A world of cytochrome P450s

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The world we live in is a biosphere influenced by all organisms who inhabit it. It is also an ecology of genes, with some having rather startling effects. The premise put forth in this issue is cytochrome P450 is a significant player in the world around us. Life and the Earth itself would be visibly different and diminished without cytochrome P450s. The contributions to this issue range from evolution on the billion year scale to the colour of roses, from Darwin to Rachel Carson; all as seen through the lens of cytochrome P450.

1. Introduction

Cytochrome P450 debuted to the world of science in 1958 as an unusual spectrum taken from rat liver microsomes with a peak of absorbance at 450 nm [1]. Fifty four years later, we know more than 18 500 examples of the P450 molecule from hundreds of species [2]. The level of detail for these proteins varies from multiple crystal structures (over 80 for cytochrome P450cam) with simulated movies of their active sites to just a fragment from a transcriptome project. Inbetween, we have glimpsed the importance of P450 for biology, and it has been applied for human benefit in the pharmaceutical industry, agriculture, biotechnology and even aesthetics.

This special issue ‘Cytochrome P450 and its impact on planet Earth’ poses the question: how would the Earth be different without cytochrome P450s? The eukaryotic cell, with its larger size and greater energy capacity due to mitochondrial function, exposed a gateway for multicellularity that has not been exploited by archaea or bacteria except for a few myxobacteria and filamentous branching cyanobacteria [3]. An essential transition to the eukaryotic cell was a change in the cell membrane to allow phagocytosis. The membrane alteration called by Cavalier-Smith the neomuran revolution was linked to the presence of cholesterol in the membranes, a eukaryotic hallmark [4]. The ability to make sterols depends directly on the P450 CYP51, so in some degree or fashion the Earth would probably not contain significant multicellular life without CYP51 impacting the composition and properties of membranes at the dawn of eukaryotes.

Moving forward in time from multicellularity to the conquest of the land, we find P450’s contributions essential in many ways. In leaving the water, plants needed a waterproof coating so that they would not dehydrate. Pathways evolved for making the cutins and suberins used in forming epicuticular waxes. Fatty acid and alkane hydroxylases in the CYP86 clan provided this function, without which the land would be surrounded by an algal pond scum and little more. A similar hydrophobic coating covers insects, or they die at hatching. This coating is provided by CYP4G1 in Drosophila and its orthologues in other species [5]. These hydrocarbons are the target of P450s of the entomopathogenic fungus Beauveria bassiana as described in the article by Kelly & Kelly [6].

If a cuticle is assumed for existence on dry land, what other key aspects of life on land are P450 dependent? Lignin is the support molecule for woody plants. The tallest plants without lignin are mosses [7]. Lignin is a product of P450 reactions in the phenylpropanoid and monolignol pathways (CYP73, CYP98 and CYP84). It is used for making tracheids and vessels for water conduction and it offers support for upward growth. The famous dictum ‘Allest ist Blat’ (all is leaf) by Goethe [8] would not be true for there would be no leaves as
we usually think of them. The planet’s flora would be limited to bryophyte-like plants, only a few inches high.

The carboniferous forests that gave rise to massive coal beds depended on the extensive biosynthesis of lignin in the bark of these early conifers. The bark was very thick and 38–58% lignin. It would not be exaggeration to say that the P450s that made lignin powered the industrial revolution. These ancient P450s continue to contribute to our economy today. In 2008, coal accounted for 28 per cent of world energy consumption: 505 quadrillion British thermal units [9].

Pollin is another major land plant innovation that is P450 dependent. Dispersal of pollen exposes the fragile male gametes to a harsh environment. The tough polymer sporopollenin evolved to protect the contents of the pollen grain, and this polymer is made partially by pollenin evolved to protect the contents of the pollen grain, and this polymer is made partially by pollenin. Dispersal of pollen exposes the fragile male units [9].

The element of chance events in the birth or death of genes is explored and rejected. The power-law relationship has been noted in many biological contexts. The implications are that stochastic forces are at work in the distribution of sizes in CYP families and selective forces such as plant–animal warfare are not necessary to explain the current spectrum of CYP genes. This result is counterintuitive but examples are given of the same power law describing CYP family distributions in animals that eat a single plant (silkworm), and those that eat hundreds of different plant species from more than 140 plant families (spider mite). If selective forces were at play one might expect a different result in these two cases. The concept of stable and unstable CYP families is explored and rejected. Even the CYP51 family, a primary case for stability over a billion years, has a documented bloom in grasses (the CYP51H subfamily) that has changed function from a sterol biosynthetic enzyme to a role in secondary metabolite synthesis. The element of chance events in the birth or death of genes may underlie the vast diversity of P450s that we see, with evolution acting on what is available at the time. There may be less selective pressure acting to create blooms than has been suggested before, but the end result is nevertheless the same, a prodigious biodiversity of cytochrome P450s in the world.

My first introduction to the biological diversity on planet Earth was from Jules Verne in the form of Prof. Pierre Aronnax, a French marine biologist with encyclopedic knowledge of every living thing in the sea. He would describe at length all the specimens of corals, molluscs, cetaceans and fish that he saw on his journey aboard the Nautilus, including latun binomial names [18]. Kelly & Kelly [6] bring that broad and detailed knowledge of microbial CYPs to this issue with mention of 26 bacteria, one archaea, six protists, 23 fungi and one animal (a sponge). CYPs from these species are discussed in relation to early P450 evolution, food security, biorefinery, pharmacuetics and industrial biotechnology. Particular attention is given to CYP51 as a drug target against human and agricultural pathogenic fungi, oomycetes, trypanosomes and related protozoa. Our food supply is partly...
dependent on application of anti-fungal CYP51 inhibitors. The cost for battling these organisms is measured in billions of dollars per annum. Other applications of microbial CYPs are represented by examples of antibiotic synthesis and semi-synthesis, industrial production of steroids, bioengineering of pathways for fine chemicals and bioremediation of toxins, gasoline additives and even explosives. The coupling of CYPs into pathways with polyketide synthases and non-ribosomal peptide synthases holds promise for bioengineering novel antibiotics and anti-cancer drugs via oxidative tailoring. Microbial CYPs are only beginning to be catalogued and characterized and the potential for exploiting these versatile catalysts seems immense. Kelly & Kelly [6] list 10 classes of P450s, partly based on their electron donors. This categorization is expanded upon with numerous examples in the contribution of Lamb & Waterman [19].

More than half a century of research on P450s has established the textbook view of these enzymes. Eukaryotic cytochrome P450s reside in the endoplasmic reticulum or mitochondrial inner membranes with different electron transport chains supplying reducing equivalents in each compartment. Bacteria have soluble P450s with a typical ferredoxin/ferredoxin reductase type of electron supply chain. However, real life is complicated. There are exceptions and the exceptions can be of the ‘everything you thought you knew is wrong’ variety. Lamb & Waterman [19] write the comprehensive P450 textbook and show us the exceptions, including novel fusions of many types and long-established motifs gone far from the accepted norms. Even the invariant EXXR motif has its deviations. Last but not least, the haem thiolate ligand itself cannot be found in the CYP408 family. This raises the philosophical and semantic issue of what constitutes a P450, if even the P450 spectrum is gone? One is reminded of the story of the Pearl of Love. A great Prince so loved his wife that when she died he built her a great mausoleum. After years of construction, it was finally done and the Prince came to view it in all its glory. Only one thing marred the Pearl. The Prince pointed to his wife’s sarcophagus and commanded: ‘Take that thing away’ [20]. It seems both life and love evolve.

Darwin’s dream was to understand the evolutionary history of life on the Earth. Today’s sequence revolution is bringing that dream close to reality as more and more detail is added to the ‘Tree of Life’. It is even being recast bringing that dream close to reality as more and more sequencing results from this search uncovered additional neighbouring genes that are also clustered (Hox genes and NK genes) and have a developmental role (Hox, NK, Wnt, ARF). The clustering of this group of genes points to a developmental locus in early animal evolution.

Inside the animals, one taxon with a disproportionately large impact on planet Earth are the insects. Extremely relevant to us is the development of insecticide resistance. David et al. [24] outline the relentless battle between human chemists and their insect targets who eventually rebuff the toxins we humans throw at them. This is a fitting contribution in the light of the 50th anniversary of Rachel Carson’s Silent Spring (27 September 1962) [25] leading to the ban of DDT (dichlorodiphenyltrichloroethane). Among the animals, insects, especially mosquitoes, have the most P450s (with the exception of the deer tick, Ixodes scapularis). However, only a handful are conserved across taxa for fundamental purposes, such as ecdysteroid metabolism and molting. The vast majority are adaptable to many functions, including detoxification of insecticides. This is particularly important to humans because the numbers of people at risk for mosquito borne diseases is in the billions. David et al. [24] document the nature of resistance to insecticides in mosquitoes with emphasis on P450s. The hope is that knowing how resistance develops will be an aid in devising better insecticides, and it could provide a useful handle to monitor the spread of resistance genes. Identification of resistance single-nucleotide polymorphisms (SNPs) allows PCR-based methods in the field. Novel methods include tracking the expression of genes responsible for resistance by fluorogenic and luminescent substrates, causing resistant mosquitoes to light up. Such knowledge can inform decisions about pesticide use and alert officials when to change tactics.

The nearly random recruitment of CYPs and their SNPs from mosquito CYPomes for resistance genes has a serious indirect impact on humanity.

A direct impact on humans is mediated especially through our own set of 57 P450s. Nebert et al. [26] present the current status of these genes and how they affect human health and disease. Human P450s tend to belong to expanded families with multiple subfamilies (CYP1, CYP2, CYP3, CYP4) or they are nearly unique, some with only one family member. These are in 14 families CYP5–CYP51. These latter families usually have well-defined substrates and functions (except for some orphans like CYP20). Substrates for P450s are typically lipids or lipophilic compounds. The range covers polycyclic aromatic hydrocarbons to sterols, steroids, fatty acids, retinoids, hundreds of drugs and more than 150 eicosanoids. Phenotypes are rare for mutants or deletions of CYP2, CYP3 and CYP4 families, but they can be quite dramatic for the more specific CYPs. Severity ranges from embryolethality to sugar control, salt balance, rickets, blindness and secondary sexual characteristics. Progress in defining the roles of human orphans may depend on research in distant animal relatives due to the conservation across hundreds of millions of years for genes like CYP20. Future progress may rest in massive data acquisition and network analysis at the genomic and epigenomic levels as practiced in the ENCODE project.

This volume is a sampling of a few threads in the magic carpet of cytochrome P450. We visit microbes, plants, animals and fungi and consider P450s’ contribution to our sense of beauty in flowers, our vulnerability to pathogens and our dependence on P450s for survival. Some of our most valuable agrichemical anti-fungals target P450s while other P450s...
inactivate our best pesticides. Still others make key antibiotics like vancomycin, antimalarials like artemisinin and anti-cancer drugs like taxol. Our own human P450s are the basis for sometimes fatal drug interactions and the beneficial conversion of codeine into morphine. Even morphine is synthesized by P450s in the opium poppy. As P450s have been on the planet for billions of years they have penetrated every ecology and nearly every eukaryote, except for a few parasites, so that if they were suddenly removed the world would regress to a primordial sea of bacterial life. And then they would be evolved again.

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References


