or crosstalks between pathways could be automated using tools available in Cytoscape or Pathway Commons (cPath). The Cytoscape merging function was evaluated for this purpose. This function compares the

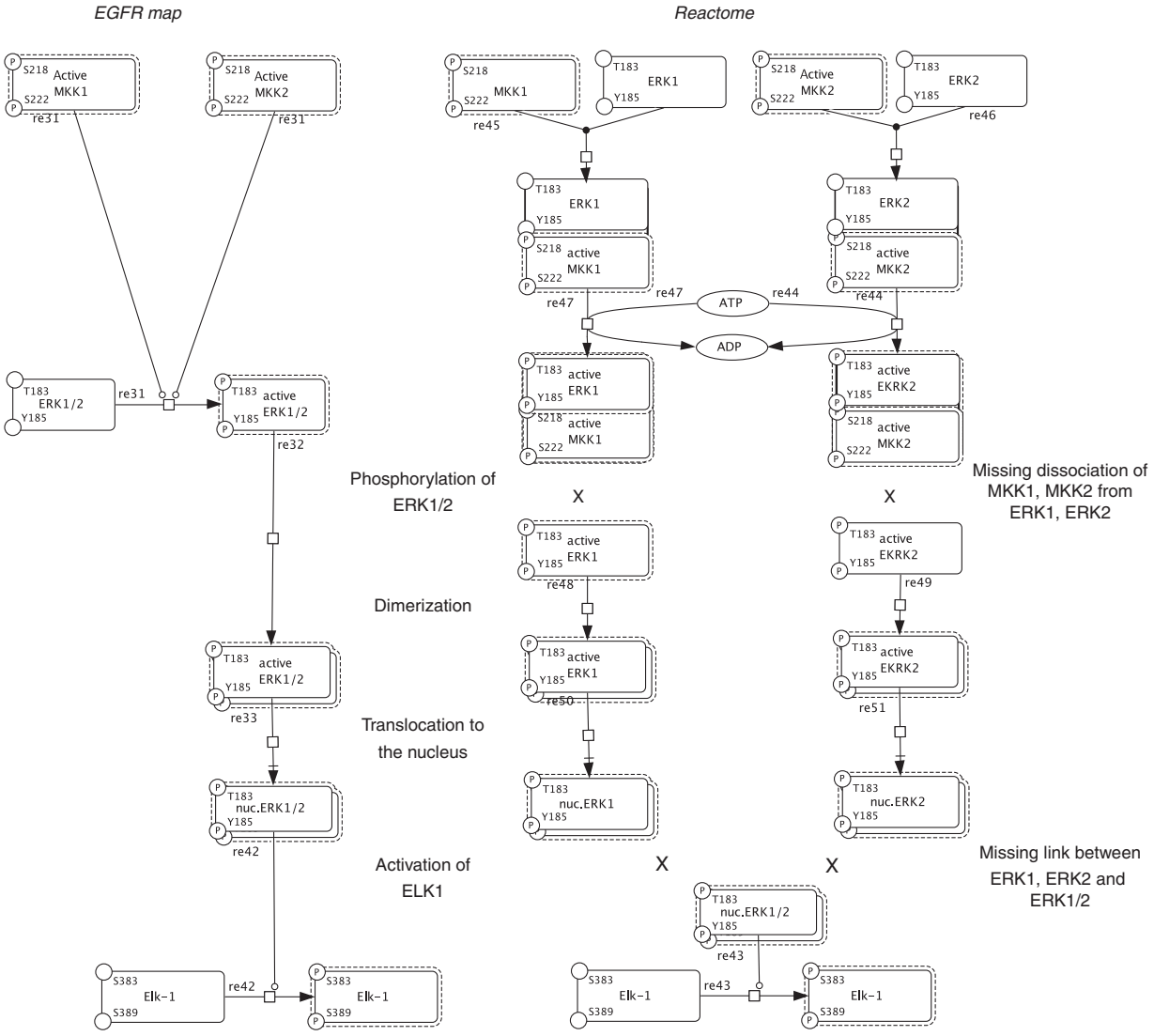


Figure 2 Comparison of ERK signalling as found in the *EGFR map* and in Reactome. In Reactome, ERK1 or ERK2 are phosphorylated by MKK1 or MKK2, respectively. The same reaction is found in the *EGFR map*, but here ERK1 and ERK2 are represented as a single entity, namely ERK1/2, which combines both proteins and can be phosphorylated by MKK1 and MKK2. In Reactome, dimerization and translocation to the nucleus are still separately described for each entity. Then, the representation switches from separate entities to one combined entity. In addition, the dissociation of MKK1 or MKK2, which is needed before the dimerization of ERK can take place, is not described in Reactome. Manual intervention is needed to correctly map both representations.

Figure 1 *EGFR map*. *EGFR map* created using CellDesigner ver.2.0 (Oda *et al*, 2005). This map has been coloured to show the entities and reactions that are in common between the *EGFR map* and information found in Reactome. Red colour denotes that the entities and reactions are equivalent, purple connotes that they are similar but differ in some description details, and white is used for entities and reactions that could not be directly found in Reactome. In a second step, the map was extended by querying Reactome with key entities appearing in the *EGFR map*, which were missing in the representation of the EGFR pathway in Reactome. After this extension process, we coloured new equivalent entities in green and new similar entities (the ones that are differently described in both resources) in turquoise. For comparing the *EGFR map* with the pathways downloaded from Reactome, the Reactome pathways have been imported into Cytoscape and the entities and reactions have been manually compared using the node and edge search functions of Cytoscape. As the SBML version of the *EGFR map* does not contain unique identifiers for the nodes (species), all names have been first matched to Entrez Gene identifiers, which have then been used for comparing entities between the *EGFR map* and Reactome. In the extension process canonical names as well as Entrez Gene and UniProt identifiers have been used to retrieve all the information available in Reactome. In some cases, when no results were obtained in this way, the search was additionally expanded. For example, to find the EGFR crosstalk with the GPCR signalling, we additionally searched for 'G protein'. GPCR signalling pathways activated by $S1P_1$, $S1P_{2/3}$, LPA_1, LPA_2, EP_3 and $EP_{2/4}$ were not found in Reactome. Instead, GPCR signalling through thrombin and glucagon receptors that are related to EGFR signalling (Prenzel *et al*, 1999; Buteau *et al*, 2003) are present in Reactome and were incorporated into the *EGFR map* to complete the missing crosstalk.

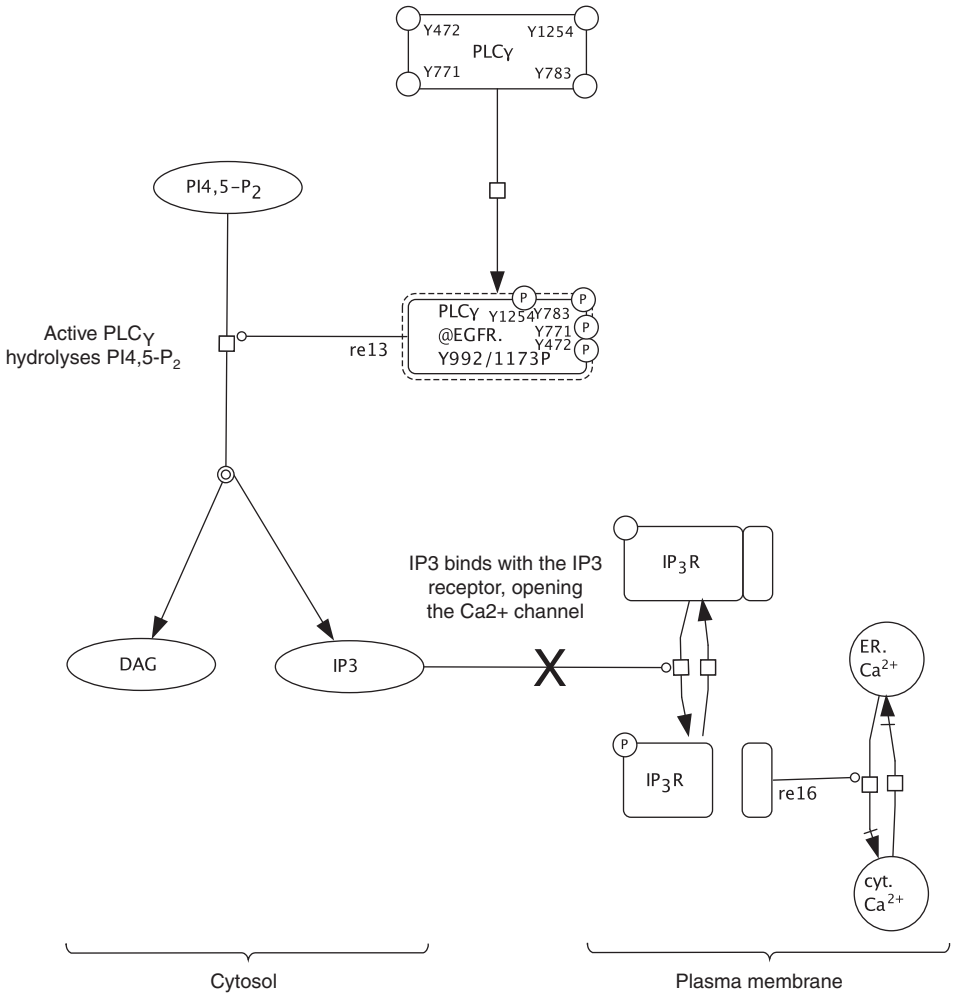


Figure 3 Example—annotation issue. The two reactions 'Active PLCγ hydrolyses PI4,5-P_2' (REACT_12078.2) and 'IP3 binds with the IP3 receptor, opening the Ca2 + channel' (REACT_12008.1) are connected through the entity IP3 as the hydrolysis of PI4,5-P_2 results in DAG and IP3, and then IP3 binds to its receptor enabling the Ca^{2+} release. However, the process in which IP3, located in the cytosol, translocates to the plasma membrane to bind to its receptor is not precisely described in Reactome, leading to differences in the annotation of both IP3 entities. This precludes the automated merging of both chains of reactions and manual intervention was needed to connect the reactions correctly.

attributes of the nodes to automatically connect reactions from different pathways. However, when tested on the reactions in the *EGFR map*, several problems arose. Most of them appeared as a result of annotation issues. For instance, Figure 3 shows two reactions in which the two IP3 entities are differently annotated. The first IP3 entity is located in the 'cytosol', whereas this cellular location annotation is missing for the second IP3, precluding the expected merging of the two reactions. Hence, an automatic integration is impeded and manual intervention is needed. Another factor that hampers finding connections between reactions or pathways is the use of combined entities. For instance, the already mentioned ERK1 and ERK2 proteins first represented as separate entities are later described as a combined ERK1/ERK2 entity (Figure 2). This problem could be solved by considering all the annotations of the nodes while deciding whether two entities are equivalent or not. This would allow comparing nodes that represent states of entities, for instance, post-translational modified proteins or proteins annotated using cellular locations. In summary, reconstruction of crosstalks between signalling pathways is difficult by means of the automatic tools currently available. Manual intervention is required to recover all reactions involved in the pathways and their crosstalks.

The case study presented here shows that a process combining automatic retrieval and manual intervention can be used to reconstruct the *EGFR map* in its main features. This shows that current pathway databases contain a lot of detailed information though in some cases this information is still incomplete and manual intervention is needed to obtain a complete and correct network representation containing different signalling pathways. This is especially critical for reactions that are part of regulatory feedback loops, as these determine the dynamic behaviour of the signalling pathway. Nevertheless, information obtained for individual reactions and even for some pathways is quite complete and would be accurate enough as a starting point for model building. A proposal for a strategy for the use of pathway data from public databases for network modelling is presented in Box 1.

Table III Pathways downloaded for extending the *EGFR map*

Extension to	Reactome pathways downloaded	Reactome identifier
MAP kinase cascade	RAF activation	REACT_2077.4
	MAP kinase cascade	REACT_634.4
	ERK1/2 activates ELK1	REACT_12406.1
	ERK1/2/5 activate RSK1/2/3	REACT_12487.1
	RSK1/2/3 phosphorylates CREB at serine 133	REACT_12622.1
Ca^{2+} signalling	Active PLCG1 hydrolyses PIP2	REACT_12078.2
	DAG stimulates protein kinase C-delta	REACT_12062.1
	IP3 binds to the IP3 receptor, opening the Ca^{2+} channel	REACT_12008.1
	Release of calcium from intracellular stores by IP3 receptor activation	REACT_12074.1
	Calcium binds calmodulin	REACT_12602.1
GPCR signaling	Thrombin-activated activation cascade	REACT_57.1
	Glucagon signalling in metabolic regulation	REACT_1665.2
Cell cycle	p53-dependent G1/S DNA damage checkpoint	REACT_85.1
	NRAGE signals death through JNK	REACT_13638.1
	Activation of BAD and translocation to mitochondria	REACT_549.2
	BH3-only proteins associate with and inactivate anti-apoptotic BCL-2 members	REACT_330.1
	Cyclin D-associated events in G1	REACT_821.2
	Cyclin E-associated events during G1/S transition	REACT_1574.2

Conclusions and perspectives

In this paper, we have reviewed the main knowledge resources of human pathways, and we have evaluated the feasibility of using this information for the reconstruction of signalling pathways in a biologically meaningful manner. Moreover, we have presented a scenario in which data from public pathway databases are directly used for modelling (see Box 1). In this regard, we have briefly discussed the main standards for representation of biological networks, BioPAX and SBML. Furthermore, we have discussed the advantages and drawbacks of current methods for pathway retrieval and integration, using the EGFR signalling as an illustrative example.

We encourage the combination of data from different pathway databases, as they are often complementary and, in this way, a better coverage of all the reactions involved in a given pathway will be achieved. However, the integration of pathways from different databases poses a challenge, as different standard formats are used and data models vary.

Even if we choose a single database as a source of pathway information, the retrieval of a signalling pathway in conjunction with its crosstalks to other signalling cascades is a difficult task. Although in this case the data model and representation formats are not an issue, there are still annotation problems that remain to be solved to allow an effective integration of reactions and pathways. In this regard, the communication of these problems to database curators by the users will be of great help to improve the completeness and quality of annotations.

There is a strong need of tools for the automatic integration of different pathways in a biological meaningful way. The analysis presented here stresses that this is not a trivial task. As several annotation problems and inconsistencies exist, manual intervention is needed to achieve the integration. Moreover, other factors have to be considered to decide whether two pathways can be merged: are the pathways found in the same cell type? Or, are they found at the same developmental stage of the cell? Accurate annotation of the reactions taking place in each signalling pathway will be required to appropriately solve these questions.

The case study on the EGFR signalling has highlighted very important issues for the practical use of pathway databases. The information obtained for individual reactions and even for particular pathways is quite complete in most of the cases and would be accurate enough as a starting point for modelling. Although we did not carry out a systematic evaluation of all the reactions found in Reactome, on the basis of the results of this case study we can conclude that public databases contain accurate and quite complete information about the main processes involved in cell signalling pathways. However, for processes for which no such level of detail on the reactions is available, the representation recovered from public databases will be less complete. For example, comparison of manually created Rb/E2F pathway with data from Reactome indicated that the latter does not cover all the reactions (Calzone *et al*, 2008). We foresee that in the following years, the coverage of the databases will grow as well as the quality of the annotations, which will benefit the scientific community in providing a source of representations of pathways for modelling purposes.

We would like to finish by stressing the importance of the annotation and data representation issues for an effective integration of data from public pathway databases. Researchers involved in pathway annotation and in pathway modelling should engage in collaborative projects to take advantage of the data already available in public databases and work together on representations that fit the needs of both communities.

Supplementary information

Supplementary information is available at the *Molecular Systems Biology* website (www.nature.com/msb).

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Conflict of interest

The authors declare that they have no conflict of interest.

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