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and Ovidiu Radulescu

Comprehensive Analysis of Parasite Biology

From Metabolism to Drug Discovery

Volume 7

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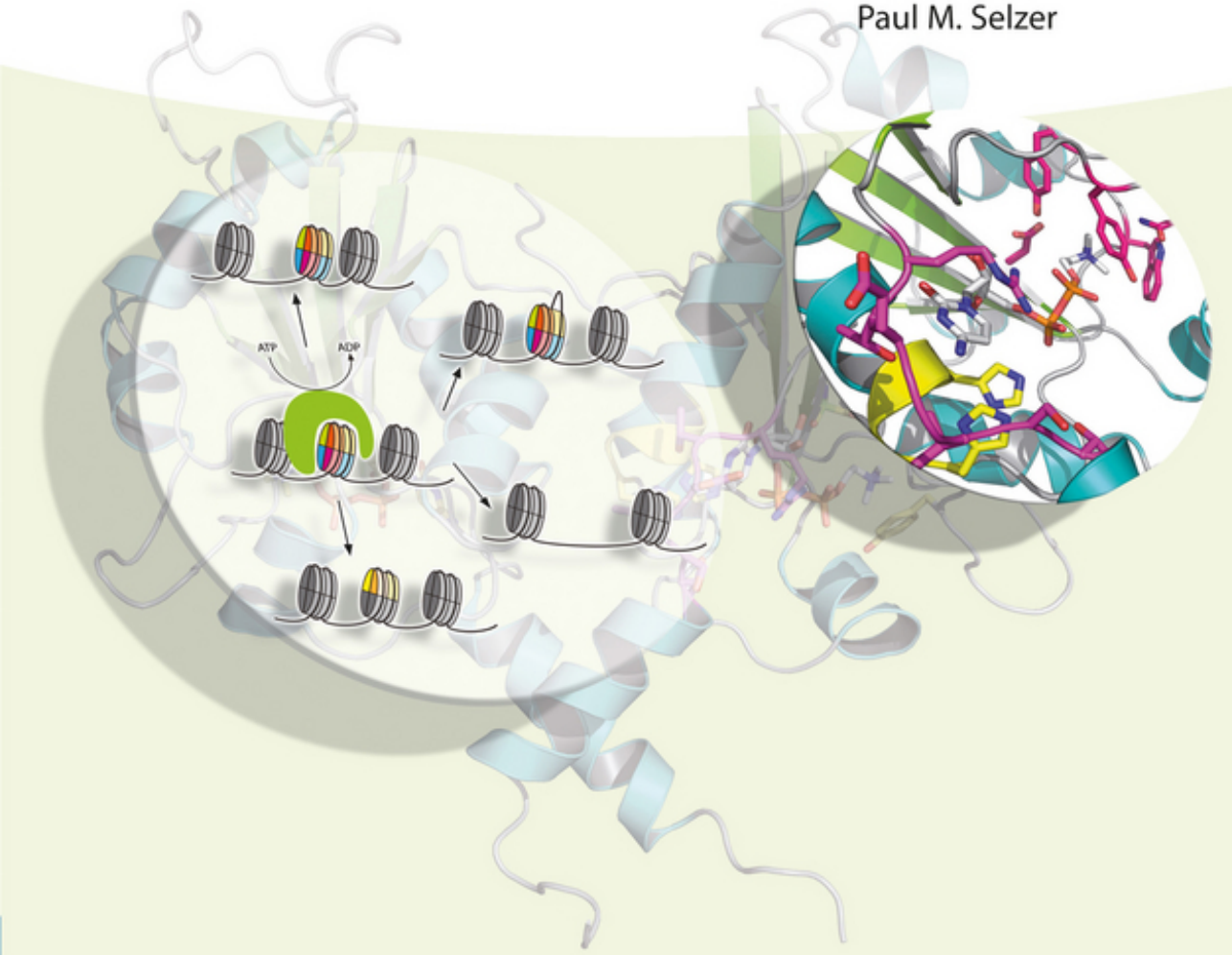
Paul M. Selzer



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Cover

Three-dimensional model of the catalytic domain of *Plasmodium falciparum* CTP:phosphocholine cytidylyltransferase - the rate-limiting enzyme of the phosphatidylcholine biosynthesis pathway - with the bound product CDP-choline. The protein is shown in ribbon representation. CDP-choline is depicted in stick representation. The inset shows a close-up view of the active site with residues coordinating CDP-choline depicted in stick representation. The structure visualization was prepared on the basis of a structural model provided by E. Guca *et al.*, Chapter 7.

The positioning of nucleosomes along eukaryotic genomes is organized by ATP-dependent chromatin remodeling complexes that can promote various changes to the nucleosome landscape, including nucleosome sliding, unwrapping, eviction, and histone exchange. These changes result in altered DNA accessibility and can affect transcriptional activity, E.M. Bunnik & G. LeRoch, chapter 18.

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Foreword

I was delighted to write this Foreword for the book for two main reasons. Firstly, the subject area fits very well with my personal scientific biases (e.g., multiple protozoan parasites rather than simply one, multidisciplinary approaches, multinational), and secondly, I think that one can express one's opinions freely in such an article in the sure knowledge that no one will be offended as few apart from the Editors are likely to read it. I shall attempt do a little bit to rectify the demise of Prefaces/Forewords later in this piece. Unfortunately, books themselves are now a much less important means of communication in science than they were; many are not easily available online and are expensive, in contrast to the enormous amount of literature available free on the Internet. Thus, Introductions to scientific papers can be easily filled with grand-sounding reviews and papers without the need to pay money (or, seemingly on occasions, even to read the articles except for the Abstract). Their lack of easy availability means that articles in books do not get cited (or found by search engines such as Pubmed) and hence cannot become high profile, even when they are excellent. Thus, in some countries, the United Kingdom being one example, they are perceived as valueless except for youngsters trying to make their way into a scientific career. This is a great sorrow and, perhaps, can be changed – as I suggest as follows.

It has not always been like this, and one message I shall try to convey is that much is being lost in the current approach to focus only on readily available and very recent articles when investigating a topic. It would indeed make life easier if this was adequate, but, unfortunately, in my opinion, one usually loses out greatly if one simply relies upon the interpretation of others of the appropriate literature; they too may not have read all the key articles. When I entered parasitology, books were a vital resource. I recall fondly scanning the pages of Biochemistry of Parasites (second edition, edited by Von Brand, 1974); it contained so much detailed facts as well as appropriate references (it saved many hours scanning through Current Contents). Many of these data are indeed still highly relevant and have not been superseded. I strongly encourage those with interests in the areas to consult such tomes (I am sure that good University libraries still have a copy of this and other similar volumes). Interestingly, some good books can now be obtained freely and in full on the Internet, such as Biochemistry and Molecular

Biology of Parasites (edited by JJ Marr and Miklos Muller in 1995; Chapter 3 appeals particularly to me), Biochemical Protozoology, and Molecular Basis of Drug Design and Resistance, a short list, which, admittedly, reflects my biases, but nevertheless containing excellent and still relevant reviews with data that sometimes have not been published elsewhere. These are readily available now, but do they get read? It would be interesting to know, but I suspect that the answer is "rarely," primarily as current young researchers probably do not know of their existence. Perhaps, the more experienced scientists should be encouraging those in the early stages of their career to remember appropriate past work rather than dismiss it as "ancient" and so irrelevant. I am sure that the Prefaces/Forewords do often not get read; hence, I (modestly) recommend them here as a good read.

Thus, it will be apparent that I believe that books should continue to have a place in research in biological science; good ones should provide the foundations upon which to build one's knowledge of a subject area. The aim of this book is to do that and to provide stimulus to those venturing into drug discovery against parasitic diseases. I hope that it succeeds and that it, too, is made available on the Internet and soon; certainly, this would facilitate its availability enormously. Drugs against protozoan parasites were the start of chemotherapy *per se* and successes were numerous. Indeed, new antiprotozoal drugs were the highlights of the early days of chemotherapy. Situations change and new antiprotozoal drugs have been very scarce in recent times, whereas the need has not decreased and, in some cases, the advent of drug resistance has increased the problems. The development of new technologies always brings with it the hope for massive improvements in health. Usually, the technologies yield large amounts of data (and many scientists keen to apply the methods, Review Boards of grant-awarding bodies being keen to support novel approaches), but translating that into useful and practical products has proved to be hard in very many cases. In some instances, I believe that the basic biology gets overlooked through ignorance or expediency in the rush to apply the new technologies. Thus, I am pleased to see that this volume focuses not only on the new but also takes into account the need to address important biological questions using biologically relevant materials. This is, in my opinion, an essential ingredient of all biological research. I recall learning early in my career the importance to use good and thorough scientific approaches and having important aims (such as providing steps along the road to new and needed medicines such as antiprotozoals). I also learnt the benefits of multidisciplinary approaches, and harnessing multiple sets of skills in one program can be so beneficial. Learning to collaborate with others is crucial, and that takes skill as well as patience. Aiding such interactions is an important role for funding bodies, especially such as the EC. I experienced first-hand how networks of scientists can work well, for example, COST Actions. This facilitates the building of collaborations as well as understanding of different approaches and attitudes; in my opinion, money well

spent even if the apparent bureaucracy of European networks and the associated administration can have its torments. This book is the result of one such network, and I hope that the contents of the book are useful not only to the current network but also to many more extensive networks in the future.

December 2015

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Preface

Infectious diseases caused by parasites are widespread in humans and their domestic animals. The human diseases strike hardest in the poorest nations and not only cause severe pain and distress but also impair child development, educational progress, and adult productivity, thus contributing significantly to poverty. Treatments currently available are limited by severe side effects, development of drug resistance, and/or high-cost and inadequate means of administration. We believe that there is a moral duty for researchers from the richest nations to work on control methods of diseases that afflict the poorest nations. There is, however, also self-interest for the developed nations. In direct terms, infection of travelers (and peacekeeping forces) is a constant threat. Moreover, there is a risk that parasites that had previously been eliminated from Europe may return from their reservoirs in the developing world, or – given global climate changes – novel parasites may be introduced into newly amenable habitats. Furthermore, alleviating parasitic diseases contributes to significant economic improvements, and this enhanced prosperity would contribute to future economic growth across the world.

Unfortunately, the lack of financial resources in the poorest countries in the world that are most affected by parasitic diseases has for a long time limited the interest of pharmaceutical industry in finding cures for the diseases. In recent years, the pharmaceutical industry has committed itself more to investing into neglected diseases caused by parasites, primarily because a genuine feeling of mission among influential decision-makers has emerged; however, such altruistic actions clearly have to be limited for a commercial enterprise. There has remained a limited core of academic scientists focusing on the problems caused by parasites, but successes with applied outcomes have been few. Some of the research efforts of academics have perhaps been naïve and divorced from the realities of the pharmaceutical industry, such that the results of their efforts can never be practically exploited.

In an effort to bring together the innovative approaches of academic scientists and the tenets of pharmaceutical industry, we created a doctoral training network that had the goal of enabling doctoral students to gain deep insights into and experience of the different and yet complementary approaches of both sectors in the area of drug discovery against parasitic diseases. The program, called

ParaMet, was funded by the European Commission through a Marie Curie Action. It provided a unique blend of interdisciplinary research projects covering areas integral to the drug discovery process in academia and industrial settings. This volume is one of the outcomes that this venture has generated.

This volume provides a comprehensive summary of the multidisciplinary approaches currently applied in drug discovery programs aimed against parasitic diseases. The volume is organized into four parts that introduce the various main aspects of the drug discovery pipelines. Its first part details individual protein groups or pathways that are specific to protozoan parasites and thus hold promise for future drug development. Several structure-based approaches that underpin the rational design of drugs are exemplified. Such rational approaches are complemented by use of empirical, phenotypical screening methods. The different screening methods are described with a particular focus on the use of natural compound libraries, showing how these will help to expand chemical diversity to identify unique and novel chemical scaffolds acting against parasites and parasite-specific features.

The second part of the volume outlines metabolomics approaches to identify parasite-specific pathways and metabolic nodes that are exploitable for drug discovery. Description of pathways, mechanisms, and targets is accompanied by presentation of metabolomics and lipidomics technologies needed for metabolic pathway reconstruction.

Regulation of gene expression is vital for parasites with complex life cycles. The third part of the volume describes how advances made in understanding the multitude of epigenetic mechanisms regulating the way parasites read their own genes lead to antiparasite drug discovery. Details of proteins determining nuclear organization and modulating transcription, but also of proteins involved in synthesis of resources for translation, are given.

The outcome of modern biology technologies is the generation of large datasets, which are ideal for analysis by bioinformatics and mathematical modeling procedures. Mathematical models can be used to fill in the gaps in knowledge and offer a better understanding of complex aspects of parasite physiology such as drug resistance. The fourth part of the volume describes such analytical approaches that have been applied in antiparasite drug discovery in particular to provide a platform that can be used to query parasite systems in order to make more informed decisions about potential drug targets and the likely mode of actions of drugs and parasite resistance to them.

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Part One
Identification and Validation of New Drugs and Targets

Comprehensive Analysis of Parasite Biology: From Metabolism to Drug Discovery,
First Edition. Edited by Sylke Müller, Rachel Cerdan, and Ovidiu Radulescu.

1
Discovery of the Mechanism of Action of Novel Compounds That Target Unicellular Eukaryotic Parasites

Daniela Begolo and Christine Clayton*

Abstract

In recent years, most new candidate antiparasitic drugs have been found by screening huge numbers of compounds for their ability to kill parasites, followed by counterscreening for toxicity to mammalian cells. Several public–private initiatives have supported this, yielding many hits each for Plasmodia and Kinetoplastids. From these, candidates are selected for further investigation. Although knowledge of the precise mode of action is not necessary for successful development, detailed understanding of the drug's uptake, activation, and target can be very useful in guiding medicinal chemistry, toxicology, and pharmacology. Knowledge of the target can also provide information for further drug discovery studies and in choosing partner drugs in combinations. A multiplicity of complementary approaches can be applied to investigate the drug mode of action. Examples include selecting drug-resistant parasites and identifying the resistance-causing mutations, reverse genetics to find genes required for drug susceptibility, metabolomics, and biochemical approaches such as affinity purification. Here, we review the myriad possibilities, including numerous examples.

Introduction

The development of new antiparasitic drugs is a necessary process, because many of the currently used drugs are unacceptably toxic and resistance is emerging [1, 2]. This review focuses in particular on compounds against *Plasmodium* spp., *Leishmania* spp., *Trypanosoma brucei*, and *Trypanosoma cruzi*. Multiple initiatives, for example, the public–private partnerships Drugs for Neglected Diseases initiative (DNDi, www.dndi.org) [3] and Medicines for Malaria Venture (MMV, www.mmv.org) [4] were founded to support discovery and approval of new drugs. There is continued discussion concerning the virtues of different drug discovery

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methods [5–7]. In the phenotypic drug discovery approach, large numbers of compounds are screened for their ability to kill the target pathogen, without regard to possible mechanism of action (MoA) [5, 7, 8]. The target-based approach, in contrast, first focuses on a particular mechanism and second considers the ability to kill the pathogen [5, 9]. A few years ago, an analysis of all approved first-in-class compounds showed that the phenotypic approach has, in practice, been more successful for the development of licensed drugs targeting infectious diseases [5]. As a consequence of these observations, efforts have been redirected toward phenotypic screens [10]. In the last few years, under the auspices of public–private partnerships, millions of chemical compounds have been tested for their abilities to kill protist pathogens without affecting the mammalian cells [11, 12]. As a result, thousands of drug-like molecules are now available for potential development.

Phenotypic screening is an agnostic approach: target, activation pathway (if relevant), and entrance route – which together are summarized as MoA – are initially not known. Indeed, drugs can achieve clinical approval even if their MoA is unknown [6, 7, 13]. Nevertheless, MoA knowledge is extremely helpful for discovery programs [6]. Target identification aids medicinal chemists, since 3D structure determination illuminates structure–activity relationships [14] and thus facilitates lead optimization [15]. MoA knowledge can also help to predict and monitor possible resistance emergence, as well as on-target side effects [16]. Even if a particular drug candidate is unsuccessful, knowledge of its MoA will suggest development of alternative compounds with the same MoA. Finally, compounds with novel MoA deepen our understanding of parasite biology [14, 16].

A multitude of techniques is available for target deconvolution. Usually, it is necessary to integrate several complementary approaches [16, 17]. Once a possible target has been identified, it must be validated by independent methods [18]. Here we give an overview of different method, and present examples – usually for molecules that target parasitic protists, but occasionally also other organisms (Table 1.1).

Principles

A drug that encounters a pathogen must be taken up first, which may involve either passive diffusion or active transport [2]. In some cases, it may need to be transferred to an organelle and/or activated by pathogen enzymes [2]. The activity of the drug in the cells may involve inhibition of a single or multiple enzymes, binding to macromolecules, or less specific toxicity [6]. In the latter case, selective toxicity of the drug for the pathogen, rather than the host, must rely either on accumulation of the drug in the pathogen or a particular compartment or on activation by a pathogen-specific pathway.