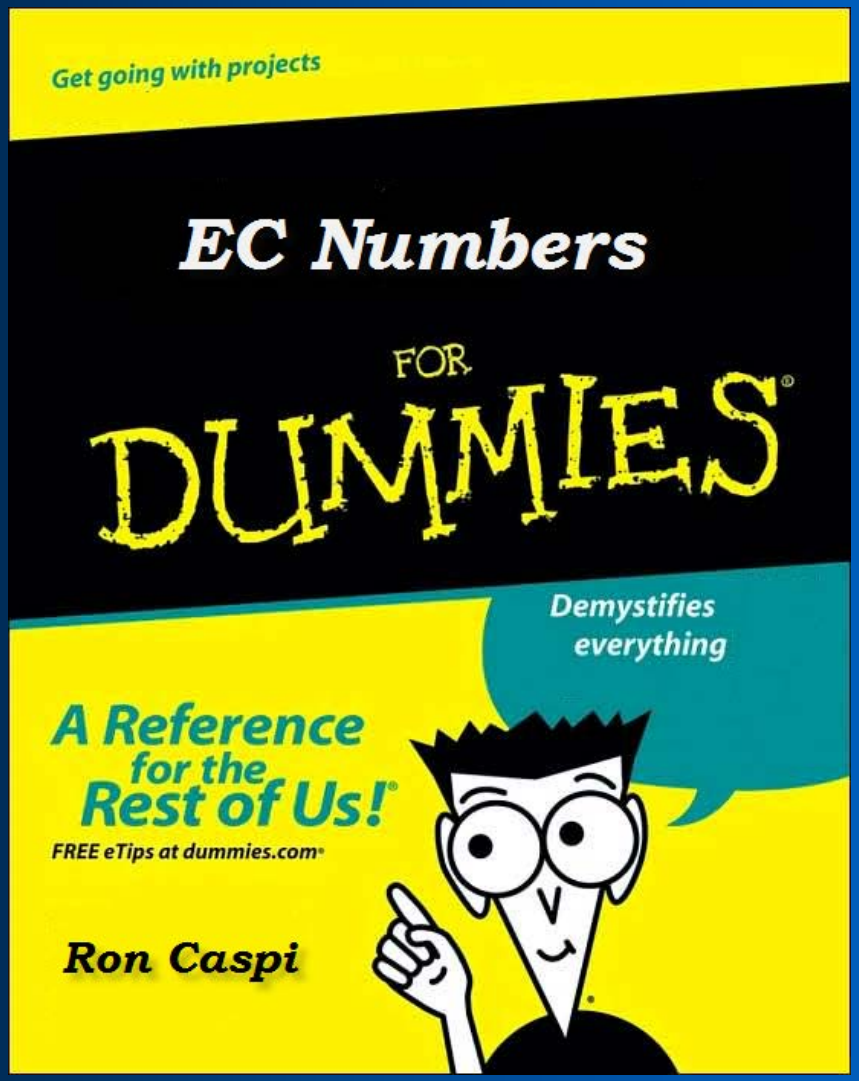
Get going with projects
EC Numbers
FOR
DUMMIES
Demystifies everything
A Reference for the Rest of Us!
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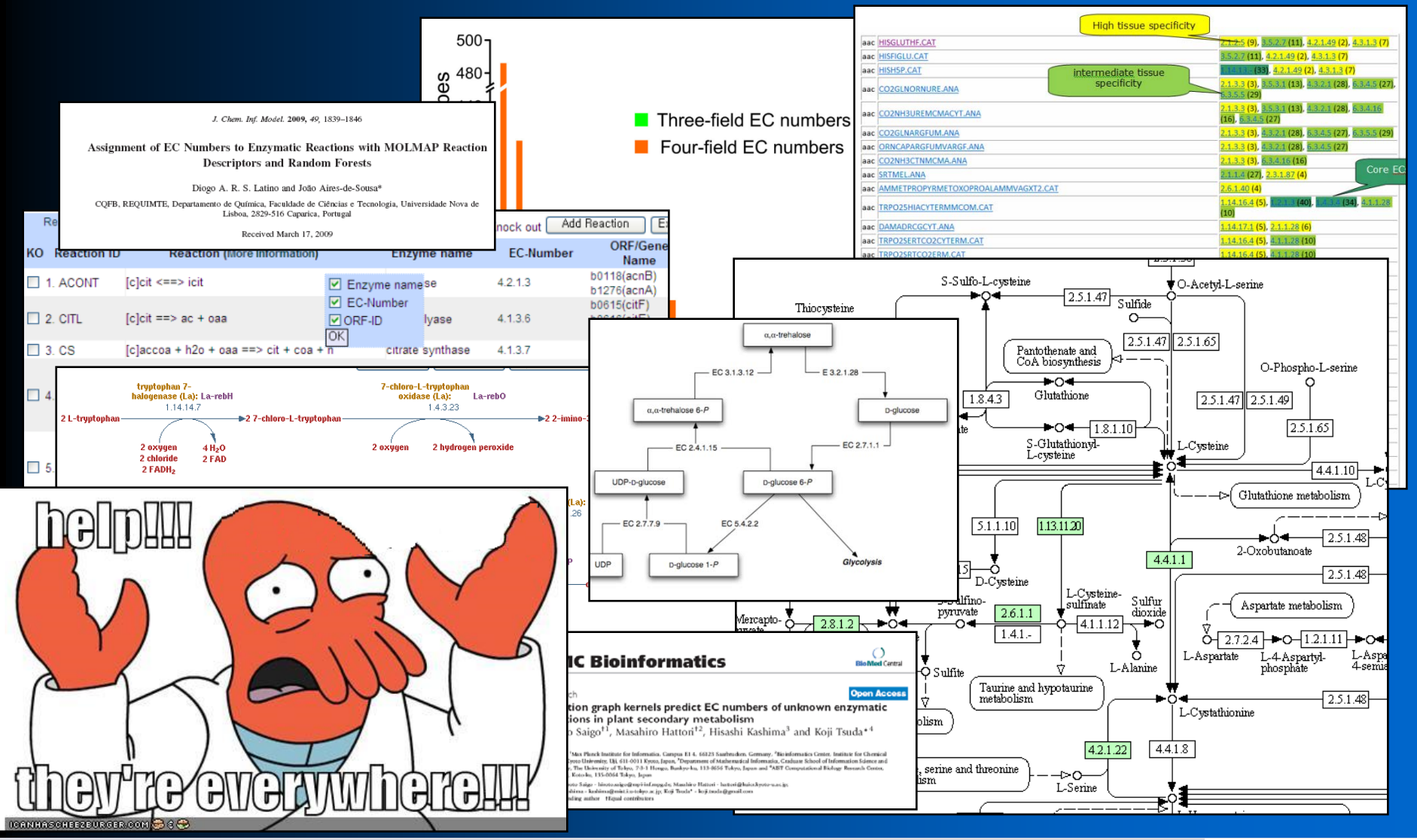
Ron Caspi

EC Numbers Are Everywhere



Historical Background

Back in the 1950s

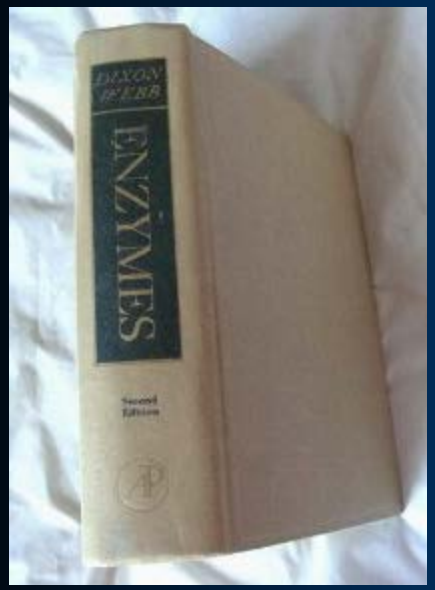
- The number of known enzymes was increasing rapidly
- No guiding authority
- The same enzymes became known by several different names, and
- The same name was sometimes given to different enzymes
- Names often conveyed little or no idea of the nature of the reactions catalyzed

The Situation Was Chaotic…

- Catalase (also known as equilase, caperase, optidase…)
- Diaphorase (dehydrogenase)
- Zwischenferment (glucose-6-phosphate dehydrogenase)

The First Enzyme Commission

In August 1955 M. Dixon and O. Hoffmann-Ostenhof convinced the president of the International Union of Biochemistry (IUB) to set up an International Enzyme Commission to tackle the problems



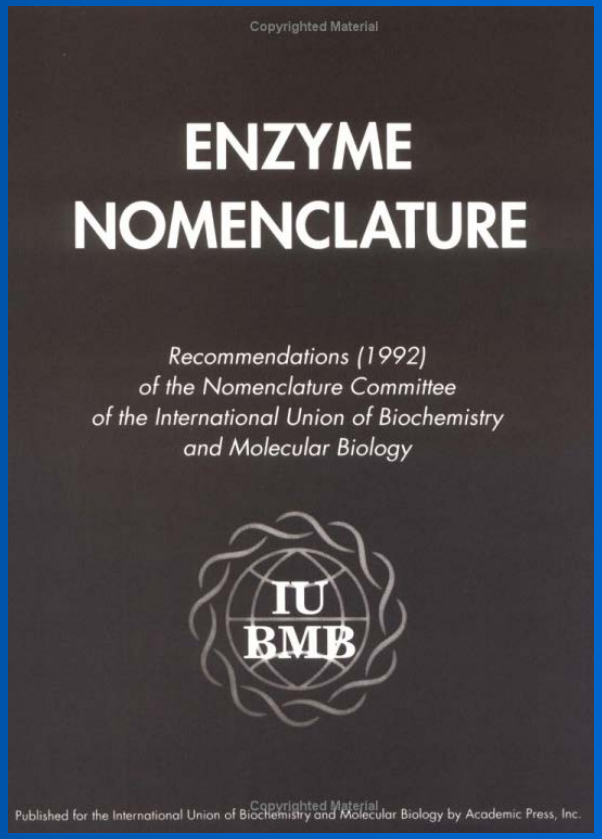
Members included:

- M. Dixon, U.K. (president)
- A.E. Braunstein, U.S.S.R.
- S.P. Colowick, U.S.A
- P.A.E. Desnuelle, France
- V.A. Engelhardt, U.S.S.R
- E.F. Gale, U.K
- O. Hoffmann-Ostenhof, Austria
- A.L. Lehninger, U.S.A.
- (K. Linderstrom-Lang, Denmark) E.C. Webb, UK
- F. Lynen, Germany

Drs. Mal Dixon and Otto Hoffmann-Ostenhof in action

The Basic Concept

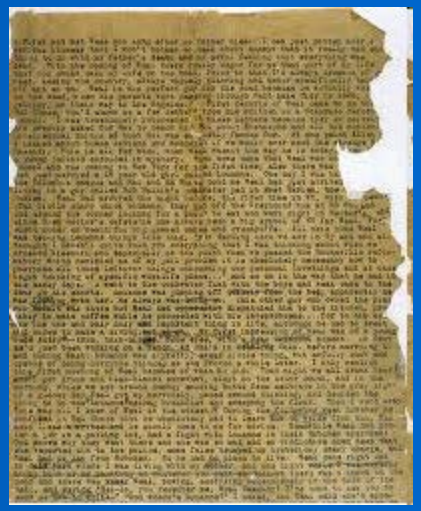
Enzymes are classified and named by the reactions they catalyze



The Reports of the First and Second Commissions

- The first EC list was presented in 1961 at the General Assembly of the IUB in Moscow
- Introduction of the Fundamental Concepts for classifications (to be discussed soon)

  712 entries



- Following this publication, the commission was dissolved, and the Standing Committee on Enzymes (only 4 of the original members) formed
- Published the second version in 1964 - 875 entries

The Expert Committee on Enzymes

- Formed in 1969 to revise the list
- Published the third document in 1972 - 1770 entries

Members:

- A.E. Braunstein, U.S.S.R.
- J.S. Fruton, USA
- O. Hoffmann-Ostenhof, Austria
- B.L. Horecker, USA
- W.B. Jakoby, USA
- P. Karlson, Germany
- B. Keil, France
- E.C. Slater, Holland
- E.C. Webb, United Kingdom
- W.J. Whelan, Australia

1977: Move to NC-IUB

- A more permanent solution was needed

- In 1977 two new nomenclature committees were formed:
  - The Nomenclature Committee of IUB (NC-IUB)
  - The IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN)
- NC-IUB (now NC-IUBMB) assumed responsibility for the EC list
- The 1978 the 4th EC list was published with 2122 entries

Current Status

- Ongoing curation by the NC-IUBMB since 1977
- Transition from print to online content
  - Last printed version (6th edition) published in 1992 (3196 entries)
  - A few supplements were published in *Eur. J. Biochem* (up to 1999)
  - All newer data is only available electronically. Currently there are 4314 entries

Current active full members:

- K.F. Tipton, Ireland (Trinity College Dublin)
- R. Cammack, UK (King's College London)
- G.P. Moss, UK (Queen Mary University of London)
- D. Schomburg, Germany (chairman) (BRENDA)

Active associate members:

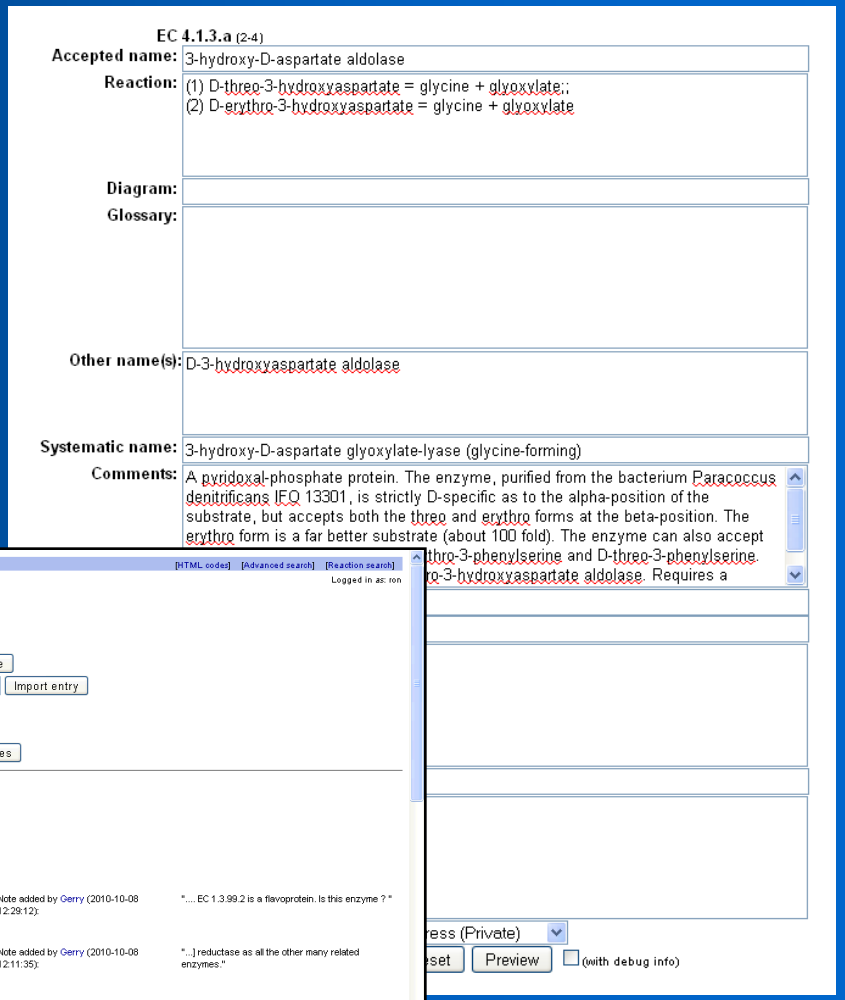
- A. McDonald, Ireland (Trinity College Dublin) – computer support
- K. Axelsen, Denmark (UniProt)
- R. Caspi, USA (MetaCyc)
- I. Schomburg, Germany (BRENDA)

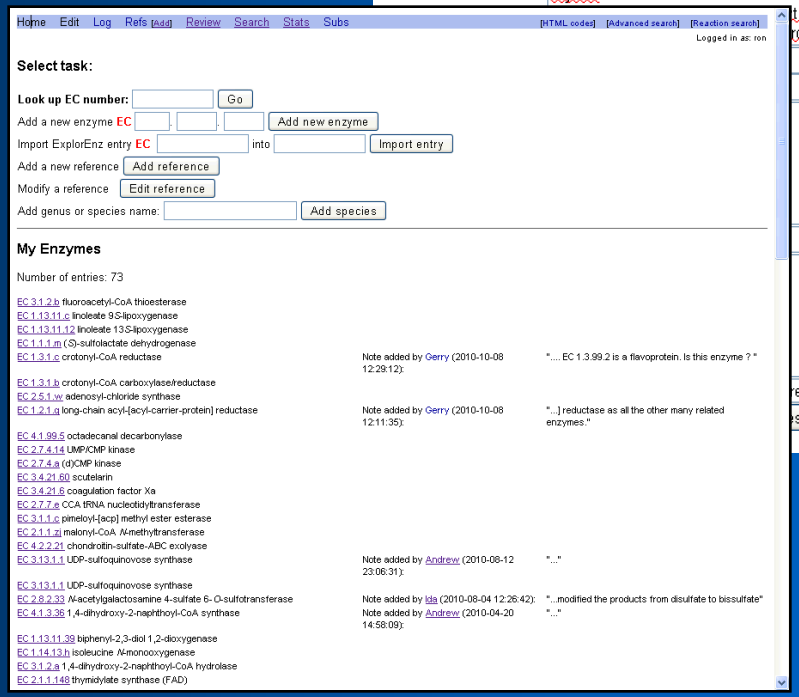
Curator (at BRENDA):

- C. Munaretto

DraftEnz

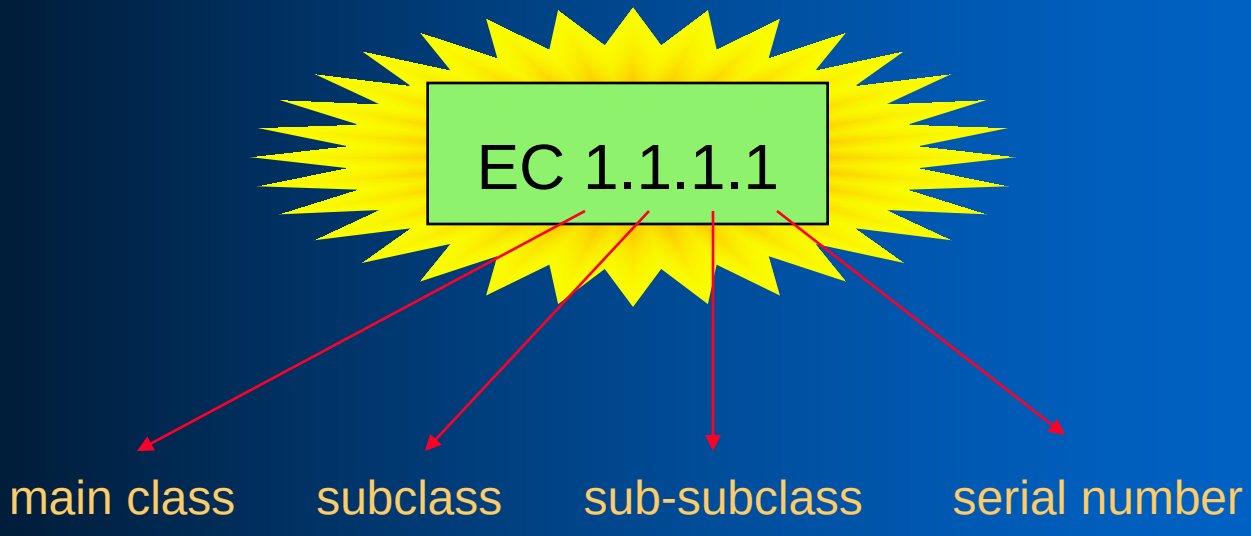
- DraftEnz is a MySQL database developed by Andrew McDonald from Trinity College that permits EC curators to enter, edit, and review enzyme entries
- Following initial curation in DraftEnz, each entry goes through a few weeks of private review and a month of public review in CurrEnz





The EC Number

Each enzyme is given a unique four-digit code, known as the Enzyme Commission, or EC, number



The Six Main Classes of Enzyme

EC 1.1.1.1

Class	Name	Reaction catalyzed
1.	Oxidoreductases	$AH_2 + B = A + BH_2$ or $AH_2 + B+ = A + BH + H+$
2.	Transferases	$AX + B = A + BX$
3.	Hydrolases	$A–B + H_2O = AH + BOH$
4.	Lyases	$A–B + X–Y = \begin{matrix} A–B \\ \vert\ \ \vert \\ X\ \ Y \end{matrix}$
5.	Isomerases	A = B
6.	Ligases	A + B + NTP = A–B + NDP + P or A + B + NTP = A–B + NMP + PP

Sub Classes and Sub-Subclasses

- Each of the six main classes is further subdivided
- The subclass generally contains information about the type of compound or group involved

EC 1.1.1.1

(e.g. 1.1. acts on the CH–OH group of donors whereas 1.3. acts on the CH–CH group of donors)

- The sub-subclass further specifies the type of reaction involved. (e.g. for the oxidoreductases, 1.-.1. indicates that NAD or NADP is the acceptor, 1.-.2. has cytochrome as the acceptor, etc

EC 1.1.1.1

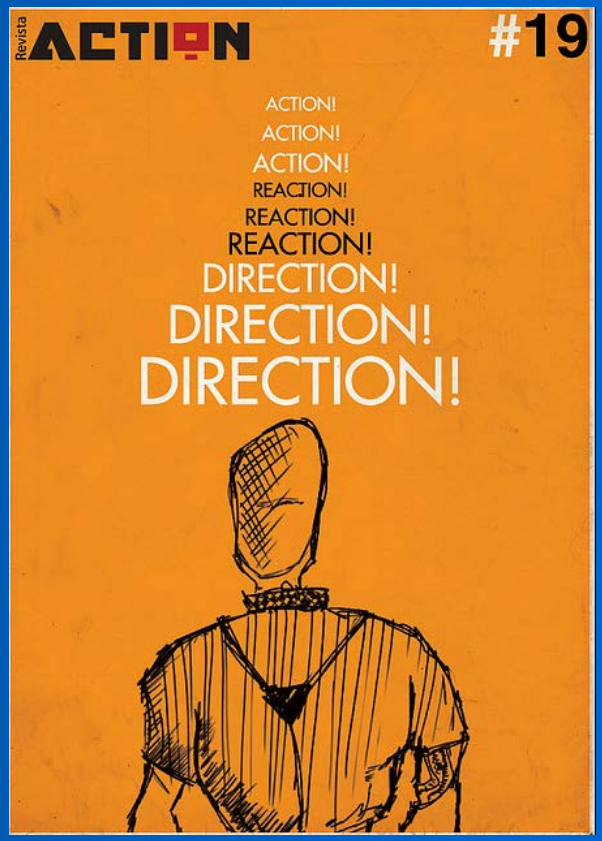
- The fourth digit is a serial number that is used to identify the individual enzymes within a sub-subclass

Sub Classes of Class 1

- **EC 1** — **Oxidoreductases**
  - EC 1.1 — Acting on the CH-OH group of donors
  - EC 1.2 — Acting on the aldehyde or oxo group of donors
  - EC 1.3 — Acting on the CH-CH group of donors
  - EC 1.4 — Acting on the CH-NH_2 group of donors
  - EC 1.5 — Acting on the CH-NH group of donors
  - EC 1.6 — Acting on NADH or NADPH
  - EC 1.7 — Acting on other nitrogenous compounds as donors
  - EC 1.8 — Acting on a sulfur group of donors
  - EC 1.9 — Acting on a heme group of donors
  - EC 1.10 — Acting on diphenols and related substances as donors
  - EC 1.11 — Acting on a peroxide as acceptor
  - EC 1.12 — Acting on hydrogen as donor
  - EC 1.13 — Acting on single donors with O_2 as oxidant and incorporation of oxygen into the substrate (oxygenases). The oxygen incorporated need not be derived from O_2
  - EC 1.14 — Acting on paired donors, with O_2 as oxidant and incorporation or reduction of oxygen. The oxygen incorporated need not be derived from O_2
  - EC 1.15 — Acting on superoxide as acceptor
  - EC 1.16 — Oxidizing metal ions
  - EC 1.17 — Acting on CH or CH_2 groups
  - EC 1.18 — Acting on iron-sulfur proteins as donors
  - EC 1.19 — Acting on reduced flavodoxin as donor
  - EC 1.20 — Acting on phosphorus or arsenic in donors
  - EC 1.21 — Acting on X-H and Y-H to form an X-Y bond
  - EC 1.22 — Acting on halogen in donors
  - EC 1.97 — Other oxidoreductases
- **EC 2** — **Transferases**
- **EC 3** — **Hydrolases**
- **EC 4** — **Lyases**
- **EC 5** — **Isomerases**
- **EC 6** — **Ligases**

Reaction Direction

- For consistency, the reaction direction is the same for all enzymes in a given class
- The *systematic* names, on which the classification and code numbers are based, may be derived from the written direction, even though only the reverse of this has been actually demonstrated experimentally
- Ideally, a comment would indicate that…



The Format

EC 1.13.12.17

Accepted name: dichloroarcyriaflavin A synthase

Reaction: dichlorochromopyrrolate + **4** O_2 + **4** NADH + **4** H^+ = dichloroarcyriaflavin A + **2** CO_2 + **6** H_2O + **4** NAD^+

For diagram of rebeccamycin biosynthesis, click here

Glossary: dichloro-arcyriaflavin A = rebeccamycin aglycone

Systematic name: dichlorochromopyrrolate,NADH:oxygen 2,5-oxidoreductase (dichloroarcyriaflavin A-forming)

Comments: The conversion of dichlorochromopyrrolate to dichloroarcyriaflavin A is a complex process that involves two enzyme components. RebP is an NAD-dependent cytochrome P_{450} oxygenase that performs an aryl-aryl bond formation yielding the six-ring indolocarbazole scaffold [1]. Along with RebC, a flavin-dependent hydroxylase, it also catalyses the oxidative decarboxylation of both carboxyl groups. The presence of RebC ensures that the only product is the rebeccamycin aglycone dichloroarcyriaflavin A [2]. The enzymes are similar, but not identical, to StaP and StaC, which are involved in the synthesis of staurosporine [3].

Links to other databases: BRENDA, EXPASY, IUBMB, KEGG

References:

1. Makino, M., Sugimoto, H., Shiro, Y., Asamizu, S., Onaka, H. and Nagano, S. Crystal structures and catalytic mechanism of cytochrome P_{450} StaP that produces the indolocarbazole skeleton. *Proc. Natl. Acad. Sci. USA* **104**:1159 (2007). [PMID: 17606921]
2. Howard-Jones, A.R. and Walsh, C.T. Staurosporine and rebeccamycin aglycones are assembled by the oxidative action of StaP, StaC, and RebC on chromopyrrolic acid. *J. Am. Chem. Soc.* **128**:1228 (2006). [PMID: 16967980]
3. Sanchez, C., Zhu, L., Brana, A.F., Salas, A.P., Rohr, J., Mendez, C. and Salas, J.A. Combinatorial biosynthesis of antitumor indolocarbazole compounds. *Proc. Natl. Acad. Sci. USA* **102**:461 (2005). [PMID: 15625109]

[EC 1.13.12.17 created 2010]

EC Numbers Define Enzymes, Not Reactions!

EC 1.5.3.17

Accepted name: non-specific polyamine oxidase

Reaction: (1) spermine + O_2 + H_2O = spermidine + 3-aminopropanal + H_2O_2
(2) spermidine + O_2 + H_2O = putrescine + 3-aminopropanal + H_2O_2
(3) N^1-acetylspermine + O_2 + H_2O = spermidine + 3-acetamidopropanal + H_2O_2
(4) N^1-acetylspermidine + O_2 + H_2O = putrescine + 3-acetamidopropanal + H_2O_2

Other name(s): polyamine oxidase (ambiguous); Fms1; AtPAO3

Systematic name: polyamine:oxygen oxidoreductase (3-aminopropanal or 3-acetamidopropanal-forming)

Comments: A flavoprotein (FAD). The non-specific polyamine oxidases may differ from each other considerably. The enzyme from *Saccharomyces cerevisiae* shows a rather broad specificity and also oxidizes N^8-acetylspermidine [3]. The enzyme from *Ascaris suum* shows high activity with spermine and spermidine, but also oxidizes norspermine [2]. The enzyme from *Arabidopsis thaliana* shows high activity with spermidine, but also oxidizes other polyamines [1]. The specific polyamine oxidases are classified as EC 1.5.3.13 (N^1-acetylpolyamine oxidase), EC 1.5.3.14 (polyamine oxidase (propane-1,3-diamine-forming)), EC 1.5.3.15 (N^8-acetylspermidine oxidase (propane-1,3-diamine-forming)) and EC 1.5.3.16 (spermine oxidase).

EC 1.5.3.16

Accepted name: spermine oxidase

Reaction: spermine + O_2 + H_2O = spermidine + 3-aminopropanal + H_2O_2

Other name(s): PAOh1/SMO; PAOh1 (ambiguous); AtPAO1; AtPAO4; SMO; mSMO; SMO(PAOh1); SMO/PAOh1; SMO5; mSMOmu

Systematic name: spermidine:oxygen oxidoreductase (spermidine-forming)

Comments: The enzyme from *Arabidopsis thaliana* (AtPAO1) oxidizes norspermine to norspermidine with high efficiency [3]. The mammalian enzyme, encoded by the SMOX gene, is a cytosolic enzyme that catalyses the oxidation of spermine at the exo (three-carbon) side of the tertiary amine. No activity with spermidine. Weak activity with N^1-acetylspermine. A flavoprotein (FAD). Differs in specificity from EC 1.5.3.13 (N^1-acetylpolyamine oxidase), EC 1.5.3.14 (polyamine oxidase (propane-1,3-diamine-forming)), EC 1.5.3.15 (N^8-acetylspermidine oxidase (propane-1,3-diamine-forming) and EC 1.5.3.17 (non-specific polyamine oxidase).

EC 1.5.3.13

Accepted name: N^1-acetylpolyamine oxidase

Reaction: (1) N^1-acetylspermidine + O_2 + H_2O = putrescine + 3-acetamidopropanal + H_2O_2
(2) N^1-acetylspermine + O_2 + H_2O = spermidine + 3-acetamidopropanal + H_2O_2

Other name(s): hPAO-1; PAO (ambiguous); mPAO; hPAO; polyamine oxidase (ambiguous)

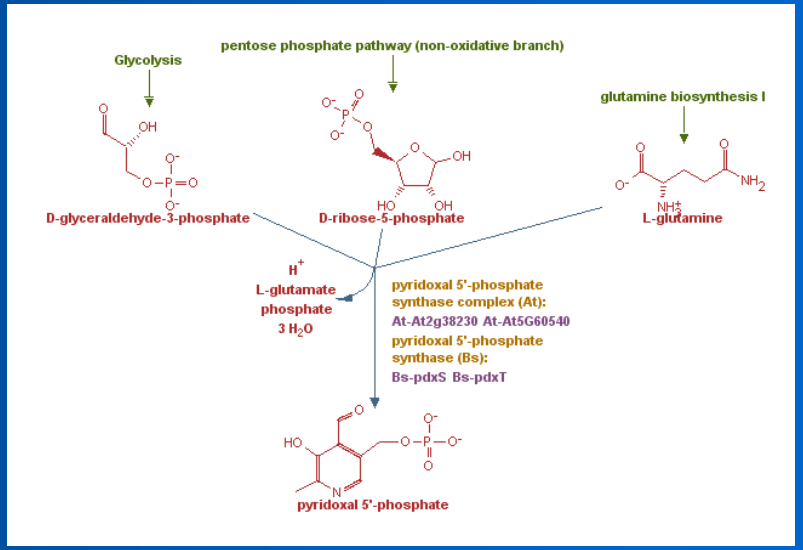
Systematic name: N^1-acetylpolyamine:oxygen oxidoreductase (3-acetamidopropanal-forming)

Comments: The enzyme also catalyses the reaction: N^1,N^{12}-diacetylspermine + O_2 + H_2O = N^1-acetylspermidine + 3-acetamamidopropanal + H_2O_2 [1]. No or very weak activity with spermine, or spermidine in absence of aldehydes. In presence of aldehydes the enzyme catalyses the reactions: 1. spermine + O_2 + H_2O = spermidine + 3-aminopropanal + H_2O_2, and with weak efficiency 2. spermidine + O_2 + H_2O = putrescine + 3-aminopropanal + H_2O_2 [2]. A flavoprotein (FAD). This enzyme, encoded by the PAOX gene, is found in mammalian peroxisomes and oxidizes N^1-acetylated polyamines at the exo (three-carbon) side of the secondary amine, forming 3-acetamamidopropanal. Since the products of the reactions are deacetylated polyamines, this process is known as polyamine back-conversion. Differs in specificity from EC 1.5.3.14 (polyamine oxidase (propane-1,3-diamine-forming)), EC 1.5.3.15 (N^8-acetylspermidine oxidase (propane-1,3-diamine-forming), EC 1.5.3.16 (spermine oxidase) and EC 1.5.3.17 (non-specific polyamine oxidase).

More accurately, an EC number stands for an active site. Enzymes with multiple active sites (e.g. if several genes fuse to encode a single polypeptide) should receive multiple EC numbers

Limitations

- No enzyme can be tested with all potential substrates…
- Enzymes that perform very complex reactions
  - pyridoxal 5'-phosphate synthase (glutamine hydrolyzing)
- Enzymes with a very broad substrate range (liver alcohol dehydrogenase)
- Old enzymes with a single reference - are they real?



Where Is the EC List?

- The primary source is

a MySQL database available at enzyme-database.org

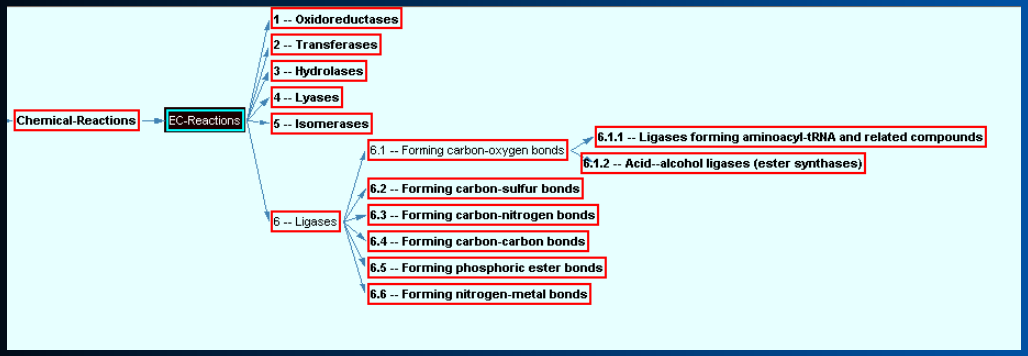
- Another database, prepared by Gerry Moss, is available at http://www.chem.qmul.ac.uk/iubmb/enzyme/

- A copy of the EC list is available via the ENZYME DB (SIB) at http://www.expasy.ch/enzyme/

- Yet another one is IntEnz at (EBI-SIB) http://www.ebi.ac.uk/intenz/index.jsp

- The EC list is also included in databases such as MetaCyc, BRENDA, KEGG etc.

EC Numbers and Pathway Tools



Each EC class, sub class and sub-sub class is implemented as a class in MetaCyc

MetaCyc Reaction: 1.97.1.3

Species Comparison

Superclasses: Reactions-Classified-By-Conversion-Type -> Simple-Reactions -> Chemical-Reactions -> EC-Reactions -> 1 -- Oxidoreductases -> 1.97 -- Other oxidoreductases -> 1.97.1 -- Sole sub-subclass for oxidoreductases that do not belong in the other subclasses

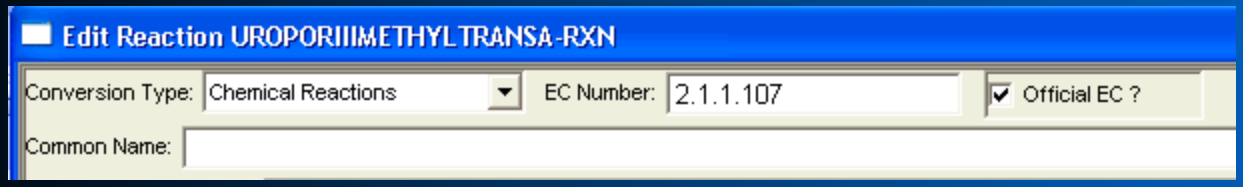
Reactions-Classified-By-Substrate -> Small-Molecule-Reactions

Enzymes and Genes:
sulfur reductase : sreC , sreB , sreA (Acidianus ambivalens)
H_2:sulfur oxidoreductase (Pyrodictium abyssi)

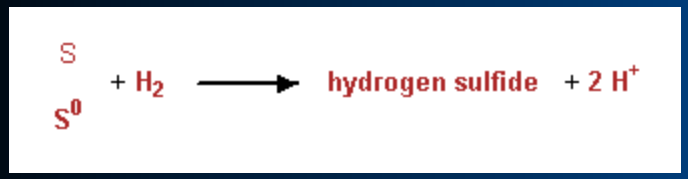
In Pathway: sulfur reduction I

Note that this reaction equation differs from the official Enzyme Commission reaction equation for this EC number, which can be found here .

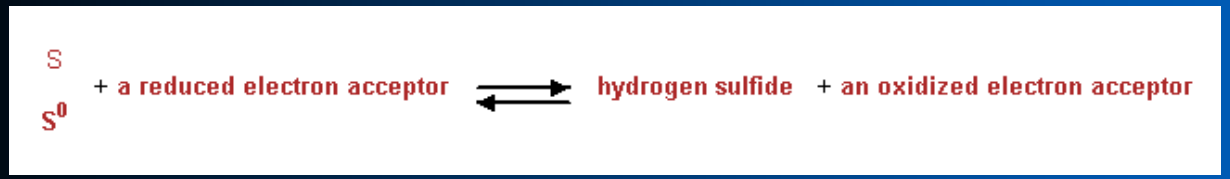
“Official” EC Numbers



- Reactions with full EC numbers can be marked “official” or “not official”
- A non-official reaction is one that matches the definition in the EC entry, yet differs from the exact reaction equation specified in the list



Non-official



Official

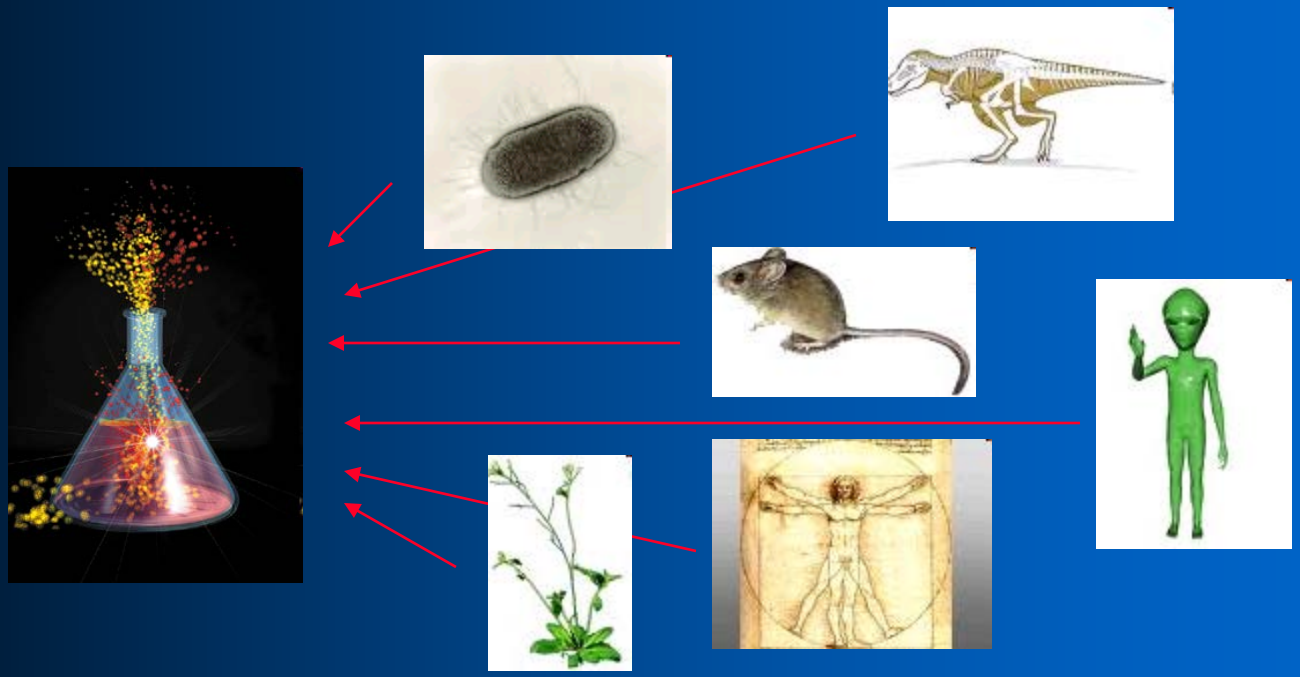
MetaCyc contains over 9000 reactions, out of which 5580 have a full EC number.

Partial EC Numbers

- Partial EC numbers look like EC numbers except the last number is replaced by a dash, e.g. 2.1.1.-
- Partial EC numbers should not be used for functional assignment!
- Partial EC numbers are used for two primary reasons:
  - Partial knowledge (2.1.1.- is the general class of methyltransferases)
  - A well characterized enzyme that has not received an EC number yet
- The use of EC 2.3.4.? Vs. EC 2.3.4.n (Green and Karp 2005)

When To Assign A Full EC Number?

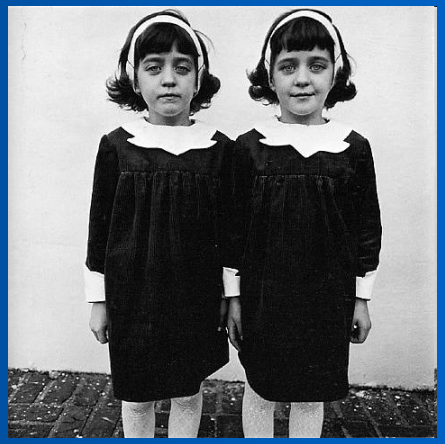
One simple rule: Assign a full EC number to a reaction only if you want the name matcher to attribute this reaction to every enzyme, in every genome, that is annotated with this number.



EC Numbers and Pathway Tools - Problems

- Currently, EC numbers are associated with Pathway Tools reactions rather than enzymes

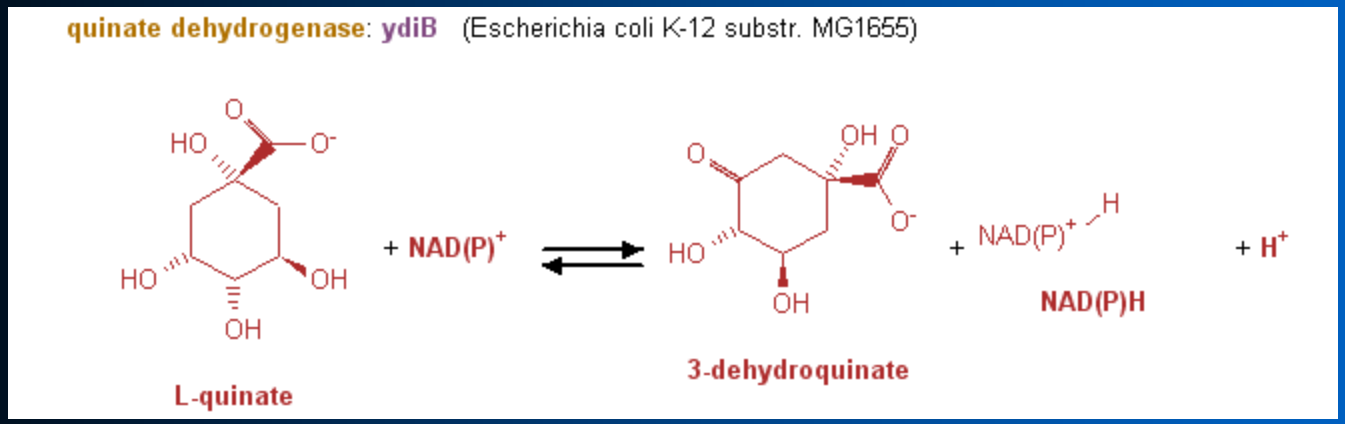
- This leads to reaction duplication



When several EC enzymes are characterized with overlapping reactions, we need to have duplicate identical reactions, each with a different EC number

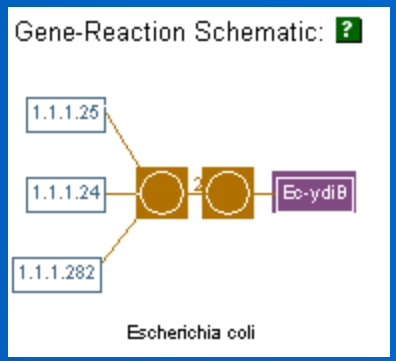
Another Problem: Incorrect Interpretation

- The E. coli YdiB protein is EC 1.1.1.282, quinate dehydrogenase



- Pathway Tools automatically expands that reaction to the two following reactions and links them to the enzyme.

L-quinate + NADP+ = 3-dehydroquinate + NADPH + H+
L-quinate + NAD+ = 3-dehydroquinate + NADH + H+



- Problem is, these two reactions are associated with the EC numbers EC 1.1.1.25 and EC 1.1.1.24, which describe other enzymes

What We Can Do About It

- Separate the reactions from the EC numbers, permitting multiple EC numbers per reaction and multiple reactions per EC number

Conclusion Remarks

- EC Numbers are very useful
- There are thousands of characterized enzymes w/o EC numbers
- Expansion of the EC list is slow
- Urgent Need to accelerate
- Why so little funding?
- Should we ask NIH to step up?