MORE THAN A VITAMIN:

WALKS ALONG AN ESSENTIAL PATHWAY

Prof Erick Strauss

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Prof Erick Strauss
Department of Biochemistry
Faculty of Natural Sciences
Stellenbosch University

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ABOUT THE AUTHOR

Erick Strauss, born on 4 November 1975 and raised in Pretoria, attended the University of Pretoria from 1994 to 1997 where he obtained his BSc (majoring in chemistry and biochemistry) and BSc(Hons) (in chemistry) degrees, both *cum laude*. He subsequently moved to the USA in 1998 to pursue his graduate studies at Cornell University in Ithaca, NY, where he worked on the biosynthesis of coenzyme A with Prof Tadhg Begley. He obtained a PhD in Chemistry and Chemical Biology from Cornell in 2003.

He returned to South Africa in the same year to accept an offer from Stellenbosch University to establish his own research group in the Department of Chemistry and Polymer Science. In 2008 he moved to the Department of Biochemistry (also at Stellenbosch University) as associate professor, and was promoted to full professor in 2013. Since the start of his independent career he has trained 11 MSc students (one as co-supervisor) and nine PhD students (one as co-promotor); he currently leads a group of four PhD students and four postdoctoral fellows.

Erick is regarded as a leading authority on the biosynthesis and enzymology of the essential metabolic cofactor coenzyme A, as well as of the design, discovery and development of antimicrobial agents that target this pathway. His publications, which have attracted a total of more than 680 citations (h-index of 12), include articles in prestigious journals such as the *Proceedings of the National Academy of the USA* (PNAS), the *Journal of the American Chemical Society* (JACS) and two articles in *Nature Chemical Biology*. One of these is a commentary piece chosen as one of ten winning entries (one of only two from outside the United States) submitted by young scientists active in the field in which they expounded their vision of the future 'Grand Challenges' in the field of Chemical Biology. Erick currently holds a B3-rating from the NRF.

Erick is a recipient of the DuPont Prize for Excellence in Teaching from Cornell University (1999); the Rector's Award for Excellence in Teaching from Stellenbosch University (2007); the President's Award from the South African National Research Foundation (2008); the Beckman-Coulter Silver Medal from the South African Society for Biochemistry and Molecular Biology (2010); and the Raikes Medal from the South African Chemical Institute (2013). In 2012 he was elected as a founding member of the South African Young Academy of Science. Since 2009 he is also husband to Suzanne and since 2012 father to Matteo – perhaps his biggest (and ongoing!) achievements to date.

ACKNOWLEDGEMENTS

Academic research is a curious endeavour. On the one hand, it is very much an ego-driven enterprise that can end up being too focused on the individual (as the previous page attests!). However, it rarely—if ever—is a one-man show, especially in the sciences, where the work is done in laboratories that have to be cleaned and maintained, where instruments have to be serviced, orders have to be placed and processed, and safety codes be enforced. My first thanks goes to all those who support us on a daily basis, very often doing thankless jobs. We don't say it enough, but your efforts are very much appreciated.

The main characters (the heroes!) in the stories that fill the next pages are the various students and postdoctoral fellows who had to perform the experiments (and when these failed, had to try again … and again). None of any of this would have been possible without the very hard and sometimes frustrating work you poured into your projects; I stand where I am because of you. To Leisl Brand, Lizbé Koekemoer, Marianne de Villiers (née van Wyk), Jandré de Villiers (the reason Marianne is not a Van Wyk anymore), Dirk Lamprecht, Ilse Rootman-La Grange, Renier van der Westhuyzen, Jaco Franken, Albert Abrie, Collins Jana, René Goosen, Tanya Paquet, Ndivhuwo Tshililo (née Muneri), Cristiano Macuamule, Leanne Barnard, Sunette Klopper, Riyad Domingo and Dave Choveaux: I feel very privileged to have you in my academic family, and look forward to seeing the outcome of the next chapter of your lives.

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But in fact all of this started at home, with parents (and an ever present Ouma) who never made me think something was not possible, and who made sure I was always challenged in some or other way. You gave me a solid foundation to build upon and you broadened my horizons. The rest of my family (close, extended and now also the in-laws) have been supportive in every way imaginable. I know you don't always understand what I do, but I'm grateful for your loyalty and keen interest. Finally, to my long-suffering wife Suzanne (the one person who had no idea what she was getting into the day she married me!): I don't know how I managed to do any of this before I met you. I don't say it enough, but thank you for being super-wife (and now super-mom) at Team Strauss. And thanks for the distraction you provide, Matteo; I was way too focused on work before you came along.

Soli Deo Gloria.

MORE THAN A VITAMIN: WALKS ALONG AN ESSENTIAL PATHWAY

1 INTRODUCTION

The fields of chemistry and biology are marvellously intertwined. The fact that one can describe the basis of biological phenomena, both those that are complex (such as inflammation, or the onset of cancer) and 'commonplace'[1] (such as the digestive process, or the transport of oxygen by the blood) in terms of the interactions of molecules, has always fascinated me. Proteins, and more particularly enzymes (i.e. proteins that catalyse chemical reactions), have held a particular attraction: since they are made up of 20 simple building blocks (called amino acids), which can be arranged in any order and any combination, they are exceedingly diverse in terms of their structures, the type of reactions they catalyse and the specific compounds that they act upon. They have to be, considering the vast range of biological processes that directly depend on their activity. Yet all of these aspects can often be explained in simple molecular terms, using chemical language that refer to the making and breaking of bonds, or the interactions between specific groups in the enzymes and those in its substrate, or to the importance of acid/base chemistry, for example. Moreover, understanding an enzyme on this level often makes it possible for its activity to be manipulated in some way. For example, its substrate specificity or the way in which it is regulated can be changed by exchanging one or more of its amino acid building blocks for another. Similarly, a compound can be designed to have a structure that mimics the specific interactions that the substrate has with the enzyme, without acting as such a substrate itself. Such compounds will act as inhibitors of that enzyme's activity—they will occupy the space that the enzyme normally uses for catalysis—and can be used in a variety of therapeutic applications: antibiotics, antipsychotics, anti-inflammatory agents and much more. Such tinkering on a molecular level—molecular engineering, in a manner of speaking—is only really made possible by a deep and fundamental understanding of the chemical principles that underlie bonding and reactivity.

Scientists that actively work on such research questions come from diverse backgrounds: some are chemists (itself a very diverse field), others are biochemists, or medical scientists—the list is quite long. However, within the last 15 years or so a group of these scientists felt the need to describe the endeavour of addressing biological problems by using chemical tools and applying chemical thinking more directly. Thus the field of **chemical biology** was coined—although, to be honest, similar kinds of studies were previously performed under names such as 'bioorganic chemistry', 'bioinorganic chemistry' or 'biological chemistry'.[2] Nonetheless, scientists are not immune to the concept of fashion, and several well-known departments of 'chemistry' (such as the one at Harvard University) were renamed as departments of 'chemistry and chemical biology' during this time. This period exactly coincided with my undergraduate studies, and by the time that I was completing my BSc(Hons) degree (in chemistry) at the University of Pretoria, my awareness of this burgeoning field had grown significantly—as did my excitement to conduct my postgraduate studies doing such work. Unfortunately, very few academic researchers in South Africa were working at the chemistry/biology interface at the time,[3] which eventually led me to pursue my studies abroad. I was accepted into the

[1] Some would argue that it is impossible to make such a distinction. Once even the 'commonplace' phenomena are studied and better understood, more often than not they turn out to be exceedingly complex!

[2] Those with lexicographic inclinations might ask how 'chemical biology' differs from 'biological chemistry'. The simplest answer is that on the deepest level, they really don't. But we can all cite examples of how the need to excite prospective funders/clients/voters gave rise to a new name for what essentially remained the same animal. In marketing this is referred to as 'rebranding', and apparently scientists are also pretty good at it.

[3] It was only after I joined Stellenbosch University that I discovered that several of the members of the Department of Biochemistry (now my colleagues) had been doing exactly this for many years!

graduate programme in the Department of Chemistry and Chemical Biology (they too succumbed to the name-changing pressures) at Cornell University in the USA, and there started my own career as a 'chemical biologist' in the group of Tadhg Begley.[4] Tadhg, who himself received postdoctoral training from both a synthetic organic chemist[5] and an enzymologist, was one of the central players in defining the field of chemical biology. In his words: "Chemical Biology can be viewed as subsuming important aspects of the traditional disciplines of bioorganic chemistry, bioinorganic chemistry, medicinal chemistry, biophysical chemistry and biochemistry, with a special emphasis on the rigorous application of the principles, tools and language of chemistry to important topics in biology".[6]

Since my return to South Africa, and especially after having started my own research group, I have been mindful of also emphasising such a "rigorous application" of chemistry in our studies. This article will describe some of the highlights of these studies, which have as their focus the biology of coenzyme A biosynthesis, with the goal of applying the new knowledge we gain to address various challenges in human health. The next section provides some context for this area of research.

[4] Tadhg is a native of Ireland who moved to the United States to pursue his PhD studies and eventually also started his academic career there. His name is Irish Gaelic, and is pronounced "Tai-gue". When I once commented to him how hard it can be to write in English when it was one's second language, he remarked that he fully understood: it was his second language as well. His schooling was completely in Gaelic.

[5] The world is indeed very small: the synthetic organic chemist in question was Wolfgang Oppolzer at the University of Geneva in Switzerland, the same person in whose group my father—a synthetic organic chemist by training—worked as a postdoctoral fellow. That was in 1981; Tadhg joined Oppolzer's group just a few years later (in 1983), by which time our family had already returned to South Africa. I very nearly first met him when I was pre-schooler!

[6] Quote cited by Prof Patrick Guiry, Director of the Centre for Synthesis & Chemical Biology, University College Dublin on 16 June 2010, as part of the introductory address on the occasion of the conferring of an honorary Doctor of Science degree on Tadhg Begley from the National University of Ireland. (http://www.ucd.ie/president/universityawards/honrorarydegree/09-10/tagdh-begley/; accessed 2014/02/03)

2 OF VITAMINS AND COFACTORS

Vitamins receive a lot of attention these days. We are told to eat certain foods because they are rich in certain vitamins, and to take multi-vitamin supplements on a daily basis to make up for the shortfall in our diet. Some vitamins are also hailed as 'anti-oxidants', or are supposed to have other special medical benefits. But what are vitamins exactly, and why do we need them?

Vitamins are organic[7] chemical compounds that are vital for the survival of a particular organism, but which the organism cannot make itself. They are therefore essential nutrients, and consequently must form part of a balanced diet—although usually only in limited amounts.[8] Lack of any particular vitamin results in a disease specifically associated with such a deficiency, and can result in death in some cases. Importantly, the requirement for vitamins is different for different organisms; plants, for example, are able to synthesise most of the vitamins, and are therefore a good dietary source of them. Currently 13 vitamins are recognised; their names and associated deficiency diseases are listed in Table 1.

Each of the vitamins has a very specific biological function. Vitamin C, for example, plays a key role in reversing damage in one of the enzymes involved in the formation of collagen, the protein that provides structure to connective tissues such as tendons, ligaments and skin. In fact, most of the vitamins serve as precursors to so-called 'cofactors', which are helper molecules that are either attached to or closely associated with some enzymes to allow them to perform chemical reactions they would otherwise be incapable of doing. As such, they expand the chemical versatility of these enzymes, since their constituent 20 amino acid building blocks are actually not very diverse from a chemical and structural point of view.

[7] While the term 'organic' is currently used to refer to foodstuffs that are grown and raised without the application or addition of man-made chemicals, in a chemical sense it refers to any compound that consists of at least the elements carbon (C) and hydrogen (H), and any combination of these with oxygen (O), nitrogen (N), phosphorus (P) and sulphur (S).

[8] The fact that vitamins only need to be taken in small quantities is often overlooked. Some people have the point of view that if a little bit of something is good, a lot of it must be better. However, consuming an excess of certain vitamins could cause a variety of adverse effects.

Table 1. The 13 vitamins, their various chemical forms, deficiency diseases and associated cofactors (Johnson, 2013).

Vitamin	Chemical form(s)	Deficiency disease	Associated cofactor(s)
Vitamin A	Retinol, retinal, other carotenoids (e.g. -carotene)	Night-blindness, hyperkeratosis, and keratomalacia	
Vitamin B_1	Thiamine	Beriberi, Wernicke-Korsakoff syndrome	Thiamine pyrophosphate (TPP)
Vitamin B_2	Riboflavin	Ariboflavinosis	Flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD)
Vitamin B_3	Niacin, niacinamide	Pellagra	Nicotinamide adenine dinucleotide (NAD^+) and its phosphate ($NADP^+$)
Vitamin B_5	**Pantothenic acid**	**Deficiency is very rare; 'pins and needles'**	**Coenzyme A (CoA)**
Vitamin B_6	Pyridoxine, pyridoxamine, pyridoxal	Anaemia, peripheral neuropathy	Pyridoxal phosphate (PLP)
Vitamin B_7	Biotin	Dermatitis, enteritis	Biotin
Vitamin B_9	Folic acid, folinic acid	Birth defects when deficient in pregnancy; megaloblastic anaemia	Tetrahydrofolic acid (THF)
Vitamin B_{12}	Cyano-, hydroxyl- and methylcobalamin	Megaloblastic anaemia	Cobalamin, methylcobalamin
Vitamin C	Ascorbic acid	Scurvy	Ascorbic acid
Vitamin D	Cholecalciferol, ergocalciferol	Rickets and osteomalacia	
Vitamin E	Tocopherols, tocotrienols	Deficiency is very rare; mild haemolytic anaemia in newborns	
Vitamin K	Phylloquinone, menaquinones	Bleeding diathesis	Menaquinone

One of the B vitamins, called vitamin B_5 or pantothenic acid,[9] is the precursor for the cofactor coenzyme A (CoA), which acts as the main acyl group carrier in biological systems. Acyl groups are the derivatised forms of organic acids, which include acetic acid (the principle component of vinegar, with two carbon atoms) and the longer fatty acids palmitic (16 carbons) and stearic acid (18 carbons). To be carried by CoA, these acids are attached to its thiol (–SH) group, as shown for palmitic acid in Figure 1.[10] This attachment increases the solubility of the acids in aqueous solutions and also makes it possible for them to be spatially manipulated; the CoA becomes a 'handle' that allows for enzymes to attach to and perform reactions on them. Additionally, the coupling of these acids to the thiol of CoA changes their chemical characteristics, which makes it possible for them to undergo chemical reactions that would otherwise not have occurred. For example, this allows for the two-carbon acetate units to be joined into longer fatty acids. It also makes the reverse reaction, i.e. the breakdown of fatty acids, possible. As such, CoA is usually associated with the biochemistry and metabolism of fatty acids, while acetyl-CoA is additionally also involved in the central metabolic processes that generate energy from various carbon sources. Several regulatory processes also depend on the acetyl group being transferred to and removed from proteins and other small molecules.

[9] The name 'pantothenic acid' is derived from the Greek word for 'from everywhere', since it is found in nearly all foods. Consequently, a clinical deficiency in the vitamin has not been described. Its alcohol derivative pantothenol (from reduction of the acid) is widely used in hair and skin care products, where it is referred to as "pro-vitamin B_5"—most likely to capitalise on the positive emotional value of vitamins in general. However, in these products its function is mainly to act as moisturiser and to give hair its shiny appearance.

[10] The 'stick' structures used in this figure is one of the ways in which the structures of organic molecules are often represented. It is a convention, just as writing from left to right is a convention, and for the uninitiated may look like total gibberish. For such people my advice is usually to treat such schemes as spot-the-difference games: compare the structures on either side of the arrow, and try to identify the differences between them. The crux of what is being shown—here, the attachment of the fatty acid to the thiol of CoA—usually becomes clear in this way.

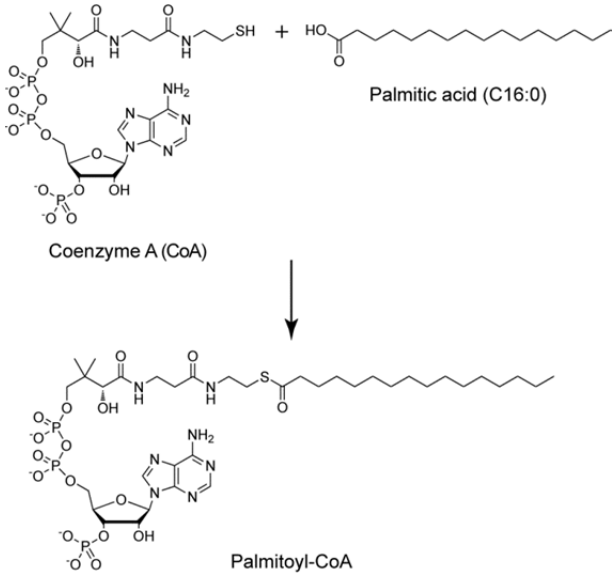

Figure 1. CoA as acyl group carrier: the fatty acid palmitic acid, which is poorly soluble in water and difficult to manipulate in enzyme reactions, is converted to palmitoyl-CoA by attachment to the thiol (–SH) group of CoA. In this way its solubility is improved, and the CoA component becomes a 'handle' that enzymes can use to hold on to the molecule while performing reactions on it.

Taken together, CoA is an extremely versatile cofactor which is involved in several central metabolic processes. By my own calculation at least 9% of all known enzyme activities make use of it directly, or rely on one of its derivatives. Consequently CoA draws much attention from researchers in diverse fields in biology. My group's main interest in CoA has been the biosynthetic process by which its vitamin precursor (pantothenic acid) is converted to CoA. A detailed description of this process follows in the next section.

3 ONE PATHWAY, MANY PATHS

3.1 The universal CoA biosynthetic pathway

Pantothenic acid is converted to CoA in five enzyme-catalysed steps, which are summarised schematically in Figure 2. For the rest of this article the enzymes will only be referred to by their abbreviated names—PanK, PPCS, PPCDC, PPAT and DPCK—and only the details that are relevant to the highlights presented here will be provided. For more detailed information on these enzymes and their characteristics interested readers are referred to a recent book chapter I authored on CoA biosynthesis and enzymology (Strauss, 2010).

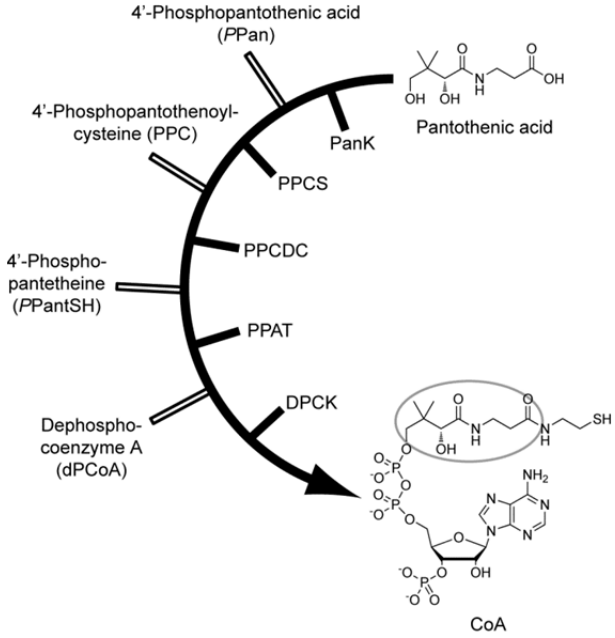

Figure 2. Schematic representation of the biosynthesis of CoA from pantothenic acid in five enzyme-catalysed steps (indicated by the black lines on the curved arrow; the abbreviation for each enzyme is given). The grey lines point to the biosynthetic intermediates. The portion of CoA that derives from pantothenic acid is circled in grey in its structure (Spry *et al.*, 2010).

To the best of our current knowledge, the CoA biosynthetic pathway is universal, meaning that all organisms convert pantothenic acid into CoA using these same five steps, with one small exception being found in the pathway active in the Archaea (Yokooji *et al.*, 2009; Tomita *et al.*, 2012; Katoh *et al.*, 2013).[11] Additionally, all organisms must synthesise CoA themselves, starting from pantothenic acid.[12] In combination, these facts have several important implications. First, it means that

[11] The Archaea are single-celled microorganisms (microbes) that are generally associated with extreme environments. They are also considered to be some of the oldest lineages on earth. In their CoA biosynthesis pathways, the first step of the universal CoA pathway does not exist. Instead, the phosphorylation occurs as part of the synthesis of pantothenic acid (the Archaea can synthesise the vitamin themselves).

[12] The mycoplasmas, primitive pathogenic bacteria that depend on their hosts for survival, are perhaps the only exception to this rule. Based on the currently available information these organisms prepare CoA from dPCoA (the penultimate biosynthetic intermediate), which would suggest that they must scavenge it from their hosts (Strauss, 2010). However, this must still be demonstrated experimentally.

studies of the pathway in simple organisms (such as the model bacterium, *Escherichia coli*) can be used to further our understanding of CoA biosynthesis in all organisms, including complex ones such as humans. On the other hand, differences that are discovered between two organisms can potentially be exploited for intervention of some kind. For example, differences between the human CoA biosynthetic pathway and the one in a pathogenic[13] bacterium that causes fatal infections in humans may offer opportunities for the discovery of new antibiotics (compounds that selectively kill the pathogen). Additionally, a better understanding of CoA biosynthesis, as well as its breakdown and utilisation, could potentially help us better manage a variety of disease states, including ones that have specifically been linked to mutations (genetic variations) in PanK, the first pathway enzyme.[14] It is with respect to all these facets of CoA biosynthesis that my group's research has been making a contribution.

3.2 Discovery of the CoA biosynthetic enzymes

Considering the central role of CoA in metabolism, and the relatively simple, linear pathway for its synthesis from pantothenic acid, it might be surprising for some to learn that when I started my PhD studies in 1998, only one of the five enzymes in the pathway (PanK) had been identified and fully characterised.[15] However, within the next two years both the PPAT and DPCK enzymes from *E. coli* had been described. The two remaining enzymes—PPCS and PPCDC—continued being elusive, and it was the first aim of my PhD research to purify and characterise the latter of these.

Initially I went about doing this based on the little information that was available on the enzyme at the time—information that later turned out to be mostly wrong. By the time we had realised this, and changed our tactics, another research group had already identified the PPCDC enzyme in *E. coli* using a completely different approach. Consequently, they published their findings within weeks of our own discovery of the enzyme, which made it a rather empty achievement. However, what seemed like a very grey cloud turned out to have a silver lining: by chance (and also by using some critical reasoning!) we uncovered that the PPCDC enzyme also had PPCS activity. This meant that in *E. coli* a single protein carried both the PPCS and PPCDC functions, which is a relatively uncommon occurrence in biosynthetic pathways. Even now, the functional relevance of this fusion is also not entirely clear. Nonetheless, this surprising finding allowed us to publish a report of the discovery and characterisation of the last remaining unidentified activity in the pathway in 2001 (Strauss *et al.*, 2001). With that, the chapter on the discovery of CoA biosynthetic enzymes seemed to have been concluded.

3.3 Variations in the CoA biosynthetic pathway

With the advent of large scale genome sequencing,[16] a vast amount of bioinformatic data[17] became available. Since CoA biosynthesis is such a conserved process (all organisms perform it in the same way), the general expectation was that the CoA biosynthetic enzymes should also not show much variation between organisms. This idea was tested by using the sequences of the five CoA biosynthetic enzymes in *E. coli* (and those subsequently discovered in other organisms) to search for genes in organisms with sequenced genomes that would encode similar proteins. In this way, a 'map' of the CoA biosynthetic enzymes in all these organisms could be drawn.

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[13] In its broadest sense, a 'pathogen' is anything that can cause disease. The name (from the Greek) literally means 'producer of suffering'.

[14] Pantothenate kinase-associated neurodegeneration (PKAN) is a rare, progressive degenerative brain disease that often causes death in early adulthood. It is estimated to affect 1 to 3 individuals in a million.

[15] The pathway, i.e. the actual transformations and biosynthetic intermediates, was discovered long before that, but only the PanK enzyme had been isolated and characterised, and the gene that encodes it in *E. coli* identified.

[16] An organism's genome is all of its hereditary information, which is usually contained in sequences of DNA. Within these sequences are the genes that code for the proteins that are active in that organism; each gene (usually) codes for a specific protein. Genome sequencing refers to the activity of determining these sequences, which is done using a variety of technologies and instruments. The speed and accuracy with which it is achieved have improved exponentially during the last decade.

[17] Bioinformatics refers to the study of the data that is obtained from genome sequencing, as well as the data that can be obtained from it either by inference (such as the identification of genes within the sequences, and the proteins that they code for) or by comparison, for example between organisms.

Step	Enzymes involved in CoA biosynthesis	EC number	Basic form (with subvariants)					Variant 1 (no *de novo* pantothenate)		Variant 2 (no Pan transport)		V. 3	V. 4
			Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Bacillus cereus*	*Homo sapiens*	*Steptococcus pneumoniae*	*Corynebacterium glutamicum*	*Mycobacterium tuberculosis*	*Mycoplasma genitalium*	*Treponema pallidum*
	Pantothenate kinase (PanK)	2.7.1.33											
1a	Type I		*coaA*	–	–	+	–	–	+	+	+	–	–
1b	Type II		–	*coaA*	–	–	+	+	–	–	–	–	–
1c	Type III		–	–	*coaX*	+	+	–	–	–	+	–	+
2	Phosphopantothenoylcysteine synthetase (PPCS)	6.3.2.5	*coaBC*	+	+	+	+	+	+	+	+	–	–
3	Phosphopantothenoylcysteine decarboxylase (PPCDC)	4.1.1.36						+	+			–	–
	Phosphopantetheine adenylyltransferase (PPAT)	2.7.7.3											
4a	Form 1 (Bacterial)		*coaD*	+	+	+	+	–	+	+	+	–	+
4b	Form 2 (Eukaryotic)		–	–	–	–	–	*coaDE*	–	–	–	–	–
5	Dephospho-CoA kinase (DPCK)	2.7.1.24	*coaE*	+	+	+	+		+	+	+	+	+

Figure 3. 'Map' of the variations in the CoA biosynthetic pathway in a range of organisms that exemplify the basic form of the pathway (with its subvariants) and several variants of it (Strauss, 2010).

Figure 3 shows a recent version of this map, and demonstrates that the situation is not entirely as simple as originally thought, with a basic form of the pathway (with some subvariations) and at least four variants of it. These distinctions are based on several factors, and also take into account whether the organism is able to synthesise its own pantothenic acid, or can obtain it from its environment by transport it into the cell. Focusing on the CoA biosynthetic enzymes themselves, the following differences are the most obvious ones: at least three different types of PanK enzymes exist, and two forms of PPAT. Additionally, as explained above, the PPCS and PPCDC activities are found on a single bifunctional protein (called CoaBC) in all bacteria (the Streptococci and Enterococci being an exception). However, in animals and plants these are separate enzymes. The situation is reversed for PPAT and DPCK: they are separate enzymes in most bacteria, but are found on a bifunctional enzyme in animals. Additionally, some organisms apparently do not have complete CoA pathways: the mycoplasmas only have a DPCK, suggesting that they must obtain dPCoA from their hosts. *Treponema pallidum*, the causative agent of syphilis, is missing both PPCS and PPCDC activities, meaning it does not have a functional CoA biosynthesis—or it manages to circumvent these enzymes in a manner that we do not yet understand.

Clearly, the diversity in the pathway exceeded all our expectations. My research group was involved in uncovering two of the more surprising variations: the discovery of a 'missing' PanK, and unravelling the reasons for the 'inactive' PPCDC in baker's yeast.

4 PANK$_{III}$: THE 'MISSING' ENZYME—THAT WASN'T

The map of CoA biosynthesis shown in Figure 3 is a recent one, and indicates that the first enzyme in the pathway in the bacterium *Pseudomonas aeruginosa* (an opportunistic pathogen that cause respiratory infections) is a type III PanK. However, prior to 2005, only two types of PanK were known: a type I, which was first described in *E. coli* and seemed to predominate in bacteria, and a type II, which was found in higher organisms such as animals and plants (strictly speaking, in all eukaryotes[18]). Curiously, at this time no PanK could be identified in the genomes of a range of mainly pathogenic bacteria, among them the causative agents of stomach ulcers (*Helicobacter pylori*), whooping cough (*Bordetella pertussis*), meningitis (*Neisseria meningitis*), antibiotic-associated diarrhoea (*Clostridium difficile*) and hospital-associated

[18] Eukaryotes are organisms that have cells with a nucleus (which contains the genetic material), and includes animals and plants, but also a range of micro-organisms: yeasts, and several different pathogenic parasites (including the one causing malaria), for example.

infections (*Acinetobacter baumannii*). This meant that in these organisms, which had all the other pathway enzymes, the PanK activity was 'missing' (Gerdes *et al.*, 2006).

A patent that appeared in 2002 seemed to hold the key to the mystery (Yocum & Patterson, 2002). As part of this patent's stated claims, it was shown that it is possible to remove the gene encoding the type I PanK in the model bacterium *Bacillus subtilis* without having a fatal effect, suggesting that an enzyme encoded by another gene also had PanK activity. This was mapped to a gene dubbed '*coaX*', with the 'X' used to denote that the corresponding enzyme's normal function was unknown at that stage. I came across this finding while doing literature searches as part of the write-up of my PhD thesis, and decided to investigate these results in more depth when I launched my own independent career.

The study and characterisation of the 'CoaX' enzymes therefore became the subject of the research project of my technical assistant and first MSc student (Leisl Brand). Together we discovered that the *B. subtilis* enzyme discovered in the patent was in fact the PanK that was 'missing' from the pathogenic bacteria. Even more importantly, the 'CoaX' PanK was apparently much more widely distributed in bacteria than the supposedly predominant type I PanKs. Additionally, we also showed that the enzyme differed from the two known PanKs in almost every respect: their sequences, their kinetics, their phosphate donor specificity, and their sensitivity to known inhibitors. We published the first description of these type III PanKs (as we later dubbed this newly discovered form of the enzyme) in 2005 (Brand & Strauss, 2005); in collaboration with Hong Zhang at UT Southwestern Medical Centre in the USA we also determined the first structure of a type III PanK, and published this in the following year (Yang *et al.*, 2006). This structure showed that while the type III PanKs belonged to the same fold as the type II enzymes, and that both types shared similar domains, the manner in which the dimer is formed in each case and their respective active site architectures are very different (Figure 4).

In combination, these studies made a seminal contribution to our knowledge of CoA biosynthesis in bacteria, and established my group as one of the leading role players in the field internationally. My group is currently continuing our work on type III PanKs, with a specific focus on them as potential targets for antibiotic development.

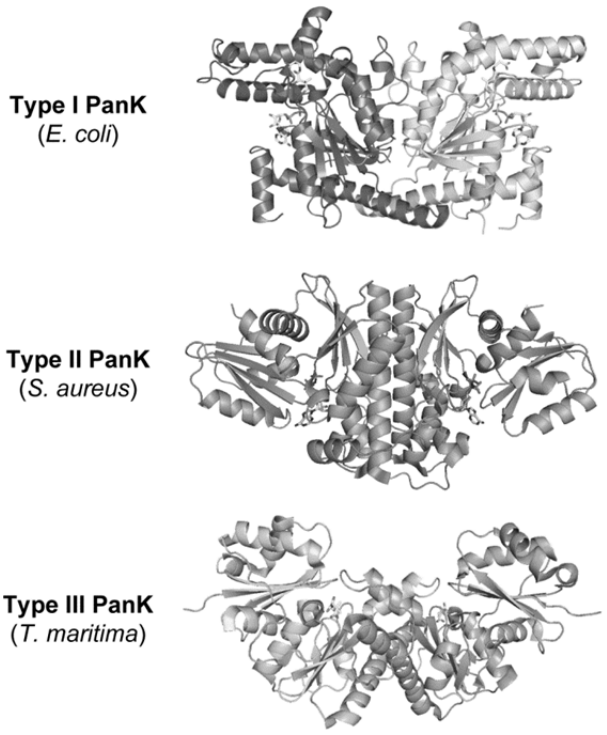

Figure 4. Structures of representative examples of the three bacterial PanK types: a type I PanK structure from *E. coli* (PDB ID: 1SQ5), the structure of a type II PanK from *Staphylococcus aureus* (PDB ID: 2 EWS), and the type III PanK from *Thermatoga maritima* (PDB ID: 3BEX). The differences between the types should be evident by comparing their respective ribbon structures. Note the similar domains in type II and III PanKs, combined with a very different interaction at the dimer interface.

5 PPCDC IN YEAST: ACTIVITY IN THE MOONLIGHT

In 2004 I was approached by a researcher in Spain, Prof Joaquín Ariño, with an interesting problem: they had isolated the protein from baker's yeast (*Saccharomyces cerevisiae*) that was proposed to have PPCDC activity (based on homology, i.e. by comparing its sequence to that of known PPCDCs), but had failed to find any evidence for such an activity. Could we help? Thus began a long and fruitful collaboration that continues to this day, and which resulted in some surprising and extraordinary findings. But first, some background is required.

5.1 The curious mechanism of PPCDCs

PPCDC catalyses the decarboxylation of its substrate, that is, it removes a molecule of CO_2 from its substrate (see Figure 5). When we first set

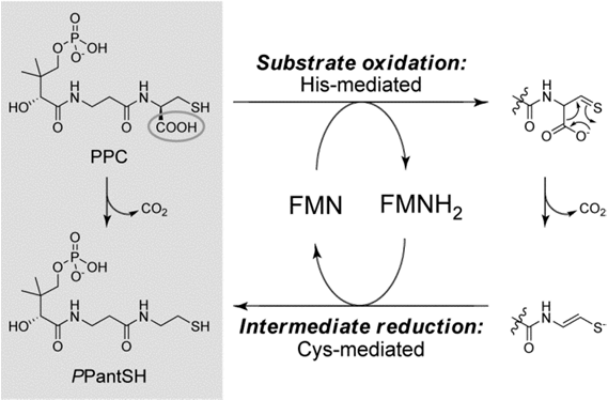

Figure 5. PPCDC-catalysed reaction (in grey box) with the carboxyl group of PPC that is removed circled. On the right is the two-step mechanism, showing that the substrate is first oxidised prior to decarboxylation, and then reduced again. These two steps depend on two specific amino acid residues, a histidine (His) and cysteine (Cys) respectively.

out to isolate the PPCDC enzyme as described earlier, we already knew that the enzyme must have an interesting mechanism from a chemical point of view. When the bond between the COOH group and the rest of the molecule is broken, negative charge builds up on the carbon backbone. However, carbon does not have the ability to deal well with such negative charges under normal circumstances, and the charge must therefore be stabilised in some manner. We envisaged several ways in which the enzyme could achieve this. In the end, reality turned out to be much more elegant than any of our proposals: the enzyme changes the substrate's thiol (–SH) group into one that can facilitate the decarboxylation by an oxidation reaction (as shown in Figure 5); once the reaction is complete, a reduction reaction ensures that the thiol is formed again. Strictly speaking the enzyme temporarily installs a new functional group to do the difficult bond cleavage, and then removes it again.

An important additional feature of this mechanism is that the oxidation and reduction reactions that make the decarboxylation possible each depend on a specific amino acid in the enzyme's active site (i.e. the space in which the reaction takes place). The oxidation relies on a conserved (i.e. all PPCDC enzymes have it) histidine (His) residue, while the reduction depends on a conserved cysteine (Cys). Readers who are interested in the details of this mechanism and how it was elucidated are referred to the respective publications that detail these discoveries (Strauss & Begley, 2001; Strauss *et al.*, 2004).

5.2 Baker's yeast's remarkable PPCDC

As remarked above, Joaquín Ariño's group had already shown that Hal3, the yeast protein which is the closest homologue to *E. coli*'s PPCDC and the PPCDC from the plant *Arabidopsis thaliana* (in essence a weed, but widely used in laboratories as a model in the studies of plant genetics), did not exhibit any evidence of having PPCDC activity. However, they had identified an important deviation in Hal3 which could be the reason for this lack of activity: it did not have the conserved Cys residue necessary for the second part of the PPCDC reaction. Interestingly, they found that yeast had two other proteins that were closely related to Hal3 and which could be considered as potential PPCDC candidates. However, these proteins also did not have all the required active site residues: the one (Vhs3) had the His, but also lacked the Cys, while in the other (Cab3) the His was missing, but the Cys was present. This meant that not one of the PPCDC candidates in yeast would be able to show PPCDC activity on their own, and experiments indicated that this was indeed the case.

The only known structure of a PPCDC enzyme—the one from *A. thaliana*, called AtHal3a—suggested a potential solution to the problem. The functional protein is a homotrimer, meaning that three identical versions of the same protein come together to form the enzyme. Additionally, an active site is found at the interface between each of the adjacent proteins, i.e. three active sites in total. Lastly, the two catalytically essential residues are found on opposite sides of the active site, meaning that the His and Cys that are involved in catalysis in a particular active site are found on different proteins. A schematic representation of the *A. thaliana* PPCDC AtHal3a is shown in Figure 6A. Based on this model, we envisaged that an active PPCDC would form in yeast if either Hal3 or Vhs3 (or both) that could provide the required His, would join with one Cab3 that has the required Cys in a heterotrimeric protein (i.e. three different proteins in the same complex). A model of such a structure is shown in Figure 6B, which shows that, unlike AtHal3a with its three active sites, only one functional active site would form in such a case. Based on this model our two groups devised and executed several biochemical and genetic experiments to establish whether it was an accurate representation of the yeast PPCDC. We were very pleased when these experiments all supported our model!

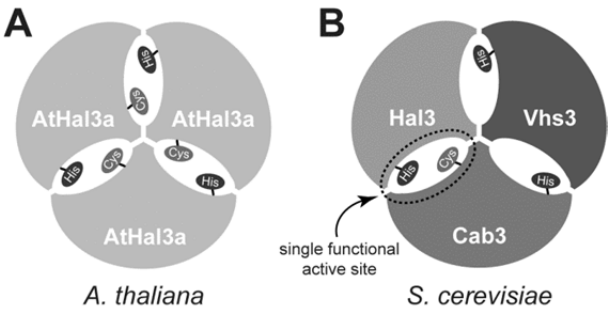

Figure 6. Schematic representations of the homotrimeric PPCDC enzyme AtHal3a from the plant *A. thaliana*, which has three functional active sites formed at the three interfaces between the proteins (Panel A), and the corresponding heterotrimeric enzyme from the yeast *S. cerevisiae*, which has only one functional active site (Panel B) (Ruiz *et al.*, 2009).

The last part of the story is perhaps the most curious: Hal3, the first PPCDC candidate to be identified, in fact already had a known and established function. It acted as an inhibitor of another enzyme (the phosphatase Ppz1) and in this way played a key role in regulating yeast's cell cycle and its ability to respond to the stress of growing in the presence of high salt concentrations. This meant that Hal3 had two completely unrelated functions: the first as a component of the active PPCDC, and the second as a regulatory protein. This characteristic qualified Hal3 as a so-called 'moonlighting' protein, i.e. a protein that has one function, but then 'moonlights' in also performing another. Several such proteins have been identified to date, but we do not yet fully understand the functional relevance of 'moonlighting' in cellular physiology.

We published this remarkable story in the journal *Nature Chemical Biology* (Ruiz *et al.*, 2009); it was also accompanied by a 'News and Views' article to highlight the significance of our findings (Osterman, 2009). Subsequent to this study, Joaquín's and my groups have continued to collaborate and have published two more articles that describe further details of this and related systems (Abrie *et al.*, 2012; Molero *et al.*, 2013); a third—on whether Hal3 could dissociate from one protein complex to join another—is currently being finalised. It has been (and continues to be!) a fascinating journey working with his group in unravelling the finer details of CoA biosynthesis in various yeasts, all while trying to grasp the significance of the system for CoA biosynthesis in higher organisms in general.

6 REVERSING THE BENEFIT: COA BIOSYNTHESIS AS A DRUG TARGET

Perhaps the most interesting prospect for research focused on CoA biosynthesis is the potential for the design and discovery of new antimicrobials,[19] i.e. compounds that could be used in the treatment of infectious diseases. Two examples of our work in this regard are highlighted in the following sections.

6.1 Pantothenamides as antivitamins

The concept of using 'antivitamins' (i.e. compounds that inhibit metabolic processes that depend on a specific vitamin) as antimicrobials is not a new one. The sulphonamides or 'sulpha drugs', the very first antibiotics that were discovered in the 1930s, were later found to impede the biosynthesis of folic acid (vitamin B_9, see Table 1). One of these sulpha drugs—sulfamethoxazole—is still widely used today. This discovery led to a rush of studies in the 1940s to discover compounds that similarly acted as antivitamins of the other vitamins, but none were as successful as the sulphonamides, and with a few exceptions research in this field has been mostly abandoned (Shapiro, 2013). However, one study published more than 40 years ago demonstrated that pantothenamides—analogues of pantothenic acid (vitamin B_5) in which its carboxyl is converted to an amide—show inhibition of the growth of several kinds of bacteria (Clifton *et al.*, 1970). In light of our interest in CoA biosynthesis we decided to investigate this result further, and in 2002 published the first follow-up study of this work: we demonstrated that when the pantothenamide with a pentyl substituent (N5-Pan) is offered to the CoA biosynthetic enzymes, it is converted into the antimetabolite ethyldethia-CoA much faster than CoA is formed from pantothenic acid (Strauss & Begley, 2002). This suggested that N5-Pan acts as a bacterial growth inhibitor by reducing CoA levels, or because the antimetabolite product cannot act

[19] Currently, the term 'antimicrobial' is used as a general term for any compound that kill or arrest the growth of any microorganism, including bacteria, viruses, fungi and protozoa (usually parasites). The more commonly used 'antibiotic' used to be synonymous with 'antimicrobial', but is now more widely used to refer to antibacterials, i.e. agents that are used in the treatment of bacterial infections.

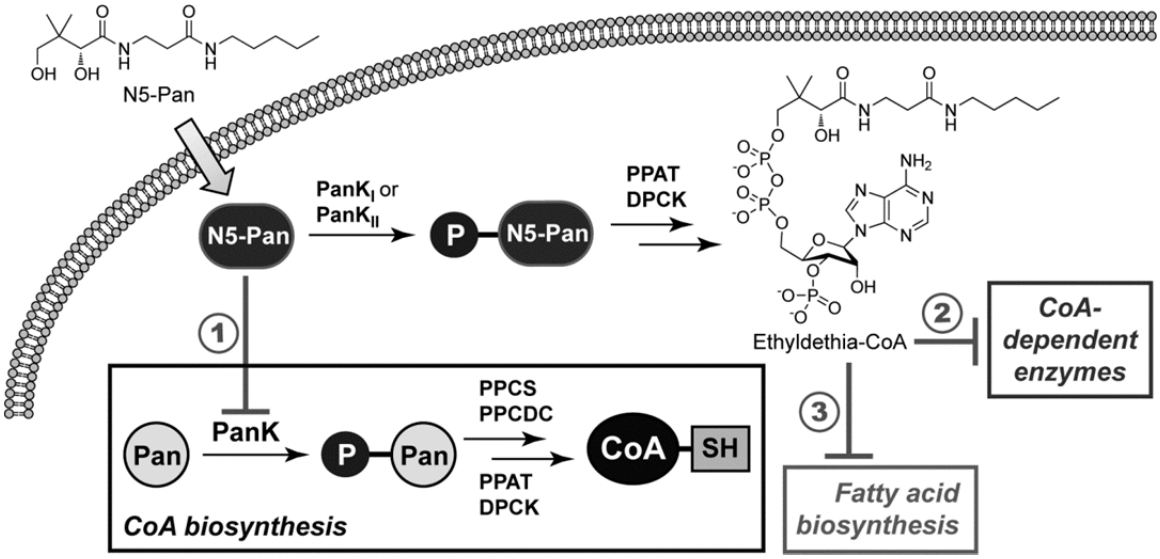

Figure 7. Potential modes of action for N5-Pan, an antivitamin of pantothenic acid (vitamin B_5): 1) Inhibition of PanK, and consequently CoA biosynthesis (leading to lowered CoA levels); 2) Conversion of N5-Pan into the antimetabolite ethyldethia-CoA by the CoA biosynthetic enzymes, causing inhibition of CoA-dependent enzymes (since the antimetabolite lacks the thiol group that is required for carrying acyl groups—see Figure 1); 3) Inhibition of fatty acid biosynthesis by inactivation of the acyl carrier proteins involved in this process by the antimetabolite.

as an acyl carrier (see Section 2) because it lacks the requisite thiol, and therefore inhibits CoA-dependent processes and/or fatty acid biosynthesis (Figure 7).

Many research groups around the world picked up on this finding, and subsequently applied it in various contexts. Some tried to establish the mode of action of the pantothenamides, leading to the now generally held belief that they act through the inhibition of fatty acid biosynthesis (Zhang *et al.*, 2004). Others used the process as a way to label proteins (Mercer & Burkart, 2007). My own group was involved in work that showed that some pantothenamides are potent antiplasmodial agents (compounds that inhibit the growth of the malaria parasite, *Plasmodium falciparum*), although the compounds were unfortunately found to be prone to degradation (Spry *et al.*, 2013). We also established a new method to prepare a small library (~150 compounds) of pantothenamides and their analogues in a simple and parallel fashion (van Wyk & Strauss, 2008) and most recently showed that the library members that have structural modifications compared to the parent compound are resistant to degradation (de Villiers *et al.*, 2013). This has re-opened the door to the development of these compounds as antimalarials, something we are currently actively pursuing. Finally, we are also revisiting the mode of antibacterial action of the pantothenamides, and have discovered that this is in fact very organism-specific, with the pathogen *Staphylococcus aureus*[20] being most sensitive. Our findings in this regard may have far-reaching implications, both for the design of new antistaphylococcal agents and for the regulation of CoA biosynthesis in general.

6.2 CJ-15,801: A surprisingly selective natural product

In 2001 a Japanese research group working with the pharmaceutical company Pfizer discovered a curiously selective antibacterial agent in extracts from a *Seimatosporium* fungus (Sugie *et al.*, 2001). This compound—which they named CJ-15,801—only inhibited the growth of *S. aureus*, but none of the other bacteria that were tested. When the structure of the compound was elucidated, it was discovered that it was identical to vitamin B_5 except for the addition of a double bond. This suggested that the compound's inhibitory action was due to some effect on CoA biosynthesis, but it was unclear how this could be so selective. We took on the challenge to solve the mystery in collaboration with two groups from UC San Diego in the USA.

[20] *Staphylococcus aureus* is known by several names and abbreviations: colloquially it is most often simply called '*Staph*', while in clinical settings it is also called 'MRSA' (pronounced 'mirsa'), using the abbreviation for 'multidrug-resistant *Staphylococcus aureus*'.

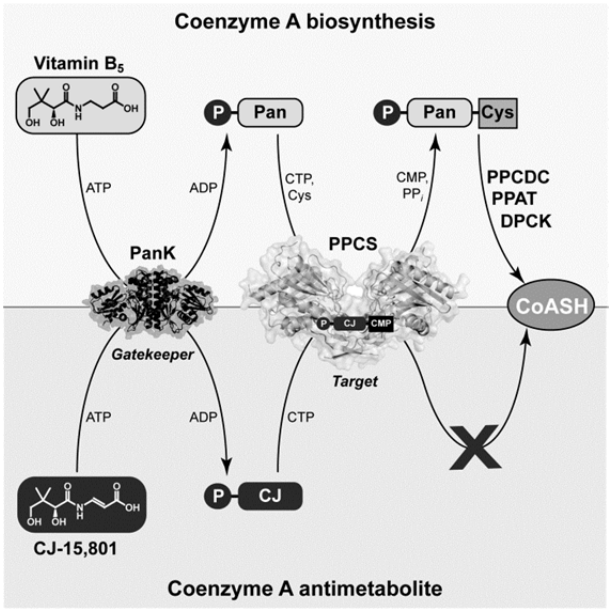

Figure 8. Mode of action and selectivity of the natural product CJ-15,801. The inhibitor is activated only by PanK enzymes that can accept it as substrate (to date, only the PanK from *S. aureus*) and that therefore act as gatekeeper to its inhibition. Subsequently, PPCS catalyses the reaction of P-CJ with CTP to form P-CJ-CMP, which binds the enzyme tightly and prevents it from performing additional catalytic cycles (van der Westhuyzen *et al.*, 2012).

By using a variety of biochemical analysis techniques we discovered that CJ-15,801 inhibits PPCS, the second enzyme of CoA biosynthesis, but only after it is activated by phosphorylation, i.e. once it can mimic the structure of the PanK reaction product. This activated form of CJ-15,801 (phospho-CJ-15,801, or P-CJ) reacts with CTP in the active site of PPCS to form P-CJ-CMP, which acts as a structural analogue of the enzyme's normal reaction intermediate. P-CJ-CMP remains tightly bound to the enzyme, and impairs its ability to perform catalysis—thereby blocking the formation of CoA. The consequent reduction in CoA levels is most likely the cause of cell growth arrest.

However, this mechanism does not explain the compound's selectivity. Since CJ-15,801 must be phosphorylated by PanK for it to act as a PPCS inhibitor, we tested the ability of various PanK enzymes to perform the reaction. We discovered that while none of the type I and type III PanKs tested could perform the reaction (see Section 4), the only known example of a bacterial type II PanK—the one from the targeted organism, *S. aureus*—was able to convert CJ-15,801 to P-CJ with an efficiency similar to that seen for pantothenic acid (its native substrate). This indicated that the PanK enzyme acted as a gatekeeper for CJ-15,801 inhibition, and that only organisms with a PanK that would accept it as a substrate would experience inhibitory effects. This mechanism (summarised in Figure 8) is reminiscent of that of the sulphonamides, and revealed CJ-15,801 as a true antibacterial antimetabolite. This discovery of a new antivitamin, which also showed potential for use as an antibiotic, drew significant interest from the chemical biology community when it was published (van der Westhuyzen *et al.*, 2012). It was highlighted in an accompanying 'Preview' article (Martinelli & Aldrich, 2012), and was also featured on the journal's cover. We are still actively pursuing further studies based on these findings in our endeavour to find clinically useful inhibitors of CoA biosynthesis.

7 FUTURE PROSPECTS

Our knowledge and understanding of CoA biosynthesis have increased significantly over the past 15 years, and it has been both a pleasure and a privilege to have contributed to this process as highlighted in this article. However, much still remains to be done: in spite of our successes in the discovery of compounds that inhibit the growth of pathogenic bacteria and the malaria parasite, these still need to be developed further before it will be possible to apply them in a clinical setting. The focus of my group is shifting more and more to exploiting the differences in the human and pathogen CoA biosynthesis pathways for the discovery, design and development of new antimicrobial agents. This work requires the continued effort of MSc and PhD students with backgrounds in both chemistry and biochemistry, and I am therefore particularly pleased to be at the only university in South Africa which currently offers a BSc degree in Chemical Biology. The continued institutional support of this field and its development will be a crucial component of any future successes that we may achieve.

In addition, my group's research interests are also starting to expand beyond CoA biosynthesis. This shift was initiated by our interest in understanding how the bacterial pathogen *S. aureus*,[21] which is rapidly becoming resistant to

[21] In the United States, more people succumb to fatal antibiotic-resistant *S. aureus* infections than to HIV/AIDS-related illnesses.

every antibiotic we have in our clinical arsenal, survives the attacks that the human immune system launches on it to fight off infection.[22] This interest was born from our curiosity about CoA, since *S. aureus* apparently uses the cofactor as part of its defence mechanisms against such attacks. This has opened new areas of study for us that have as their focus the importance and relevance of low molecular weight thiols (i.e. small molecules with –SH groups, such as CoA) in oxidative stress resistance, and in the enzymes that act on these thiols. We have already made some headway in this regard, with the design of the first inhibitors of the *S. aureus* CoADR enzyme that ensures that CoA remains reduced (van der Westhuyzen & Strauss, 2010); in collaboration with research groups in the USA we have also managed to determine the structures of this protein in complex with our inhibitors (Wallace *et al.*, 2012). This work is continuing as we now branch out our interest to enzymes that do not act on CoA, but which seem vital to *S. aureus*'s survival upon challenge with oxidative stress.

The first 11 years of my independent career have been filled with excitement, frustrations, discoveries, challenges and immense pride in our achievements. I am thrilled to be able to build on these, and to be working with a team of postgraduate students that share my enthusiasm and optimism about what lies ahead as we continue to walk the paths along the CoA pathway, and beyond.

[22] One of the first ways in which the human immune system respond to bacterial infections is to recruit certain types of white blood cells that try to engulf the bacteria (i.e. overtake them and then isolate them in a little vesicle, or bag, inside the white blood cell) where they are subsequently bombarded with oxidants (such as the equivalent of domestic bleach). This leads to the bacteria being killed in most cases, but some survive such kinds of oxidative stress, without any apparent affect.

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