PIERO ontology for analysis of biochemical transformations: Effective implementation of reaction information in the IUBMB enzyme list

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Genomics is faced with the issue of many partially annotated putative enzyme-encoding genes for which activities have not yet been verified, while metabolomics is faced with the issue of many putative enzyme reactions for which full equations have not been verified. Knowledge of enzymes has been collected by IUBMB, and has been made public as the Enzyme List. To date, however, the terminology of the Enzyme List has not been assessed comprehensively by bioinformatics studies. Instead, most of the bioinformatics studies simply use the identifiers of the

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enzymes, i.e. the Enzyme Commission (EC) numbers. We investigated the actual usage of terminology throughout the Enzyme List, and demonstrated that the partial characteristics of reactions cannot be retrieved by simply using EC numbers. Thus, we developed a novel ontology, named PIERO, for annotating biochemical transformations as follows. First, the terminology describing enzymatic reactions was retrieved from the Enzyme List, and was grouped into those related to overall reactions and biochemical transformations. Consequently, these terms were mapped onto the actual transformations taken from enzymatic reaction equations. This ontology was linked to Gene Ontology (GO) and EC numbers, allowing the extraction of common partial reaction characteristics from given sets of orthologous genes and the elucidation of possible enzymes from the given transformations. Further future development of the PIERO ontology should enhance the Enzyme List to promote the integration of genomics and metabolomics.

Keywords: Enzyme; reaction; transformation; ontology.

1. Introduction

Recent development of high-throughput techniques has promoted large-scale analysis of molecular biological data, including genomic and metabolomic data. However, much of these data have been computationally predicted, but have not been verified experimentally.¹

First, genomics studies have identified a vast number of putative genes, but many of these genes have only been partially annotated. For many putative enzyme-encoding genes, annotations are given using Enzyme Commission (EC) numbers² or Gene Ontology (GO) identifiers.³ Molecular functions in GO correspond completely to EC numbers; thus these identifiers are not substantially different. However, EC numbers are not applicable for annotating the genes (or gene products) that mediate unknown reactions, because they are assigned only to those enzymes for which overall reaction equations have been experimentally verified.

Second, metabolomics studies have implicated many biochemical compounds (metabolites) but how they are synthesized remains unknown.⁴ Putative pathways have been considered for such compounds in many studies, where the putative intermediate compounds are connected to each other by partial (incomplete) reaction equations. However, EC numbers are not applicable for the partial reactions either, for the same reason as described above.

It is important to clarify what is meant by “*partial reaction*” in this paper. A reaction may be divided into multiple reaction steps, where the product of one step becomes the substrate of the next step. Such reaction steps are not the focus of this paper. The *partial reaction* described in this paper is the substrate–product relationship, also referred to as the “*transformation*”, in which the role of the other molecules is unclear (Fig. 1(a)).

Here, we propose that organizing the terminology of enzyme reactions in the framework of computer-aided ontology⁵ would help not only to characterize and identify the enzymes that possibly mediate partial reactions of interest, but also to systematically extract the common biochemical characteristics from a group of enzyme-encoding genes.

(a) a transformation (partial reaction)

(b) reactions (overall reactions)

Fig. 1. A transformation and the corresponding reactions. The use of “a transformation” only concerns a change in the substrate, whereas the use of “a reaction” concerns all of the substrates and all of the products. (a) In organic chemistry, this *transformation* describes the change in the substrate, irrespective of the acetylating reagent. In metabolomics, this *transformation* describes the substrate–product relationship, or the partial reaction, for which the acetylating reagent is not known yet. (b) These *reactions* share the same *transformation*. The difference lies in the acetylating reagents.

1.1. Related works

The International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry and Molecular Biology (IUBMB) provide systematic nomenclature for naming chemical reactions⁶ and enzymes,² respectively. The IUBMB’s Enzyme List is the most valuable resource of experimentally verified enzymes, and it provides EC numbers, four-digit identifiers (IDs), to distinguish enzymes. IUBMB classifies enzymes into six EC classes, i.e. *EC1 oxidoreductases*, *EC2 transferases*, *EC3 hydrolases*, *EC4 lyases*, *EC5 isomerases*, and *EC6 ligases*, which are indicated by the first digit of the EC numbers. The purpose of establishing the Enzyme List was the development of a standard vocabulary for naming enzymes. Originally, EC numbers were given only to the enzymes for which reactions had been characterized experimentally. With the increase in the number of genome projects, EC numbers have also been used for the annotation of putative genes on the basis of homology information, and not only on experimental characterization.

BioPortal⁵ contains several ontologies that deal with enzymatic functions, but only *via* EC numbers. GO, on the other hand, is the most widely used dictionary for terminology related to gene functions.³ GO classifies gene functions, among which enzymatic functions are described using EC numbers. ChEBI and REX ontologies⁷ also deal with processes that involve chemistry, but they are based on GO (i.e. based on EC numbers). Enzyme Mechanism Ontology (EMO) describes enzyme reactions

and their reaction steps, but does not deal with the partial reactions that we describe in this study. Name Reaction Ontology (RXNO) considers organic name reactions but does not deal with enzyme reactions.

1.2. Scope of PIERO

Suppose a situation in which two compounds are possibly converted to each other via enzymatic catalysis (Fig. 1(a)), but for which the enzyme has not yet been identified. In fact, three enzymes may be involved in catalyzing this biochemical transformation (Fig. 1(b)). However, in the supposed situation, it is not known which of the three is involved, and it is also possible that none of the three is actually involved (an unknown reaction may occur).

It is important to note that EC numbers are not given to partial reactions (or transformations), and also that partial EC numbers do not necessarily describe the full characteristics of such transformations. For example, the partial reaction in Fig. 1(a) can be described in a sentence such as “*This reaction involves an acetylation of an amine group, yielding an N-acetyl group*”. Although the three reactions in Fig. 1(b) are actually known, if we assume that they are unknown, they are assigned partial EC numbers *EC2.3.1* or *EC3.5.1*. However, an annotation such as “*This reaction is possibly catalyzed by the enzymes belonging to EC2.3.1 or EC3.5.1*” causes loss of much information, because *EC2.3.1* or *EC3.5.1* do not provide any specific terminology such as *acetylation*, *amine group*, or *N-acetyl group*. The EC numbers only provide broader meanings; *EC2.3.1* is defined as “*transferases that transfer acyl groups other than aminoacyl groups*”, and *EC3.5.1* is defined as “*hydrolases acting on carbon-nitrogen bonds, other than peptide bonds, in linear amides*”. It is therefore challenging to describe the characteristics of enzymes with such incomplete equations.

Suppose another situation in which three enzymes mediate three reactions (Fig. 1(b)), from which we want to obtain some common characteristics. It is clear that the three reactions have a substrate–product relationship in common (Fig. 1(a)), however, this would be much more difficult to find if the number of enzymes (or reactions) was larger. The possible answer using EC numbers would be “*Two of them belong to EC2.3.1, and one of them belongs to EC3.5.1*”, which may suggest erroneously that there are no common characteristics among the three reactions.

The reason for the failure to extract these characteristics, which may occur frequently in bioinformatics studies, is that we only use EC numbers. EC numbers are merely the identifiers of enzymes, and greater detail is provided in the Enzyme List in natural language.

We thus propose an ontology, named PIERO (Enzyme Reaction Ontology for annotating Partial Information of biochemical transformation), which deals with the terminology corresponding to overall and partial biochemical changes in enzymatic reactions, using biochemical terms (such as *acetylation*, *hydrolysis*, *amine group*,

amide bond), the names of compounds (such as those in the substrate–product relationship), and the relationships among such terms. The concept of this study follows the IUPAC recommendations of 1988,⁶ which distinguish a “*transformation*” (substrate–product relationship) from a “*reaction*” (overall reaction) clearly for the purpose of naming organic chemical transformations. In this study, we used this concept, incorporated the terminology in the IUBMB’s Enzyme List (which is slightly different from that of IUPAC), and also separated the terminology into that representing *reactions* and *transformations*. This ontology was mapped onto KEGG reactions and the related entries, accompanied by linking with GO and EC numbers, thereby allowing the extraction of the common partial characteristics from the given sets of orthologous genes and the elucidation of possible enzymes from the given partial reactions.

2. Materials and Methods

Enzyme reactions, chemical structures of compounds and other enzyme-related data were retrieved from the KEGG database.⁸

The substructure–product relationship was taken from KEGG RPAIR database,⁹ where substrate–product relationships describe the *transformations* in the *reactions*. Taking a reaction $\text{Glc} + \text{ATP} = \text{Glc-6-P} + \text{AMP}$, for example, the “*main*” types of *transformations* are “*Glc -> Glc-6-P*” (conserving glucose residue) and “*ATP -> AMP*” (conserving AMP residue). The pair “*ATP->Glc-6-P*” is defined as a “*trans*” type of *transformation*, representing the transferred phosphate group. The pair “*Glc -> AMP*” is not defined as a *transformation* because it does not conserve any atoms.

The relationships between the *transformations* and the corresponding *transformation patterns* were taken from the KEGG RCLASS database.¹⁰

The relationships between biochemical substructures and the corresponding names were retrieved from the BiSSCat database¹¹ using KEGG Chemical Function-and-Substructures (KCF-S).¹²

These data were converted to the Resource Description Framework (RDF), where the relationships were described by the triad of subjects, predicates, and objects.¹³

3. Results and Discussion

3.1. Vocabulary appearance in the Enzyme List

Generally, an enzyme has a systematic name and more than one accepted names, and enzymes are named according to the reactions they catalyze. The Enzyme List terminology of the *reactions* and the *transformations* is represented with the suffix “-ase” when the terminology represents the main characteristics of the reaction (e.g. *decarboxylase*). When the terminology does not represent the main characteristics, but when it is important to distinguish its action from that of other enzymes, the suffix “-ing” is used (e.g. *decarboxylating*).

We counted the appearance of EC numbers that contain the terms including the suffixes *-ase* and *-ing* (Tables 1 and 2, respectively). Some terms (such as *phosphohydrolase*, *amidohydrolase*, *proteinase*, and *dioxygenase*) only appear in one EC class (indicated by the first digit of EC numbers). However, many terms (such as *cyclase*

Table 1. Top 40 terms with the suffix “-ase” in the Enzyme List.

	Total	The first digit of the EC numbers					
		EC1	EC2	EC3	EC4	EC5	EC6
Oxidoreductase	1480	1474	0	0	4	2	0
Synthase	748	113	295	16	202	67	55
Lyase	618	9	37	4	545	21	2
Dehydrogenase	580	572	5	3	0	0	0
Reductase	399	389	6	3	1	0	0
Hydrolase	283	1	3	264	14	1	0
Methyltransferase	269	1	268	0	0	0	0
Synthetase	263	12	97	3	22	10	119
Phosphotransferase	260	0	257	1	0	2	0
Transferase	216	5	203	6	2	0	0
Kinase	206	0	203	3	0	0	0
Oxidase	196	194	0	0	1	1	0
Phosphohydrolase	191	0	0	191	0	0	0
Monooxygenase	179	178	1	0	0	0	0
Ligase	176	0	6	3	3	0	164
Hydroxylase	171	170	1	0	0	0	0
Glucosyltransferase	128	0	124	1	0	3	0
Amidohydrolase	118	0	0	118	0	0	0
Proteinase	116	0	0	116	0	0	0
Dioxygenase	111	111	0	0	0	0	0
Phosphatase	106	0	4	102	0	0	0
Aminotransferase	100	1	96	0	1	1	1
Acetyltransferase	99	2	95	2	0	0	0
Decarboxylase	95	10	1	0	84	0	0
Isomerase	95	2	4	2	3	84	0
Transaminase	89	0	87	0	2	0	0
Dehydratase	87	0	0	1	86	0	0
Protease	82	0	0	82	0	0	0
Mutase	71	1	0	0	1	69	0
Peptidase	70	0	0	70	0	0	0
Acyltransferase	69	0	67	1	1	0	0
ATPase	67	0	0	67	0	0	0
Oxygenase	58	54	2	0	2	0	0
Cyclase	55	2	5	1	31	14	2
Deaminase	53	4	1	38	10	0	0
Endopeptidase	52	0	0	52	0	0	0
Aldolase	48	0	4	0	44	0	0
Epimerase	45	3	0	2	0	40	0
Esterase	45	0	0	45	0	0	0
Aminohydrolase	45	0	0	44	1	0	0

Note: The numbers of EC numbers that contain the respective “-ase”-form terms were counted. If an EC number had two names and both contained “*decarboxylase*”, the term “*decarboxylase*” was counted once only.

Table 2. Top 20 terms with the suffix “-ing” in the Enzyme List.

	Total	The first digit of the EC numbers					
		EC1	EC2	EC3	EC4	EC5	EC6
Hydroxylating	225	225	0	0	0	0	0
Cyclizing	164	14	26	4	89	29	2
Phosphorylating	115	7	104	0	1	0	3
Decarboxylating	103	63	26	7	7	0	0
Deaminating	74	49	1	3	21	0	0
Hydrolysing	68	5	27	14	5	3	14
Decyclizing	60	32	0	8	1	19	0
Transporting	44	4	0	40	0	0	0
Cleaving	35	21	0	12	1	1	0
Pyruvate-forming	32	2	0	0	30	0	0
Reducing	25	9	7	7	2	0	0
Phosphate-forming	25	0	0	1	24	0	0
Acetylating	19	9	8	0	2	0	0
Demethylating	17	17	0	0	0	0	0
Thioester-hydrolysing	17	0	17	0	0	0	0
Alpha-hydroxylating	15	15	0	0	0	0	0
Acylation	15	13	2	0	0	0	0
Isomerizing	13	3	3	3	2	2	0
Diphosphate-forming	13	0	3	3	1	0	6
Transferring	12	5	4	2	1	0	0

Note: The numbers of EC numbers that contain the respective “-ing”-form terms were counted. If an EC number had two names and both contained “decarboxylating”, the term “decarboxylating” was counted once only.

and cyclizing), even those that define the six EC classes (*oxidoreductase*, *transferase*, *hydrolase*, *lyase*, *isomerase*, and *ligase*), appear in two or more EC classes.

This result implied that using partial EC numbers causes loss of information. For example, the partial EC number *EC4.1.1* is defined as *carboxy-lyases* (synonym of *decarboxylases*) and it contains 84 decarboxylases. However, when looking up enzymes that have decarboxylase activity, it is not advisable to simply use *EC4.1.1*, because there are 11 other decarboxylases (Table 1) and 96 *decarboxylating* enzymes (Table 2). One of the enzymes that are not included in *EC4.1.1*, but which has *decarboxylase* activity, is *EC1.1.1.42*, which is named an “*isocitrate dehydrogenase (NADP+)*”, and has many synonyms including “*oxalosuccinate decarboxylase*”, and a systematic name “*isocitrate:NADP+oxidoreductase (decarboxylating)*”.

Another example that shows the importance of this analysis is *EC3.2.1.183*. An accepted name of this enzyme is “*UDP-N-acetylglucosamine 2-epimerase (hydrolysing)*”, and the systematic name is “*UDP-N-acetyl-α-D-glucosamine hydrolase (2-epimerising)*”. This enzyme is classified into *EC3 hydrolases* because of its *hydrolysis* activity, but the biological importance of this enzyme is its *epimerisation* activity. The main purpose of the Enzyme List is to give a unique name for a single catalytic entity. The unique name must end with the suffix *-ase*, and the suffix must not

appear twice in a name. Therefore, when an enzyme has two activities, one of them is used with the suffix *-ase*, and the other is used with the suffix *-ing*.

To sum it up, the difference in the first digits of the EC numbers does not necessarily mean that the enzymes have no characteristics in common. In fact, however, many bioinformatics studies frequently rely on EC numbers, possibly resulting in erroneous interpretation in looking up the enzymatic function of interest, and in finding common characteristics from groups of enzymes or reactions. Here, we emphasize that the full value of the Enzyme List lies in the natural language descriptions, not in the IDs (i.e. EC numbers). We believe that this is a critical issue for a wide range of bioinformatics studies, including genome annotation and pathway analysis.

3.2. Standardization and mapping of terminology

The IUPAC recommendations of 1988⁶ indicate the nomenclature for organic chemical transformations and state that a *transformation* should be distinguished from a *reaction*; the full description of a *reaction* concerns all the reactants and all the products, whereas a *transformation* only concerns the changes in a particular molecule (Fig. 1). These recommendations use terminology that is different from that of the Enzyme List. For example, “*dehydrogenation*” (as *dehydrogenase* in the Enzyme List) is equivalent to “*dihydroelimination*” in the IUPAC recommendations of 1988. Taking another example from Fig. 1, “*acylation*” refers to a process in which a hydrogen atom of a substrate is replaced by an *acyl group*, irrespective of where the *acyl group* comes from or what the substrate is. This terminology is equivalent to the terminology “*acyl-de-hydro-substitution*” in IUPAC. These terms do not represent *reactions* but represent *transformations*.

On the other hand, the IUBMB's Enzyme List contains many concepts that are not included in the IUPAC recommendations of 1988. Some of the vocabulary does not represent *transformations*, but rather represent *reactions* (e.g. *hydrolysis* and *phosphorolysis*). Other terms have the structure “*product + -forming*”, where the product is represented as the name of the compound (e.g. *pyruvate-forming*) or the functional group involved (e.g. *aldehyde-forming*). These terms do not represent *transformations* or *reactions*.

We collected the terms in the Enzyme List, and categorized them into those that represent *reactions*, *transformations*, and others. Consequently, standardized terms containing the suffixes *-sis* and *-ation* were mapped to *reactions* and *transformations*, respectively. Table 3 shows representative examples of this terminology mapping.

3.3. Construction of the PIERO ontology

The PIERO ontology was constructed by following the IUPAC recommendations of 1988, distinguishing between the descriptive terms for *reactions* and *transformations*. Among the objects already defined in KEGG, the *REACTION* and the *RPAIR* entries are equivalent to *reactions* and *transformations*, respectively. Some

Table 3. Terminology mapping.

PIERO	Enzyme List
(a) Reactions	
Hydrolysis	Hydrolase
Hydrolysis of peptide bond	Peptidase, protease, Proteinase, etc.
Hydrolysis of glycosyl bond	Glycosylase, glucosidase, etc.
Hydrolysis of phosphate ester	Phosphatase
Hydrolysis of ATP	ATPase
Phosphorolysis	
Phosphorolysis of glycosyl bond	Glycosyltransferase
Dehydration synthesis	Ligase
Synthesis	Ligase, synthase, synthetase
Lysis	Lyase
(b) Transformations	
Oxidoreduction	Oxidoreductase, reductase
Hydrogenation/dehydrogenation	Dehydrogenase
Oxygenation/deoxygenation	Oxygenase, dioxygenase
Hydroxylation/dehydroxylation	Hydroxylase
Phosphorylation/dephosphorylation	Kinase, phosphatase, phospho-transferase, phosphorylase
Pyrophosphorylation/depyrophosphorylation	Pyrophosphorylase, diphosphatase
Methylation/demethylation	Methyltransferase
Amination/deamination	Aminotransferase, deaminase, Transaminase
Acylation/deacylation	Acyltransferase
Acetylation/deacetylation	Acetyltransferase
Hydration/dehydration	Hydratase, dehydratase
Cyclization/decyclization	Cyclase
Aldol reaction	Aldolase
Glycosylation/deglycosylation	Glycosyltransferase
Galactosylation/degalactosylation	Galactosyltransferase
Phosphorylation of hydroxy group	
Phosphorylation of carboxy group	
Carboxylation/decarboxylation	Carboxylase, decarboxylase,
Isomeration	Isomerase, epimerase
Intramolecular-migration	Mutase
(c) Product compound formation	
Pyruvate formation	Pyruvate-forming
Phosphate formation	Phosphate-forming
(d) Product substructure formation	
Aldehyde-formation	Aldehyde-forming
Carboxymethyl-formation	Carboxymethyl-forming

Note: Vocabulary in the Enzyme List was mapped to (a) those representing overall reactions (*reactions*), (b) those representing partial reactions (*transformations*), (c) those representing the product compounds, and (d) those representing the product functional groups, and their suffixes were standardized into “-ation” or “-sis”.

Fig. 2. Proposed ontological relationships in PIERO. The rectangles represent the classes, and the arrows represent the predicates that connect the subjects and the objects. “piero:”, “ec:” and “kegg:” are the prefix of the classes and the predicates defined in PIERO, EC numbers, and KEGG.

additional concepts in KEGG,⁸ GO,³ and BiSSCat¹¹ were incorporated into PIERO, as follows (Fig. 2).

- A *compound* is a molecule whose structure can change during enzymatic reactions. Equivalent to the KEGG COMPOUND and other compound database entries.
- A *substructure* is a substructure or a functional group in a *compound* that has name(s) defined in IUPAC or IUBMB. Equivalent to the BiSSCat FGROUPE entry.
- An *rclass* is a substructure that changes during the *transformation*, equivalent to the KEGG RCLASS entry.
- An *enzymatic function* is a subclass of a *molecular function* defined in GO, which is an experimentally characterized catalytic activity and the associated conditions mediated by the *gene products*. Equivalent to the EC number.
- A *gene product* is a substance (often a protein) with verified enzymatic function(s), and is encoded by a *gene*. A *gene* is equivalent to the KEGG GENES entry.
- An *ortholog* is a group of genes that share the same evolutionary origin and the same *molecular function*. Equivalent to the KEGG ORTHOLOGY entry.

The arrows between the classes represent the predicates, e.g. the arrow between *gene product* and *reaction* is “*catalyzes*”, representing “A *gene product* *catalyzes* a *reaction*”. In PIERO, the *gene products* have *enzymatic functions*, which are associated with EC numbers (and GO biological functions). We separated *enzymatic*

Fig. 3. Specialization of transformation terminology using substructures. The rectangles and arrows represent the classes and predicates, respectively. The rounded rectangles represent the specialized transformation terminology or substructures. “rdfs:” represent the prefix of the predicates defined in the RDF schema.

functions and *reactions* because some enzymes catalyze more than one reaction, and we attempted to deal with the common characteristics of those reactions, regardless of EC numbers.

A *reaction* consists of one or more *transformations*, and *transformations* are characterized by *compounds*, *substructures*, and *transformation patterns* (Fig. 2). The *transformation* terminology can be modified by using additional information (Fig. 3). For example, the *transformation* in Fig. 1(a) involves an *acetylation*, which is a specific term related to *acylation*. This relationship corresponds to that between the substructures *acetyl group* and *acyl group*. The use of the *substructure* or *compound* as a target group or a generated group gives a more specific description of the *transformation*. For example, the *transformation* in Fig. 1(a) can be regarded as a subclass of “*acetylation on an amino group to yield an N-acetyl group*”.

3.4. Identifying putative enzymes from substrate-product relationships

Here, we demonstrate some examples of applications of the PIERO ontology. The first situation mentioned in the Introduction section was formulated as a problem in identifying enzymes that putatively catalyze the transformation of *L-lysine* into *N6-acetyl-L-lysine*. These compounds are given the compound IDs *C00047* and *C02727* in KEGG, respectively.

When we simply want to identify the known *reactions*, exploring the path *compound* -> *transformation* -> *reaction* would be sufficient (Fig. 2). The *transformation* that contain both *C00047* and *C02727* is given the ID *RP00263* in KEGG. The

reactions that contain the transformation *RP00263* are *R00454*, *R00458*, and *R01620*, which are equivalent to the reactions in Fig. 1(b).

When we required further reactions that include transformations such as “acylation of an amino group to yield an *N*-acyl group,” exploring the path *transformation* -> *substructure* (Fig. 3) -> *transformation pattern* -> *transformation* -> *reaction* (Fig. 2) returned 299 reactions. When we specified more detailed information, such as “acetylation of an amino group to yield an *N*-acyl group,” 64 reactions were obtained.

3.5. Extracting common characteristics from multiple genes

The second situation in the Introduction was formulated as the problem of finding common transformations in given reactions, and it is as simple as exploring the path *reaction* -> *transformation*. When the given reactions were *R00454*, *R00458*, and *R01620* (Fig. 1(b)), the obtained transformation was *RP00263* (Fig. 1(a)).

As a further demonstration of the usefulness of PIERO, we analyzed the common characteristics of enzymatic reactions that are catalyzed by enzymes belonging to the same orthologous group. Table 4 shows some examples, where the orthologs were classified into those (a) sharing the same type of transformations, (b) catalyzing sequential reactions that share the same type of transformations, (c) catalyzing

Table 4. Common characteristics obtained from the orthologous enzymes mediating multiple functions.

Common characteristics	Ortholog	EC number	Enzyme function
(a) Sharing the <i>transformations</i> for the same type of bonds			
Phosphate esters	K01103	2.7.1.105	6-phosphofructo-2-kinase
		3.1.3.46	Fructose-2,6-bisphosphate 2-phosphatase
	K02203	2.7.1.39	Homoserine kinase
		3.1.3.3	Phosphoserine phosphatase
	K08073	2.7.1.78	Polynucleotide 5'-hydroxyl-kinase
Glycosyl group	K01196	3.1.3.32	Polynucleotide 3'-phosphatase
		2.4.1.25	4-alpha-glucanotransferase
		3.2.1.33	Amylo-alpha-1,6-glucosidase
Glycosyl group	K05343	3.2.1.1	Alpha-amylase
		5.4.99.16	Maltose glucosylmutase
Nucleotidyl group	K12506	2.7.7.60	MEP cytidylyltransferase
		4.6.1.12	MECDP-synthase
Amide bond	K01460	3.5.1.78	Glutathionylspermidine amidase
		6.3.1.8	Glutathionylspermidine synthase
Carbamoyl group	K11540	2.1.3.2	Aspartate transcarbamylase
		3.5.2.3	Dihydroorotase
		6.3.5.5	Carbamoyl-phosphate synthetase 2
Deamination	K00816	2.6.1.7	Kynurenine — oxoglutarate transaminase
		2.6.1.64	Glutamine — phenylpyruvate transaminase
		4.4.1.13	Cysteine-S-conjugate beta-lyase
Thioester bond	K00659	2.3.1.65	Bile acid-CoA:amino acid N-acyltransferase
		3.1.2.2	Palmitoyl-CoA hydrolase
	K05939	2.3.1.40	Acyl-[ACP] — phospholipid O-acyltransferase
		6.2.1.20	Acyl-acyl carrier protein synthetase

Table 4. (Continued)

Common characteristics	Ortholog	EC number	Enzyme function
Formyl group	K14652	3.5.4.25	GTP cyclohydrolase II
		4.1.99.12	DHBP synthase
Thiol/selenothiol group	K11717	2.8.1.7	Cysteine desulfurase
		4.4.1.16	Selenocysteine lyase
Hydrogen sulfide	K13034	2.5.1.47	Cysteine synthase
		4.4.1.9	L-3-cyanoalanine synthase
Aldol reaction	K16305	2.2.1.11	6-deoxy-5-ketofructose 1-phosphate synthase
		4.1.2.13	Fructose-bisphosphate aldolase
Triphosphate of ATP	K01947	2.7.1.33	Type III pantothenate kinase
		6.3.4.15	Biotin-[acetyl-CoA-carboxylase] ligase
(b) Catalyzing sequential reactions that share the same type of <i>transformations</i>			
Hydrogenation of porphyrins	K02302	1.3.1.76	Precorin-2 dehydrogenase
		2.1.1.107	Uroporphyrin-III C-methyltransferase
		4.99.1.4	Sirohydrochlorin ferrochelatase
Oxygenation	K17863	1.13.11.60	Linoleate 8R-lipoxygenase
		5.4.4.5	Fructose-bisphosphate aldolase
Cyclization of terpenes	K12928	4.2.3.42	Aphidicolan-16beta-ol synthase
		5.5.1.14	Syn-copalyl-diphosphate synthase
(c) Catalyzing sequential reactions that do not share the same type of <i>transformations</i>			
Fatty acid metabolism	K01825	1.1.1.35	3-hydroxyacyl-CoA dehydrogenase
		4.2.1.17	Enoyl-CoA hydratase
		5.1.2.3	3-hydroxybutyryl-CoA epimerase
		5.3.3.8	Enoyl-CoA isomerase
Starch metabolism	K16055	2.4.1.15	Trehalosephosphate-UDP glucosyltransferase
		3.1.3.12	Trehalose-phosphatase
(d) Acting on similar substrates without sharing the same type of <i>transformations</i>			
DNA/RNA modification	K15719	3.6.4.12	DNA helicase
		5.99.1.3	DNA topoisomerase
Protein modification	K03170	2.3.1.48	Histone acetyltransferase
		3.2.1.169	Protein O-GlcNAcase
Shikimate pathway	K13829	2.7.1.71	Shikimate kinase
		4.2.3.4	3-dehydroquinate synthase

sequential reactions that do not share the same type of *transformations*, and (d) acting on similar substrates without sharing the same type of *transformations*. Here, the same type of *transformation* means those *transformations* that generate or cleave the same type of bonds. Among the 102 orthologs that mediate the reactions with different EC numbers at the first digit, 38 and eight orthologs were classified as (c) and (d), respectively, and no common characteristics was found in five of the orthologs. The remaining 51 orthologs were found to have some common characteristics related to the type of *transformations*.

In this paper, we only showed representative examples, i.e. the common characteristics of *reactions* that have different EC classes (the first digits of EC numbers), but which are mediated by the same orthologs. However, this analysis could be applied to many other groups of reactions, e.g. the reactions that have different EC

numbers (or subclasses), but which are mediated by the same orthologs or by the same protein domains, etc.

4. Conclusion

In this study, we introduced the concept of *transformation* based on the IUPAC recommendation regarding the characterization of the Enzyme List of IUBMB, which facilitates identification of possible *reactions* from a *transformation*, and for finding common characteristics from a given set of *reactions*. Although we used the KEGG database to design the proposed PIERO ontology, it has links to genes, orthologs, EC numbers, and compounds in other databases. We intend to further develop the PIERO ontology, effectively using the IUBMB's Enzyme List that is carefully managed in the form of natural language, and which should allow elucidation of the relationship between genomics and metabolomics from the biochemical changes of interest to users.

The PIERO ontology has been implemented as an OWL ontology and is available via a GenomeNet SPARQL endpoint (<http://reactionontology.org/>), where the users can make queries using the SPARQL language.¹³ Recent years, RDF is gathering more attention in the bioinformatics community to describe various types of data, and some RDF-related tools (including SPARQL endpoints) are becoming available.^{14–16} To date, these are still developing technologies, and many studies attempt to improve the calculation efficiency and the usability.^{17–19} Our SPARQL endpoint also leaves room to improve, although the continuing development of the ontology data and the related technologies would enable more complex queries with better usability in the foreseeable future.

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