Update

Letters

iPath: interactive exploration of biochemical pathways and networks

Ivica Letunic[1], Takuji Yamada[1], Minoru Kanehisa[2] and Peer Bork[1]

[1] EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany
[2] Bioinformatics Center, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan

iPath is an open-access online tool (http://pathways.embl.de) for visualizing and analyzing metabolic pathways. An interactive viewer provides straightforward navigation through various pathways and enables easy access to the underlying chemicals and enzymes. Customized pathway maps can be generated and annotated using various external data. For example, by merging human genome data with two important gut commensals, iPath can pinpoint the complementarity of the host–symbiont metabolic capacities.

New era of pathway exploration

The recent publication of the KEGG (http://www.genome.jp/kegg/) global overview map of metabolic pathways in Scalable Vector Graphics format [1] represents an important step towards large-scale visualization and interpretation of various data regarding metabolic activities. The global map has been manually constructed using 123 classical KEGG maps with an average of 17 reactions each; the result is an overview of a large proportion of all metabolic reactions known to date, collected from various biological systems (see Figure 1 for details).

To access and digest this vast amount of information, we have developed an interactive Pathways Explorer, iPath, which provides powerful tools to visualize, navigate, explore and analyze all, or a subset of, various pathway maps. Pathway maps in iPath are accessed through an interactive online viewer, which provides simple zooming and panning controls, with different levels of map detail corresponding to various zoom levels. Detailed information is available for various nodes and edges (lines) in the map, such as for the enzymes, reactions and compounds involved. Further information can be accessed via hyperlinks to other online resources, for example, KEGG [1], PubChem (http://pubchem.ncbi.nlm.nih.gov/), 3DMET (http://www.3dmet.dna.affrc.go.jp/) and ChEBI [2].

iPath can export the maps into various graphical formats, which can be further modified or included in various documents and publications.

Mapping data onto pathways and customizing maps

iPath provides a simple way of creating customized pathway maps through data mapping. Several types of data can be used to change every part of the map, such as pathway and compound identifiers, protein accession numbers, COG [3], eggNOG [4], KEGG orthologous group identifiers and enzyme EC numbers. In addition, colors, opacity and width can be specified for any node or edge in the map. Queries can also be performed using enzyme names, enabling easy highlighting of various pathways where individual or groups of enzymes occur.

These features create a powerful framework for the exploration and analysis of particular metabolic pathways or overall metabolism, and for comparative analyses of various genomics, transcriptomics or proteomics datasets. The many new display and analysis opportunities provided by iPath include overviews of the metabolic capacity encoded by a single (meta)genome, exploration of metabolic differences in various spatial and temporal series datasets, comparative and evolutionary studies with many organisms in addition to species-complementarity studies. The latter is illustrated by a straightforward analysis to identify pathways in abundant human gut bacteria that complement those in human (Figure 1).

Figure 1 shows all known, categorized metabolic pathways of *Homo sapiens* and the complementary bacterial pathways of *Bifidobacterium longum* and *Methanobrevibacter smithii*. These two bacteria inhabit the human intestine, and are an integral part of our intestinal microflora [5–7]. Among the many pathways that are specific to *B. longum* and *M. smithii*, are those of peptidoglycan and alternative isoprenoid biosynthesis (Figure 1). Most importantly, *B. longum* and *M. smithii* synthesize five compounds that are missing in human metabolism, namely, vitamins B1, B2, B5, B9 and H, which can all be absorbed in the human large intestine [8]. The respective complementary pathways that are responsible for the synthesis of these vitamins are clearly visible in iPath (Figure 1). iPath further reveals that the two bacteria also encode, in contrast to humans, the enzymes needed for the synthesis of several other vitamins (such as B12, K2 and B3); whether these vitamins can also be absorbed by the human large intestine still needs to be determined.

It is well known that bacteria provide various effective and toxic compounds in the human intestine. However, it is still unclear which compounds are synthesized by human endosymbiotic bacteria. The simple analysis shown here demonstrates the power of iPath in hypothesis generation, comparative pathway analysis and many other applications.

Exploring the metabolism of various species

In addition to the global overview map, iPath provides a set of species-specific metabolic pathways maps, which were created using ortholog definitions for enzymatic proteins of each of the 183 species present in the global map (21

Corresponding author: Bork, P. (bork@embl.de).

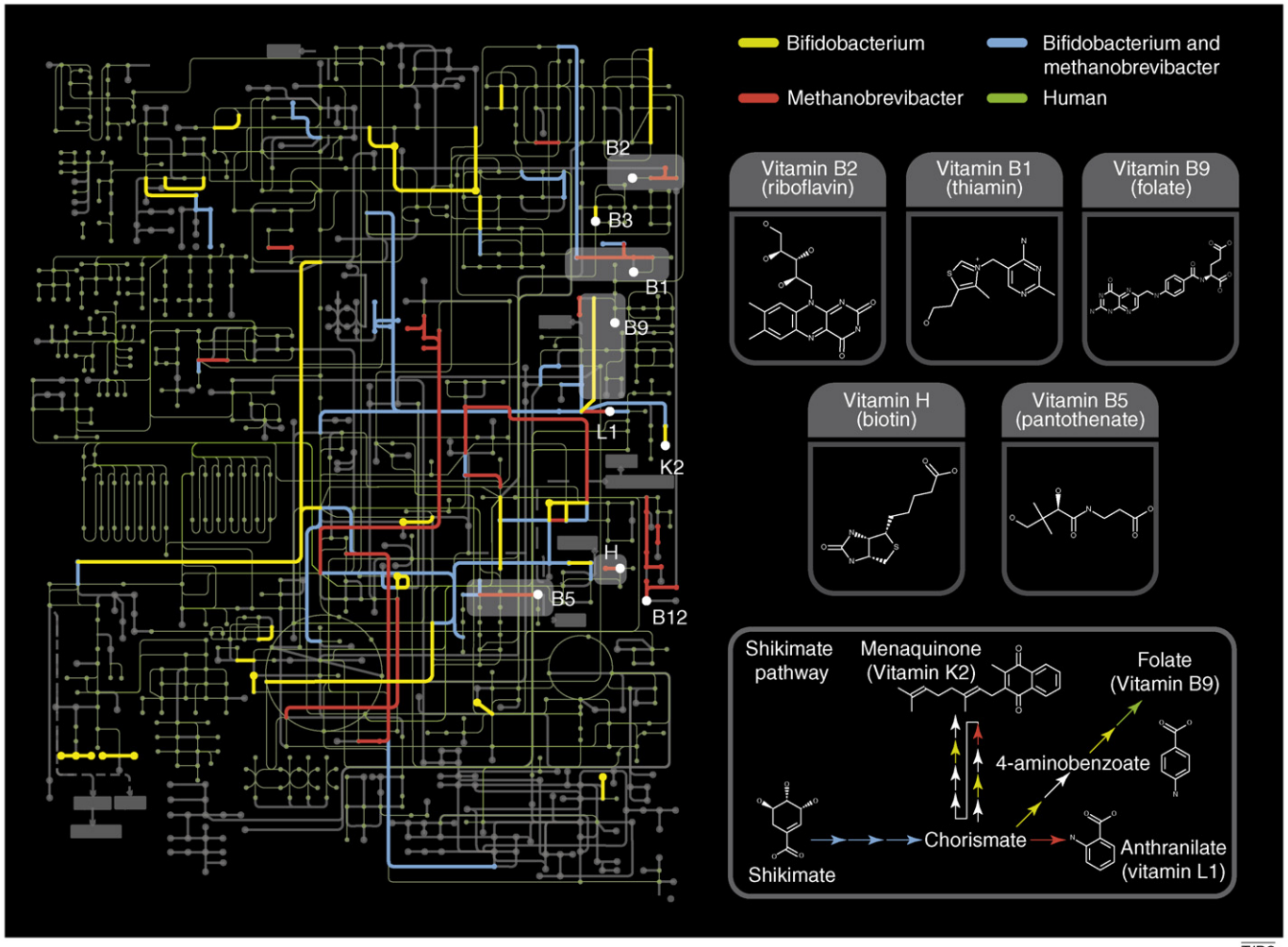

Figure 1. Bacterial complement pathways in the human intestine. The custom metabolic map shown here was generated using iPath. Protein orthologous groups defined by KEGG were used to detect pathway presence and complementarity in *Homo sapiens, Bifidobacterium longum* and *Methanobrevibacter smithii*. Nodes in the map correspond to various chemical compounds and edges (lines) represent series of enzymatic reactions. A total of 1973 orthologous groups for enzymes catalyzing 2354 reactions are fused into the edges and 1789 chemicals are present in the nodes of the map, all of which can be accessed interactively. **Left:** Metabolic pathways of *H. sapiens, B. longum* and *M. smithii*. The latter two species are considered key commensals in the human intestinal microflora [5–7]. Pathways present in *H. sapiens* are shown in green. Red and yellow lines correspond to pathways that are specific to *Methanobrevibacter* and *Bifidobacterium*, respectively. Pathways that are present in both bacteria, but not in *H. sapiens*, are shown in blue. White circles mark the metabolic locations of several human vitamins. **Right:** Vitamins that are synthesized by bacteria and that can be absorbed in the large intestine of humans [8] have been extracted by iPath. The pathways that synthesize these vitamins are highlighted in the map (left) by pale white squares. Also missing in *H. sapiens*, but found in the two bacteria, are various enzymes of the Shikimate pathway. This pathway is one of the branch points for vitamins that are synthesized by bacteria, such as folate (vitamin B9), menaquinone (vitamin K2) and anthranilate (vitamin L1).

Eukaryota, 154 *Bacteria* and 18 *Archaea*). These can be accessed through an interactive phylogenetic tree, generated using iTOL [9]. In addition to the standard map that has been directly generated from orthology mapping, each species has a filtered version, wherein certain enzymes are removed. The filtering procedure removed enzymes that are part of incomplete pathways; exceptions are made in cases where products of an enzymatic reaction are used by neighboring enzymes.

Future directions

With the ever increasing amounts and types of molecular biological data, new tools are needed for their complex analyses. iPath is hopefully one of them and it is intended that the number of intuitive and powerful tools, which simplify the analysis and navigation of large metabolic pathways maps and which can uncover various as-yet unknown correlations and complementarities, continue to increase.

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