Research Article

Specific and Nonhomologous Isofunctional Enzymes of the Genetic Information Processing Pathways as Potential Therapeutical Targets for Tritryps

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Received 15 January 2011; Revised 22 March 2011; Accepted 5 May 2011

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Leishmania major, Trypanosoma brucei, and Trypanosoma cruzi (Tritryps) are unicellular protozoa that cause leishmaniasis, sleeping sickness and Chagas’ disease, respectively. Most drugs against them were discovered through the screening of large numbers of compounds against whole parasites. Nonhomologous isofunctional enzymes (NISEs) may present good opportunities for the identification of new putative drug targets because, though sharing the same enzymatic activity, they possess different three-dimensional structures thus allowing the development of molecules against one or other isoform. From public data of the Tritryps’ genomes, we reconstructed the Genetic Information Processing Pathways (GIPPs). We then used AnEnPi to look for the presence of these enzymes between Homo sapiens and Tritryps, as well as specific enzymes of the parasites. We identified three candidates (ECs 3.1.11.2 and 6.1.1.-) in these pathways that may be further studied as new therapeutic targets for drug development against these parasites.

1. Introduction

Recent estimates indicate that more than one billion people, living in tropical and subtropical regions of developing countries, are at the risk of contracting diseases (which are mostly endemic at these places) caused by the protozoans Leishmania major, Trypanosoma brucei, and Trypanosoma cruzi (Tritryps) are unicellular protozoa that cause leishmaniasis, sleeping sickness and Chagas’ disease, respectively. Most drugs against them were discovered through the screening of large numbers of compounds against whole parasites. Nonhomologous isofunctional enzymes (NISEs) may present good opportunities for the identification of new putative drug targets because, though sharing the same enzymatic activity, they possess different three-dimensional structures thus allowing the development of molecules against one or other isoform. From public data of the Tritryps’ genomes, we reconstructed the Genetic Information Processing Pathways (GIPPs). We then used AnEnPi to look for the presence of these enzymes between Homo sapiens and Tritryps, as well as specific enzymes of the parasites. We identified three candidates (ECs 3.1.11.2 and 6.1.1.-) in these pathways that may be further studied as new therapeutic targets for drug development against these parasites.

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Leishmania major, Trypanosoma brucei, and Trypanosoma cruzi (Tritryps) are unicellular protozoa that cause leishmaniasis, sleeping sickness and Chagas’ disease, respectively. Most drugs against them were discovered through the screening of large numbers of compounds against whole parasites. Nonhomologous isofunctional enzymes (NISEs) may present good opportunities for the identification of new putative drug targets because, though sharing the same enzymatic activity, they possess different three-dimensional structures thus allowing the development of molecules against one or other isoform. From public data of the Tritryps’ genomes, we reconstructed the Genetic Information Processing Pathways (GIPPs). We then used AnEnPi to look for the presence of these enzymes between Homo sapiens and Tritryps, as well as specific enzymes of the parasites. We identified three candidates (ECs 3.1.11.2 and 6.1.1.-) in these pathways that may be further studied as new therapeutic targets for drug development against these parasites.

1. Introduction

Recent estimates indicate that more than one billion people, living in tropical and subtropical regions of developing countries, are at the risk of contracting diseases (which are mostly endemic at these places) caused by the protozoans Leishmania major, Trypanosoma brucei, and Trypanosoma cruzi [1–3]. These three microorganisms, together known as the Tritryps (family Trypanosomatidae, order Kinetoplastida), also cause the death of thousands of people every year [4]. Despite all these facts, these infirmities are still considered as neglected diseases by the health agencies [5].

The control of the diseases caused by these parasites depends nowadays on chemicals, vaccines not being commercially available so far. Besides, there is a very limited set of pharmaceuticals available at this moment: most of them were discovered at approximately 50 years ago, and they also have disadvantages like high toxicity, low efficacy, or high costs; the development of resistance is also a possibility [6–8]. However, with the recent publication of the Tritryps’ genomes [9–11], new opportunities allowed a better understanding of several biological processes that, up to this point, were poorly understood or even unknown in these organisms [7, 12].

Cellular functions are based on complex networks of chemical reactions that interact producing observable results. The rapid development of DNA sequencing techniques provided a huge amount of information leading to a new comprehension about the organization of cellular processes. First, by using annotation data, genes are classified in groups in accordance with their functions. Part of the gene products are enzymes, proteins that catalyze cellular reactions, making part of complex biochemical pathways. In the postgenome era, the study of these processes is gaining an importance, to improve the comprehension of the dynamics and regulation of these pathways, as well as the discovery of previously unknown steps [13, 14].

The reconstruction of biochemical pathways is considered to be one essential step in the study of cellular processes [15]. Applications of these reconstructions may vary from
the drawing of the biological system to the generation of testable hypotheses about the structure and working of the pathway and from the elucidation of complex properties not inferred by the simple description of the individual components to the recognition of potential drug targets against pathogenic organisms via the identification of essential steps in these processes [16]. Several methods and databases are available for the reconstruction of said pathways from genome information; one of the main resources for this task is the KEGG database [13, 17, 18]. One way to link the biological processes to the genomic information is through the EC numbers, which represent the reaction each enzyme catalyzes. There are other types of functional classifications, (reviewed by Ouzounis and collaborators [19]), but the EC classification system is certainly one of the most used by the scientific community.

Enzymes have a high degree of specificity for their substrates and are fundamental for any biochemical process. They act in an organized sequence, catalyzing successive reactions in enzymatic pathways, guaranteeing the maintenance of life in all organisms [20]. A particular group of enzymes, the nonhomologous isofunctional enzymes (NISE or analogous enzymes), executes the same function in different organisms, but without detectable similarity between their primary structures and, possibly, between their tertiary structures as well. Once analogy is detected between a pathogen’s enzyme and its human counterpart, it may be possible to use this analog as a potential target for drug development, provided it belongs to an essential biochemical component to the recognition of potential drug targets against pathogenic organisms via the identification of essential steps in common between these parasites and other species. A different study in which enzymes are considered analogous (i.e., with different evolutionary origins) according to different functions comprising a certain pathway were extracted from these descriptions as a collection of EC numbers and were used as templates for the reconstruction of the correspondent pathways in Tritryps. Each pathway is associated with a set of proteins, usually a list of enzyme families with their EC numbers. KEGG has a total of 10 maps distributed among these pathways: 6 maps representing replication and repair; 2 maps symbolizing the transcription, but only one with an associated EC number; 2 translation maps of which only one has an associated EC number.

2. Methodology

2.1. Predicted Protein Sequences of Tritryps. The dataset of predicted proteins of Leishmania major, Trypanosoma brucei, and Trypanosoma cruzi was obtained from TritrypDB (http://tritrypdb.org/tritrypdb/) as shown in Table 1.

2.2. Pathways and Enzyme Classes. A set of pathways (maps) referring to the replication and repair, transcription and translation processes was obtained from KEGG (http://www.genome.jp/kegg/pathway.html#genetic). This dataset contains a complete biochemical description of the pathways related to genetic information processing observed in different organisms. Functions comprising a certain pathway were extracted from these descriptions as a collection of EC numbers and were used as templates for the reconstruction of the correspondent pathways in Tritryps. Each pathway is associated with a set of proteins, usually a list of enzyme families with their EC numbers. KEGG has a total of 10 maps distributed among these pathways: 6 maps representing replication and repair; 2 maps symbolizing the transcription, but only one with an associated EC number; 2 translation maps of which only one has an associated EC number.

2.3. Clustering. To group homologous enzymes with the same activity, we used the AnEnPi pipeline (http://www.dbmm.fiocruz.br/AnEnPi/) [22], which was based on a previous study in which enzymes are considered analogous (i.e., with different evolutionary origins) according to differences in their primary structures [24]. After clustering, enzymes within a given cluster are considered homologous, while enzymes in different clusters (of the same function) are considered analogous. As the cut-off parameter used in AnEnPi is based on experimental data obtained from enzymes, other values should probably be employed for other types of proteins.

2.4. Protein Function Inference. Using another module of AnEnPi, we were able to infer function of the predicted proteins of trypanosomatids using the groups (or clusters) obtained after clustering. In this module, the EC number assignment is based on the sequence similarity report from a BLASTP [28] procedure: predicted proteins of Tritryps (query) against the sequences of each individual AnEnPi cluster (subject), as described in detail in [22]. The cutoff employed for functional inference was the e-value of $e^{-20}$.

2.5. Genetic Information Processing Pathways Reconstruction and Search for NISE and Specific Enzymes. The reconstruction of the GIPPs was performed using the data inferred by the AnEnPi pipeline. After functional inference, enzymatic activities shared by Tritryps were disclosed using scripts written in Perl language. NISE and specific enzymes were obtained through an examination of the groups (or clusters) produced after clustering, where sequences of Tritryps and
**3. Results and Discussion**

3.1. **KEGG, Clustering, and Enzymatic Activity Inference.** The Tritryps' genomes were first sequenced in 2005 [9–11], with all chromosomes well characterized (with the exception of *T. cruzi* due to the high degree of repetitions in its genome). However, some of the GIPPs still present gaps [29]. The computational reconstruction of these processes, in this work, is an attempt to obtain a better representation of them, with emphasis on the analogous and specific enzymes. These analogs are enzymes that, even with a small or no significant similarity between their primary structures (which reflect in differences in their 3D structure), are able to catalyze the same reaction [24]. For these reasons, recent efforts have been made to include this phenomenon in the functional annotations [21, 22, 30]. Inference of function, if based only on sequence similarity, may be insufficient since they are usually not able to detect nonhomologous isofunctional enzymes.

Tritryps share a series of features, like the presence of subcellular structures such as the kinetoplast and glycosomes. Each trypanosomatid is transmitted by a different vector, possessing distinct life cycles, tissue specificity, and pathogenies in their mammal host [31, 32]. In addition, they are considered “ancient” from an evolutionary perspective; in fact, they present peculiar mechanisms in some of the genetic information transmission processes. Many of these still have gaps to be filled [33]. In this context, we have compared the number of enzymatic activities shared among the three microorganisms (taking into account all pathways) and the unique activities based on the results obtained after clustering (Figure 1). It may be worth noticing that some activities found have the same isoform (or, more precisely, analog form) in the three microorganisms; this may serve as a basis (ideally and depending on several other factors) for one unique drug for the three pathogens or (much more likely) a family of related/similar molecules as drugs.

KEGG has its own annotation protocol, which to our knowledge is not described in detail anywhere; only its general lines are known [17, 34]. We opted to make a functional inference from all the predicted proteins of Tritryps, in order to have a unified and comparable data. For this, we performed a BLASTP of the available predicted proteins in the TriTrypDB against the obtained clusters. From this it was possible to infer functions not detected by KEGG, in almost all pathways studied. Even using a very restrictive cut-off (*e*-value < $10^{-20}$), more enzymes were identified (data not shown), indicating the validity of this approach. In fact, even after using more restrictive *e*-values, like $10^{-30}$ or $10^{-40}$, results did not differ for several ECs (data not shown). With these information, some of the GIPPs were reconstructed. The description of the enzymatic activities found by AnEnPi for each Tritryp is listed in Table 2.

3.2. **Computational Reconstruction of the GIPPs.** Figure 2 displays the computational reconstruction of the GIPPs using the map representing the aminoacyl-tRNA biosynthesis (map 00970) as an example. The other 7 maps, as well as the tables with the description of the enzymes highlighted in each map, are available in the Supplementary Material available online at doi:10.4061/2011/543912. In this map, all enzymatic activities detected by KEGG were also identified by AnEnPi for the Tritryps, with the exception of SepRS (EC 6.1.1.27). This enzyme participates of the alternative formation of Cys-tRNACys linking O-phosphoserine, a precursor of the aminoacid cysteine, to tRNACys. Then SepCysS (Sep-tRNA:Cys-tRNA synthetase—EC 2.5.1.73) converts O-phosphoseryl-tRNACys in cysteinytl-tRNACys. This alternative formation of Cys-tRNACys has been only detected in methanogenic archaea so far, where in some species the enzyme cysteinyl-tRNA synthetase (EC 6.1.1.16), which catalyses the direct production of Cys-tRNACys, is lacking [35, 36]. However, we could not identify the second enzyme which completes the alternative formation of Cys-tRNACys,
Table 2: List of additional ECs found in each process from GIPPs.

| Pathway number | Map description                | T. cruzi | T. brucei | L. major | EC description                          |
|----------------|--------------------------------|----------|-----------|----------|-----------------------------------------|
| Map00970       | Aminoacyl-tRNA biosynthesis    | 2.1.2.9  | a         | b        | Methionyl-tRNA formyltransferase         |
|                |                                | 6.1.1.   | 6.1.1.    | 6.1.1.   | O-phosphoseryl-tRNA synthetase          |
| Replication and repair |
| Map03030       | DNA replication               | 3.6.1.-  | 3.6.1.-   | 3.6.1.-  | Hydrolases acting in phosphorus-containing anhydrides |
| Map03410       | Base excision repair          | 3.1.11.2 | 3.1.11.2  | 3.1.11.2 | Hydrolases acting on ester bonds         |
|                |                                | 2.7.11.22 | 2.7.11.22 | 2.7.11.22 | Cyclin-dependent kinase                  |
| Map03420       | Nucleotide excision repair    | 3.1.11.2 | 3.1.11.2  | 3.1.11.2 | Hydrolases acting in phosphorus-containing anhydrides |
| Map03430       | Mismatch repair               | 3.1.11.2 | 3.1.11.2  | 3.1.11.2 | Hydrolases acting on ester bonds         |
| Map03440       | Homologous recombination      | 3.1.11.2 | 3.1.11.2  | 3.1.11.2 | Hydrolases acting on ester bonds         |
| Map03450       | Nonhomologous end-joining     | 2.7.11.22 | 2.7.11.22 | 2.7.11.22 | DNA-directed DNA polymerase             |
|                |                                | 3.1.11.2 | 3.1.11.2  | 3.1.11.2 | Hydrolases acting on ester bonds         |
|                |                                | 4.2.99.- | 4.2.99.-  | 4.2.99.- | Other carbon-oxygen lyases              |

*Previously identified by KEGG as entry “Tb11.01.7110”.  
*bPreviously identified by KEGG as entry “LmjF32.2240”.

SepCysS. One possible explanation is that, while this pathway is essential to archaea (that do not possess the direct pathway for Cys-tRNACys formation), it is not for the Tritryps. Or yet, this enzyme has a particular gene sequence or structure, not yet examined experimentally.

The enzymatic activity represented by EC 2.1.2.9 (methionyl-tRNA formyltransferase), which is also part of the aminoacl-tRNA biosynthesis map, was identified by KEGG only for L. major and T. brucei; this activity was identified by AnEnPi in T. cruzi. This enzyme is responsible for adding the formyl radical to tRNAMet, which serves as the tRNA initiator of the polypeptide chain during translation in bacteria. It has the same function in eukaryotes, acting in mitochondria [27]. Since mitochondria have a bacterial evolutionary origin, their translational apparatus follow the bacterial model. Genomic data of the organisms studied in this work consists mainly of nuclear DNA. The occurrence of this enzyme in nuclear DNA is in agreement with the observed absence of tRNA genes in the mitochondrial DNA of Tritryps (kDNA), which are imported from the cytoplasm [37–39].

DNA in cells is often under attack by mutagens, oxygen radicals, and ionizing radiation, and even cellular processes can create mutagenic and cytotoxic DNA lesions which can be lethal to the cell. Organisms possess broad mechanisms of DNA repair to fix damaged DNA and in order to keep viability and genomic stability [40]. In this context, we identified four enzymatic activities with complete EC numbers (four digits) from three DNA repair pathways: base excision repair (EC 3.1.11.2), nucleotide excision repair (EC 2.7.11.22), and nonhomologous end-joining (EC 2.7.11.1 and EC 2.7.7.7) (Table 2).

The enzyme exodeoxyribonuclease III (EC 3.1.11.2—Figure S3 and Table S4) is responsible to catalyze the degradation of double-stranded DNA acting progressively in a 3′ to 5′ direction, releasing 5′-phosphomononucleotides on base excision repair (BER) pathway. The enzymes of this pathway are conserved from bacteria to man, but mammalian enzymes frequently add in, within a larger structural framework, the catalytic core domains of bacterial enzymes [40, 41].

Cyclin-dependent kinase (EC 2.7.11.22) from nucleotide excision repair (NER) is linked to a complex called holo-TFIIF complex (Figure S4 and Table S5). This is a multiprotein complex required not only for transcription but also for nucleotide excision repair. This enzyme is responsible for the phosphorylation of the carboxy-terminal domain (CTD) of RNA polymerase II in the absence of promoter opening [42].

Nonhomologous end-joining (NHEJ) is a kind of recombination that links the ends from broken nonhomologous chromosomes. The core NHEJ components are conserved from yeast to mammals and consist of the XRCC4/DNA-Ligase IV complex and the Ku70/Ku80 heterodimer. Both protect exposed DNA of degradation. First, the catalytic subunit, formed by DN-APKcs (EC 2.7.11.1—nonspecific serine/threonine protein kinase) and Artemis, is recruited. The DNA-PKcs phosphorylate the Ku heterodimer and also the Artemis complex which corresponds to a nuclease. Interactions between such protein complexes approximate
the chromosomal ends. Another enzyme whose participation is essential in such complex is the DNA-directed DNA polymerase (EC 2.7.7.7) which fills in the gaps when the ends are joined (Figure S6 and Table S7) [43–45].

### 3.3. Specific Enzymes and Functional Analogs between the Tritryps and Homosapiens as Potential Therapeutic Targets.

Data produced by the genome projects of the Tritryps allowed researchers to establish new strategies to solve the problems caused by these diseases, which affect a great percentage of the world’s population [46]. The majority of the proposed drugs so far were discovered many years ago and several of them are toxic, have low efficacy, and the risk of resistance development is also a possibility [7]. To search for functional analogs that could serve as potential candidates as drug targets, we looked for the presence of these enzymes between the Tritryps and *H. sapiens* as Potential Therapeutic Targets.

Exodeoxyribonuclease III is an exonuclease that cleaves the 5′ side of an AP (apurinic/apyrimidinic) site, acting in...
the repair pathway by base excision [47]. In *Escherichia coli*
this enzyme is a DNA-modifying enzyme, very frequently
used in molecular biology, which degrades single-stranded
DNA as a substrate. We searched for more information
about the inhibitors of this enzyme in the BRENDA
database (http://www.brenda-enzymes.org/). According to
Hoheisel [48], double-stranded DNA was found to be a
competitive inhibitor of the enzyme activity. Other known
inhibitors are EDTA (Ethylenediamine tetraacetic acid) [49],
Mn\(^{2+}\) at concentrations above 5 mM [50], NaCl [48], p-
chloromercuribenzoate [51], PNA (Peptide nucleic acids)
[52], and ZnCl\(_2\) [51, 53].

Apurinic/apyrimidinic sites are very toxic to cells if not
repaired. These sites can be generated by normal aerobic
metabolism, UV light, or H\(_2\)O\(_2\). Exodeoxyribonuclease III
(*xthA* gene) can be considered a relevant target for Tritryps
because it plays an essential role in the BER pathway, a key
repair system to neutralize DNA oxidative stress. *E. coli* *xthA*
mutant strains hold a residual AP endonucleolytic activity
due to the protein encoded by the *nfo* gene, the endonuclease
IV (Endo IV). Mutants of *nfo* or *xthA* genes are generally
sensitive to oxidizing agents [54]. Some authors pointed out
that Exo III is involved in the protection of *E. coli* cells against
the toxic effects of UV light, H\(_2\)O\(_2\) [54–57] and is necessary
to induce DNA damage repair [58].

Moreover, we have also identified a potential therapeutic
target unique for *L. major*, the DNA 3-methyladenine
glycosilase II (EC 3.2.2.21). This enzyme consists in a glycosilase
which breaks the bond between alkylated nitrogenated bases
and their phosphate group, removing it and leaving an AP
site [59, 60].

O-phosphoseryl-tRNA synthetase (EC 6.1.1.-), assigned
to the Aminoacyl-tRNA biosynthesis map, was identified as a
specific activity in Tritryps when compared with *H. sapiens.*
This enzyme, today designated by the EC number 6.1.1.27,
catalyzes the alternative formation of Cys-tRNACys [61], as
previously described.

The TDR Targets database (http://tdrtargets.org/) inte-
grates genetic and biochemical information to pharmacolog-
data, all related to (primarily) tropical pathogens. The
main objective is to assist the search for targets using an
integrative platform [62]. None of the two ECs identified
(EC 3.1.11.2 and EC 6.1.1.27) had any information related to
the Tritryps in this database. This suggests that the approach
used in this work may increase the number of possible drug
targets. However, exodeoxyribonuclease III (EC 3.1.11.2) is
assigned as a potential target in this database, but for other
organisms. In addition, DNA 3-methyladenine glycosilase
II (EC 3.2.2.21), which in this work was identified only in
*L. major*, is also assigned as a potential target (again, for other
organisms, not for *Leishmania*).

None of the enzymatic functions disclosed in this work
has a resolved 3D structure in the PDB database for any of
the Tritryps. Use of resolved 3D structures, as well as other
types of information like functional studies, is paramount to
advance research on these enzymes, to ensure that they are
indeed possible targets for drug development. In the present
work, we have studied only a part of the pathways assigned
to the GIPPs in KEGG. We have left aside other important
pathways such as those related to protein folding, sorting,
and degradation, consisting in about 7 additional maps with
several enzymes. Moreover, KEGG has already integrated
more information and maps to the GIPPs, since it is updated
weekly. In the future, a thorough reevaluation of the available
data may disclose new cases of analogy and/or new specific
enzymes.

The utilization of computers is constantly increasing in
the field of drug discovery, because of the great potential
in speeding up the identification of suitable targets and
useful compounds and also (arguably the most important
feature) in reducing costs. In this work, the development and
utilization of computational methods allowed us to identify,
in the genetic information processing pathways of Tritryps,
specific and nonhomologous isofunctional enzymes (NISE).
The identification of NISE allowed the construction of an
enriched list of proteins (containing not only organism-
specific enzymes) that must be further studied to be validated
as drug targets. Among these studies, we can cite (i) the
obtention of crystals of the selected proteins to allow the
construction of 3D models by molecular modeling, (ii)
molecular dynamics and docking studies, to obtain a refined
representation of their structure, including movement and
possibly other interacting molecules as well, and (iii) a series of
functional studies to determine their kinetics, expression
patterns, stability, essentiality, and so forth.

**Acknowledgments**

The authors thank CAPES, CNPq, FAPERJ, PDTIS/Fiocruz,
and PAPES/Fiocruz for financial support. They are grateful
to Dr. Wim Degrave for the valuable discussions and support.

**References**

[1] U. González, M. Pinart, M. Rengifo-Pardo, A. Macaya, J.
Alvar, and J. A. Tweed, “Interventions for American cutaneous
and mucocutaneous leishmaniasis,” *Cochrane Database of*
*Systematic Reviews*, no. 2, article CD004834, 2009.

[2] World Health Organization, “African trypanosomiasis (sleep-
ing sickness),” Fact sheet N°259, October 2010, http://www.
who.int/mediacentre/factsheets/fs259/.

[3] World Health Organization, “Chagas disease (American try-
panosomiasis),” Fact sheet N°340, June 2010, http://www.who.
int/mediacentre/factsheets/fs340/.

[4] M. P. Barrett, R. J. S. Burchmore, A. Stich et al., “The trypano-
somiases,” *The Lancet*, vol. 362, no. 9394, pp. 1469–1480, 2003.

[5] WHO, *Global Plan to Combat Neglected Diseases 2008–2015*,
WHO, Geneva, Switzerland, 2007, WHO/CDS/NTD/
2007.003.

[6] M. P. Barrett and I. H. Gilbert, “Perspectives for new drugs
against trypanosomiasis and leishmaniasis,” *Current Topics in
Medicinal Chemistry*, vol. 2, no. 5, pp. 471–482, 2002.

[7] C. R. Caffrey and D. Steverding, “Recent initiatives and strate-
gies to developing new drugs for tropical parasitic dis-eases,”
*Expert Opinion on Drug Discovery*, vol. 3, no. 2, pp. 173–186,
2008.
[8] M. P. Barrett, G. H. Coombs, and J. C. Mottram, “Recent advances in identifying and validating drug targets in trypanosomes and leishmanias,” Trends in Microbiology, vol. 7, no. 2, pp. 82–88, 1999.

[9] M. Berri man, E. Ghedin, C. Hertz-Fowler et al., “The genome of the African trypanosome Trypanosoma brucei,” Science, vol. 309, no. 5733, pp. 416–422, 2005.

[10] N. M. A. El-Sayed, P. Myler, D. C. Bartholomeu et al., “The genome sequence of Trypanosoma cruzi, etiologic agent of Chagas disease,” Science, vol. 309, no. 5733, pp. 409–415, 2005.

[11] A. C. Ivens, C. S. Peacock, E. A. Worthey et al., “The genome of the kinetoplastid parasite, Leishmania major,” Science, vol. 309, no. 5733, pp. 436–442, 2005.

[12] S. Kaur, A. V. Shivange, and N. Roy, “Structural analysis of trypanosomal sirtuin: an insight for selective drug design,” Molecular Diversity, vol. 14, no. 1, pp. 169–178, 2010.

[13] H. Ma and A. P. Zeng, “Reconstruction of metabolic networks from genome data and analysis of their global structure for various organisms,” Bioinformatics, vol. 19, no. 2, pp. 270–277, 2003.

[14] B. Ø. Palsson, Systems Biology: Properties of Reconstructed Networks, Cambridge University Press, New York, NY, USA, 1st edition, 2006.

[15] A. M. Feist, M. J. Herrgård, I. Thiele, J. L. Reed, and B. Ø. Palsson, “Reconstruction of biochemical networks in microorganisms,” Nature Reviews Microbiology, vol. 7, no. 2, pp. 129–143, 2009.

[16] J. A. Papin, N. D. Price, S. J. Wiback, D. A. Fell, and B. Ø. Palsson, “Metabolic pathways in the post-genome era,” Trends in Biochemical Sciences, vol. 28, no. 5, pp. 250–258, 2003.

[17] M. Kanehisa, S. Goto, M. Furumichi, M. Tanabe, and M. Hira kawa, “KEGG for representation and analysis of molecular networks involving diseases and drugs,” Nucleic Acids Research, vol. 38, no. 1, pp. D355–D360, 2010.

[18] P. D. Karp, M. Krummenacker, S. Paley, and J. K. Wagg, “Integrated pathway-genome databases and their role in drug discovery,” Trends in Biotechnology, vol. 17, no. 7, pp. 275–281, 1999.

[19] C. A. Ouzounis, R. M. R. Coulson, A. J. Enright, V. Kunin, and J. B. Pereira-Leal, “Classification schemes for protein structure and function,” Nature Reviews Genetics, vol. 4, no. 7, pp. 508–519, 2003.

[20] D. L. Nelson and M. M. Cox, Lehninger—Principles of Biochemistry, W. H. Freeman & Company, New York, NY, USA, 4th edition, 2004.

[21] A. C. Guimarães, T. D. Otto, M. Alves-Ferreira, A. B. de Miranda, and W. M. Degrave, “In silico reconstruction of the amino acid metabolic pathways of Trypanosoma cruzi,” Genetics and Molecular Research, vol. 7, no. 3, pp. 872–882, 2008.

[22] T. D. Otto, A. C. Guimarães, W. M. Degrave, and A. B. de Miranda, “AnEnPi: identification and annotation of analogous enzymes,” BMC Bioinformatics, vol. 9, p. 544, 2008.

[23] P. V. Capriles, A. C. Guimarães, T. D. Otto, A. B. Miranda, L. E. Dardenne, and W. M. Degrave, “Structural modelling and comparative analysis of homologous, analogous and specific proteins from Trypanosoma cruzi versus Homo sapiens: putative drug targets for chagas’ disease treatment,” BMC Genomics, vol. 11, p. 610, 2010.

[24] M. Y. Galperin, D. R. Walker, and E. V. Koonin, “Analogous enzymes: independent inventions in enzyme evolution,” Genome Research, vol. 8, no. 8, pp. 779–790, 1998.

[25] M. V. Omelchenko, M. Y. Galperin, Y. I. Wolf, and E. V. Koonin, “Non-homologous isofunctional enzymes: a systematic analysis of alternative solutions in enzyme evolution,” Biology Direct, vol. 5, p. 31, 2010.

[26] Y. I. Pavlov, P. V. Scherbakova, and I. B. Rogozin, “Roles of DNA polymerases in replication, repair, and recombination in eukaryotes,” International Review of Cytology, vol. 255, pp. 41–132, 2006.

[27] B. Lewin, Genes IX, Jones & Bartlett, Sudbury, Mass, USA, 9th edition, 2007.

[28] S. F. Altschul, T. L. Madden, A. A. Schäffer et al., “Gapped BLAST and PSI-BLAST: a new generation of protein database search programs,” Nucleic Acids Research, vol. 25, no. 17, pp. 3389–3402, 1997.

[29] M. M. Klingheil, P. Burton, R. Barnes, and R. McCulloch, “The three R’s of the trypanosomal genomes: replication, recombination and repair,” in Trypanosomes: After the Genome, D. Barry, R. McCulloch, J. Mottram, and A. Acosta-Serrano, Eds., pp. 133–175, Horizon Bioscience, Norfolk, UK, 1st edition, 2007.

[30] M. Alves-Ferreira, A. C. Guimarães, P. V. Capriles, L. E. Dardenne, and W. M. Degrave, “A new approach for potential drug target discovery through in silico metabolic pathway analysis using Trypanosoma cruzi genome information,” Memorias do Instituto Oswaldo Cruz, vol. 104, no. 8, pp. 1100–1110, 2009.

[31] K. Vickerman, “The diversity of the kinetoplastid flagellates,” in Biology of the Kinetoplastida, W. H. R. Lumsden and D. A. Evans, Eds., pp. 1–34, Academic Press, London, UK, 1976.

[32] C. R. Davies, P. Kaye, S. L. Croft, and S. Sundar, “Leishmaniasis: new approaches to disease control,” The British Medical Journal, vol. 326, no. 7385, pp. 377–382, 2003.

[33] J. E. Donelson, M. J. Gardner, and N. M. El-Sayed, “More surprises from Kinetoplastida,” Proceedings of the National Academy of Sciences of the United States of America, vol. 96, no. 6, pp. 2579–2581, 1999.

[34] M. Kanehisa, M. Araki, S. Goto et al., “KEGG for linking genomes to life and the environment,” Nucleic Acids Research, vol. 36, no. 1, pp. D480–D484, 2008.

[35] R. Fukunaga and S. Yokoyama, “Structural insights into the first step of RNA-dependent cysteine biosynthesis in archaea,” Nature Structural and Molecular Biology, vol. 14, no. 4, pp. 272–279, 2007.

[36] S. I. Hauenstein and J. I. Perona, “Redundant synthesis of cysteinyl-tRNACys in Methanosarcina mazei,” Journal of Biological Chemistry, vol. 283, no. 32, pp. 22007–22017, 2008.

[37] F. Charrière, T. H. Tan, and A. Schneider, “Mitochondrial initiation factor 2 of Trypanosoma brucei binds imported elongator-type tRNA(Met),” Journal of Biological Chemistry, vol. 280, no. 16, pp. 15659–15665, 2005.

[38] S. Adhya, “Leishmania mitochondrial tRNA importers,” International Journal of Biochemistry and Cell Biology, vol. 40, no. 12, pp. 2681–2685, 2008.

[39] T. J. Vickers, S. M. Murta, M. A. Mandell, and S. M. Beverley, “The enzymes of the 10-formyl-tetrahydrofolate synthetic pathway are found exclusively in the cytosol of the trypanosomatid parasite Leishmania major,” Molecular and Biochemical Parasitology, vol. 166, no. 2, pp. 142–152, 2009.

[40] C. D. Mol, D. J. Hosfield, and J. A. Tainer, “Abasic site recognition by two apurinic/apyrimidinic endonuclease families in DNA base excision repair: the 3’ ends justify the means,” Mutation Research, vol. 460, no. 3–4, pp. 211–229, 2000.
[41] A. Memisoglu and L. D. Samson, “Base excision repair in yeast and mammals,” *Mutation Research*, vol. 451, no. 1-2, pp. 39–51, 2000.

[42] J. M. Egly, “The 14th Datta Lecture. TFIIH: from transcription to clinic,” *FEBS Letters*, vol. 498, no. 2-3, pp. 124–128, 2001.

[43] L. C. Chen, K. M. Trujillo, W. Ramos, P. Sung, and A. E. Tomkinson, “Promotion of Dn14-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hfd1/Hfd2 complexes,” *Molecular Cell*, vol. 8, no. 5, pp. 1105–1115, 2001.

[44] T. A. Dobbs, J. A. Tainer, and S. P. Lees-Miller, “A structural model for regulation of NHEJ by DNA-PKcs autophosphorylation,” *DNA Repair*, vol. 9, no. 12, pp. 1307–1314, 2010.

[45] C. Kühne, M. L. Tjörnhammar, S. Pongor, L. Banks, and A. Simoncsits, “Repair of a minimal DNA double-strand break by NHEJ requires DNA-PKcs and is controlled by the ATM/ATR checkpoint,” *Nucleic Acids Research*, vol. 31, no. 24, pp. 7227–7237, 2003.

[46] E. A. Worthey and P. J. Myler, “Protozoan genomes: gene identification and annotation,” *International Journal for Parasitology*, vol. 35, no. 5, pp. 495–512, 2005.

[47] L. H. Harrison, K. L. Brame, L. E. Geltz, and A. M. Landry, “Closely opposed apurinic/apyrimidinic sites are converted to double strand breaks in *Escherichia coli* even in the absence of exonuclease III, endonuclease IV, nucleotide excision repair and AP lyase cleavage,” *DNA Repair*, vol. 5, no. 3, pp. 324–335, 2006.

[48] J. D. Hoheisel, “On the activities of *Escherichia coli* exonuclease III,” *Analytical Biochemistry*, vol. 209, no. 2, pp. 238–246, 1993.

[49] Z. Yang, A. M. Sismour, and S. A. Benner, “Nucleoside alphathiotriphosphates, polymerases and the exonuclease III analysis of oligonucleotides containing phosphorothioate linkages,” *Nucleic Acids Research*, vol. 35, no. 9, pp. 3118–3127, 2007.

[50] W. Sun and A. W. Nicholson, “Mechanism of action of *Escherichia coli* ribonuclease III. Stringent chemical requirement for the glutamic acid 117 side chain and Mn2+ rescue of the Glu117Asp mutant,” *Biochemistry*, vol. 40, no. 16, pp. 5102–5110, 2001.

[51] C. C. Richardson, I. R. Lehman, and A. Kornberg, “A deoxyribonucleic acid phosphatase-exonuclease from *Escherichia coli*. II. Characterization of the exonuclease activity,” *Journal of Biological Chemistry*, vol. 239, pp. 251–258, 1964.

[52] A. Slaitas, C. Ander, Z. Földes-Papp, R. Rigler, and E. Yeheksiel, “Suppression of exonucleolytic degradation of double-stranded DNA and inhibition of exonuclease III by PNA,” *Nucleosides, Nucleotides and Nucleic Acids*, vol. 22, no. 5–8, pp. 1603–1605, 2003.

[53] D. M. Kirtikar, G. R. Cathcart, and D. A. Goldthwait, “Endonuclease II, apurinic acid endonuclease, and exonuclease III,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 73, no. 12, pp. 4324–4328, 1976.

[54] L. L. Souza, I. R. Eduardo, M. D. Pádua, and A. C. Leitão, “Endonuclease IV and exonuclease III are involved in the repair and mutagenesis of DNA lesions induced by UVB in *Escherichia coli*,” *Mutagenesis*, vol. 21, no. 2, pp. 125–130, 2006.

[55] T. Takemoto, Q. M. Zhang, Y. Matsumoto et al., “‘3’-blocking damage of DNA as a mutagenic lesion caused by hydrogen peroxide in *Escherichia coli*,” *Journal of Radiation Research*, vol. 39, no. 2, pp. 137–144, 1998.

[56] D. M. Serafini and H. E. Schellhorn, “Endonuclease III and endonuclease IV protect *Escherichia coli* from the lethal and mutagenic effects of near-UV irradiation,” *The Canadian Journal of Microbiology*, vol. 45, no. 7, pp. 632–637, 1999.

[57] N. R. Asad, L. M. Asad, A. B. Silva, I. Felzenszwalb, and A. C. Leitão, “Hydrogen peroxide effects in *Escherichia coli* cells,” *Acta Biochimica Polonica*, vol. 45, no. 3, pp. 677–690, 1998.

[58] A. P. Guedes, V. N. Cardoso, J. C. De Mattos et al., “Cytotoxic and genotoxic effects induced by stannous chloride associated to nuclear medicine kits,” *Nuclear Medicine and Biology*, vol. 33, no. 7, pp. 915–921, 2006.

[59] H. E. Krokan, R. Standal, and G. Slupphaug, “DNA glycosylases in the base excision repair of DNA,” *Biochemical Journal*, vol. 325, no. 1, pp. 1–16, 1997.

[60] M. D. Wyatt, J. M. Allan, A. Y. Lau, T. E. Ellenberger, and L. D. Samson, “3-Methyladenine DNA glycosylases: structure, function, and biological importance,” *BioEssays*, vol. 21, no. 8, pp. 668–676, 1999.

[61] K. Sheppard, J. Yuan, M. J. Hohn, B. Jester, K. M. Devine, and D. Söll, “From one amino acid to another: tRNA-dependent amino acid biosynthesis,” *Nucleic Acids Research*, vol. 36, no. 6, pp. 1813–1825, 2008.

[62] F. Agüero, B. Al-Lazikani, M. A. Aslett et al., “Genomic-scale prioritization of drug targets: the TDR Targets database,” *Nature Reviews Drug Discovery*, vol. 7, no. 11, pp. 900–907, 2008.
