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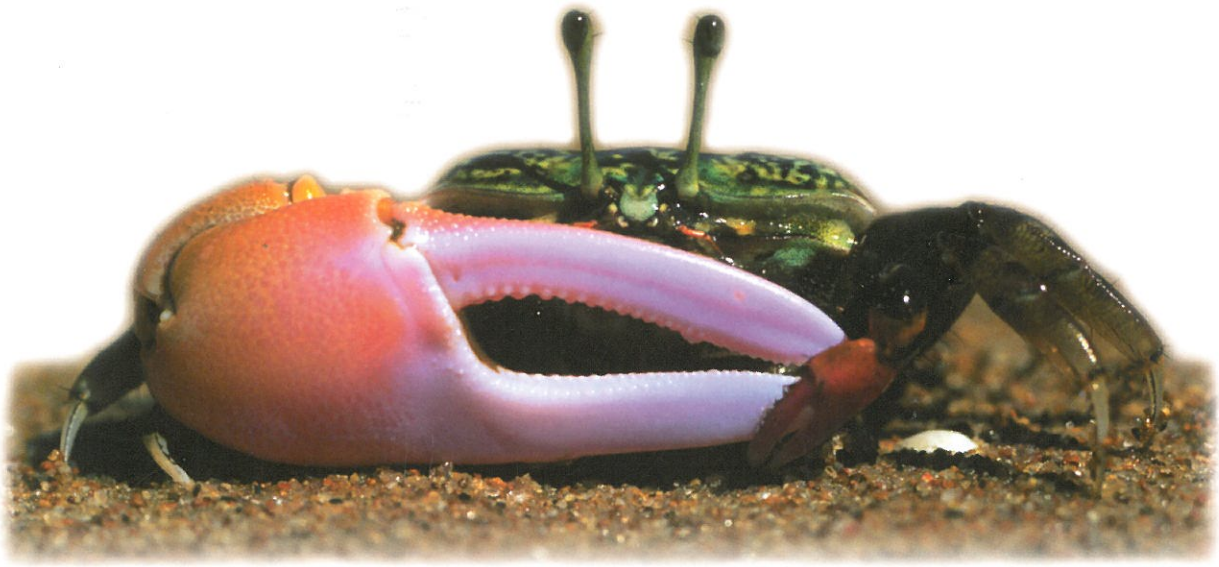

A glimpse through crab eyes

It's not easy being green

Getting more wheat for your water

Alternative oxidase: essential safety valve?

Corals yield clues to the mysteries of development

Contents . . .

The Research School of Biological Sciences is one of Australia's leading centres for basic biological research and graduate training. Since its inception in 1967, it has focussed on three domains: plant science, genetics, and neuroscience. This work is carried out in 11 research groups, currently organised as follows:

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Editorial . . .

Welcome to the thirteenth edition of Biologic...

Unlike previous editions, this Biologic does not have a theme. Instead, the stories on the following pages reflect the broad spectrum of research taking place within the Research School of Biological Sciences.

A glimpse of the world through crab eyes, page 2, is an example of science learning from the incredible elegance of Nature. Using the visual systems of soldier crabs as a model, scientists are piecing together basic information about environmental perception and navigation that will help shape the eyes of tomorrow's robots and mechanical devices. In many ways, it is a lesson in humility as researchers discover how much more sophisticated the eyes of a tiny crab are than the most advanced computational systems of modern science.

It's not easy being green, on page 6, covers research on photosynthetic bluegreen algae that is aimed at explaining one of the most puzzling mysteries of modern plants – the remarkable *in*efficiency of photosynthetic CO_2 fixation. Despite being the first crucial link in practically all of the world's food chains, photosynthesis is quite an inefficient reaction. The controversial question facing scientists today is, can we borrow components of the cynobacterial CO_2 concentrating mechanism in an attempt to improve photosynthetic CO_2 fixation in crop plants?

Corals yield clues to the mysteries of development, on page 10, looks at some colourful and intriguing research being carried out jointly by scientists from RSBS, Southern Cross University in NSW, and James Cook University in Queensland. The team are looking at corals – some of the most simple animals known – and using them to uncover genes that initiate the first steps of development, and the subsequent chain of events that shape fully-formed animals with tissues, limbs and organs out of single fertilised cells.

Getting more wheat for your water, on page 14, looks at some extremely practical research being carried out in collaboration with CSIRO Plant Industry, aimed at breeding wheat plants that grow better in Australia's arid climate. Already the group has demonstrated that their plants have a yield advantage of around 10 per cent in dry conditions – an improvement which could be worth millions to the Australian economy, especially in drought years. And they believe that the same technique, which is based on fundamental scientific research, could be adapted to make other crops more suited to the environments in which they are grown.

Alternative Oxidase – evolutionary mistake or essential safety valve, page 18, deals with another of Nature's little mysteries: a plant protein which does everything from initiating steamy plant sex in the Indonesian jungle to protecting tomato plants from frost damage. And although the function of the alternative oxidase protein in plants is not fully understood, it has been implicated in several important facets of plant life. Potential applications of the project, being conducted jointly by scientists at RSBS and the ANU Division of Biochemistry and Molecular Biology, are slow-ripening tomatoes which last longer on the shelf. Long-term benefits of the work, like those of all basic research, are still hard to predict.

We hope you enjoy the edition.

Damon Shorter

A glimpse of the world through CRAB EYES

Everybody, at one time or another, has been fooled by their eyes – seen an imaginary shape lurking in a shadow or mistaken a passing stranger in the street. Yet apart from these rare mistakes, our eyes are rarely wrong. The visual system is remarkably good at interpreting the stream of signals that floods into the brain each time we open our eyes. Together, the eyes and brain sort through this mad jumble of light, lumping information together into shapes and images and leaving us with our familiar visual picture of the world. In effect, our eyes filter out the noisy mess of visual reality and construct for us an orderly version of our environment containing the most useful information.

The most advanced computer systems are only now beginning to grapple with visual tasks we find mundane, and the eyes of a fly are easily more effective than the best robotic eyes. Trying to understand and ultimately emulate the incredible interpretative skills of natural visual systems motivates Dr Jochen Zeil in his research at the RSBS Department of Visual Sciences. His goal has taken him back in evolutionary history to investigate some of the most simple visual systems, and in the process he has become an unlikely expert on crabs.

Vision in crabs is relatively easy to study because, compared with humans, crabs have simple eyes that perform only a limited range of visual tasks. As Dr Zeil explains it, working with the "stripped-down visual system" of a crab gives researchers an opportunity to discover the essential elements of vision. But a close look at even the primitive visual system of a crab is a humbling experience.

"I see a crab first as something I just go overboard with admiration about," Dr Zeil confesses, leaning back in his chair in his ground-floor office in RSBS. "What they can do, how they live, how entertaining they are, and I think that I could *never* have invented them. My first reaction is just awe," he exclaims. "The second, is then the trained eye of someone with an interest in visual mechanisms. How do they do it? What sorts of processing do they use, what sort of visual cues do they need, what sort of control systems do they need? And how can we experimentally try to find out?"

To understand *how* a crab uses vision, Dr Zeil needed to first work out *what* a crab can see and, more specifically, what tasks it uses its eyes for. Dr Zeil has spent weeks on end studying the habits and behaviour of fiddler crab populations on mud flats in Kuwait and, more recently, around Townsville in north Queensland. Yet as it turns out, some of the most important clues about the way crabs see can be guessed from the way their eyes are built.

The construction of an eye gives a good indication of the sort of visual information it conveys to the brain. Different eyes are designed to see different things. Insects and crabs, for instance, can recognise the polarisation (plane of vibration) of light, something that we, humans can not. While information about

the polarisation of light might be useless to us, dragonflies, for example, use this special feature of their vision to locate flat water surfaces they need to breed. Some animals can only see in black and white, while others can see colours even in the ultraviolet and infrared regions of the visual spectrum. "For any one animal, the eye is constructed like a filter that is matched to the likelihood of events occurring in its visual world," says Dr Zeil. Just by examining the eyes of animals, you can get a pretty good idea of what is important to them.

Life in a flat world

"The interesting thing about fiddler crabs is that their eyes seem to be completely shaped by the flat world they are living in," Dr Zeil explains. As humans, our eyes have a central area of best vision (the fovea) that is small and round which we point at places of interest. Our eyes are also far apart, facing forward on our faces, and this makes them good at judging distances, examining objects in detail, recognising faces and doing other important human tasks. But our forward-facing eyes are less good at detecting predators sneaking up from behind.

Fiddler crabs, on the other hand, don't have a central area of high resolution, but instead have a 360° visual field and a thin horizontal band of high resolving power centred on the horizon.

There are good reasons why crab eyes have evolved this way. "On flat ground, to decide whether something is larger than yourself can be done by asking 'does it stick up above the horizon?'," Dr Zeil explains. "If it does, it is larger than you, irrespective of how far away it is." A crab with good visual resolving power along the horizon can quickly decide if something is bigger or smaller than itself and that translates directly into the time it has to run away if something approaches. "There must be a very powerful selection for getting your visual system tuned to that kind of cue," he explains.

Apart from needing their eyes to recognise approaching predators, crabs also use vision to recognise each other. Fiddler crabs get their name from their elaborate territorial and mating displays. Each male has one of its colourful claws disproportionately enlarged and uses it for fighting and signalling. These claws are waved up and down in a way that reminded early biologists of a person playing a fiddle, and each species has a slightly different choreography – some waving their claws more vertically, others more horizontally.

For Dr Zeil, the fascinating question is how a crab (with a brain a few cubic millimetres in size) can distinguish these highly stereotyped claw wavings from background movements caused by, for instance, a bird running across their field of vision. No computer gets even close to the abilities of a humble crab in distinguishing this sort of visual information. To understand how a crab does it, Dr Zeil plans to reconstruct the crab's visual world – in effect, to look at the world through crab eyes.

Thinking Like a Crab

"We do what is called a natural scene analysis," Dr Zeil explains. "We analyse quantitatively the spatial, spectral, polarisation and temporal characteristics of the crab's environment by recording them with video cameras, spectrographic imagers, polarisers, and UV cameras. We then look for animal-relevant information." The challenge is then to find ways of interpreting the information in a way that would be useful for a crab. "We have started with these crabs partly because it is a very simple, structured world they are living in," he says. "That means you can specify the

Figure 1. A fiddler crab, waving its large, brightly coloured claw which is used to signal mates and mark its territory. The erratic waving patterns reminded early biologists of the frantic movements of a fiddler's bow.

types of behavioural decisions these animals need to make visually. It is a setting that you might hope to be able to describe and understand."

Currently, Dr Zeil is looking at detection problems which involve just one aspect of vision – contrast. "If I were to design a crab," he asks, "where would I allocate its sensitivities for certain visual tasks?" For example, if a crab is to recognise the horizon, the contrast between the sky and the ground will differ depending on whether the crab looks in the ultraviolet, the blue, or the red region of the visual spectrum. While Dr Zeil can not directly measure the way a crab sees, he can do the next best thing – record the horizon through ultraviolet, blue, and red filters from a crab's perspective and then look at the different levels of contrast himself. The greater the contrast between the sky and the ground, he argues, the better chance the crab would have of detecting the horizon, and the more likely the crab would be to look at the horizon in that region of the spectrum.

To analyse more difficult visual tasks associated with movement, Dr Zeil collaborates with another researcher working in the department, Dr Johannes Zanker, an expert on motion vision. Up until now, Dr Zanker's research has focussed primarily on how simple algorithms can be used to model the ways humans perceive motion. But Dr Zanker has found the computational models he has developed can also be used to interpret images in Dr Zeil's video footage of crabs.

When the videos are analysed using Dr Zanker's computer algorithms, a glimpse of how the world might look to a crab begins to take shape. The digitised image sequences are sent through a network of small elements analogous to the eye's motion detectors. "If you analyse the output at the individual level of these motion detectors," says Dr Zeil, "it is extremely noisy and you can not see any sort of pattern. But when you do the first simple operations, such as pooling the outputs of neighbouring motion detectors or taking a longer integration time, then the noise disappears and out comes this choreography of movement directions. That tells us that using this kind of simple processing, the crab's brain could, in principle, distinguish a succession of different motion directions."

Although no one can be sure, this sort of visual analysis is probably similar to that carried out in the

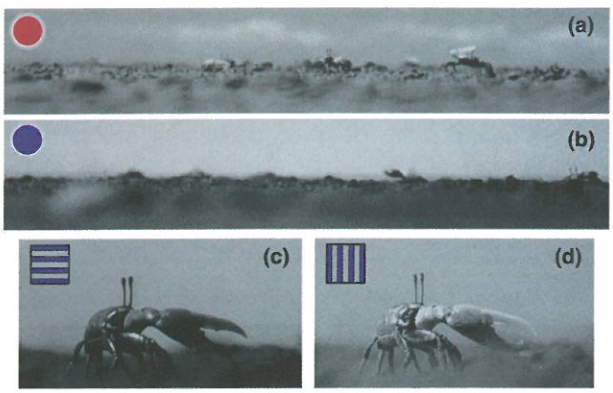

Figure 2. A view of the mudflat seen through a red filter (a), and a blue filter (b) where the horizon is more clearly visible. The outline of a crab is more easily distinguished through a horizontal polariser (c) than through a vertical polariser (d), and the remote-controlled video camera (e) Dr Zeil uses to record the reactions of crabs to an approaching "predator".

minuscule brain of a crab every time it sees a moving object. And the adequacy of the computer models can in future be tested by comparing their outputs with the activity of neurons recorded directly from a crab's brain, Dr Zeil says.

Robotic Eyes of the Future

The insights Dr Zeil is gaining about how animals extract visual information may at some stage become relevant to engineers designing artificial eyes for the machines of the future.

"There isn't a robot in existence that would be able to navigate out there yet," Dr Zeil says, pointing out the window of his office. "The real world is simply too complicated. But if we can learn something about what natural scenes are made up of, what dynamics they have, and where important information can be found in terms of spatial resolution, spectral resolution, and motion, then I think that it will also interest the robotics people."

Particularly relevant to robotics is the problem of egomotion, which relates to the way visual images change when the head and eyes move. Whenever we walk, for instance, stationary objects around us appear to move. Although the problem is overcome by even the most primitive flying insects, egomotion creates massive difficulties for scientists trying to design robots that can interpret the visual world as they move about.

"Even seemingly simple animals still have millions of years of experience with information processing," Dr Zeil says. "Whenever you look at animals like bees or crabs, you very quickly realise that they cope with problems that are still way beyond our ability to duplicate them with a machine. So there is still plenty for us to learn from them," he laughs.

Working with three other RSBS researchers, Dr Javaan Chahl , Dr Martin Hofmann and Professor Srinivarsan, Dr Zeil now plans to apply his ideas of visual reconstruction to the problem of egomotion. By mounting a video camera on a robotic gantry (a device that allows the camera's movement to be controlled in three dimensions), the team will try to record and reconstruct the views seen by flying insects.

"Animals work out from the pattern of image motion on their eyes what their orientation in space is and also their direction of movement," he explains. "But they also extract information about the three dimensional layout of the world. That is something computer vision people are really interested in – what are the algorithms that do this reliably and robustly under real world conditions"

One can not help but wonder, as scientists such as Dr Zeil begin to unpick the complex visual processes that help us make sense of the world, whether the distinction between animal and robot ever begins to blur. For Dr Zeil, does he ever think of his fiddler crabs as merely clever biological robots? "I come close to this image of a robot," he replies, "but only as an analytical tool for me. I might make hypotheses about what the animal's mechanism of orientation is, for instance, and the most potent way of testing them is trying to build a machine. But I don't see them as robots," he laughs. "Not at all. They are much too beautiful in their own right for that."

If you want to know more ...

• The variation of resolution and of ommatidial dimensions in the compound eyes of the fiddler crab *Uca lactea annulipes* (Ocypodidae Brachyura, Decapoda).

Zeil J and Al-Mutairi M. J. Exp. Biol, 199, 1569-1577 (1996)

• A glimpse into crabworld.

Zeil J and Zanker JM. Vision Res, 37, 3417-3426 (1997).

Dr Jochen Zeil

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it's not easy being green

looking to solve the problems of photosynthesis

Photosynthesis has been around on earth for about three billion years. It harnesses energy from the sun through the green chlorophyll pigments, pulls carbon dioxide from the atmosphere, replaces it with oxygen, and builds the carbon into living organisms that feed all the world's food chains.

But photosynthetic carbon fixation has proven a difficult reaction for plants to get right. Despite evolutionary improvements, photosynthesis in modern plants is still surprisingly inefficient. For decades scientists have been intrigued by this odd quirk of nature. Why is photosynthesis so slow and clumsy when compared to other biological reactions? Is it a fundamental flaw of evolution? And could photosynthesis, a process so vital to life on earth, ever be improved?

The heart of the problem

At the heart of the photosynthetic reaction is the enzyme Rubisco, whose name is an acronym for its more impressive title, Ribulose-1,5-bisphosphate carboxylase/oxygenase (see: *The most abundant enzyme.* Biologic, July 1993). Rubisco is the catalyst that takes chemical energy derived from sunlight and uses it to fuse carbon dioxide into sugar chains, trapping the carbon in a biologically useful form. Atom by atom, Rubisco and the photosynthetic machinery draw carbon from the atmosphere and build it into fuel molecules plants need to live and grow.

For eons Rubisco has been catalysing this fundamental reaction, creating organic molecules to support the world's mass of food webs and biological organisms. Yet despite its ubiquitous and essential role in nature, the enzyme is fundamentally flawed. Not only is it slow in fixing carbon dioxide gas (orders of magnitude slower than most other enzymes), it can only poorly distinguish between its intended target, carbon dioxide, and another far more prevalent gas, oxygen.

The two gases react with Rubisco in conflicting ways. When Rubisco encounters an oxygen molecule, a wasteful product is produced and carbon dioxide is lost through photorespiration. This retrograde oxygenation reaction wastes a large proportion of the plants energy – up to a third according to some estimates – and appears to have few, if any, beneficial effects. Photorespiration, as the oxygenation reaction is called, appears to be a classic case of three steps forward, one step back. Plants are forced to manufacture enormous quantities of Rubisco to compensate for its low effectiveness, diverting precious nitrogen away from other parts of the growing plant. Ironically, the very ineffectiveness of Rubisco as an enzyme has made it the most abundant protein on earth.

The best of a bad situation

Nature has evolved some ingenious ways to increase the efficiency of the Rubisco reaction and minimise photorespiratory losses. The most elaborate of these is found in a group of plants known as C_4 plants, which include tropical grasses like sugarcane. C_4 plants minimise the risk of a stray oxygen molecule reacting with Rubisco by surrounding the enzyme with high concentrations of carbon dioxide. Rubisco is cordoned-off in specialised cells in the leaf called bundle sheath cells and the plant pumps carbon dioxide into these cells via a complicated series of chemical shunts. The result is a build-up of carbon

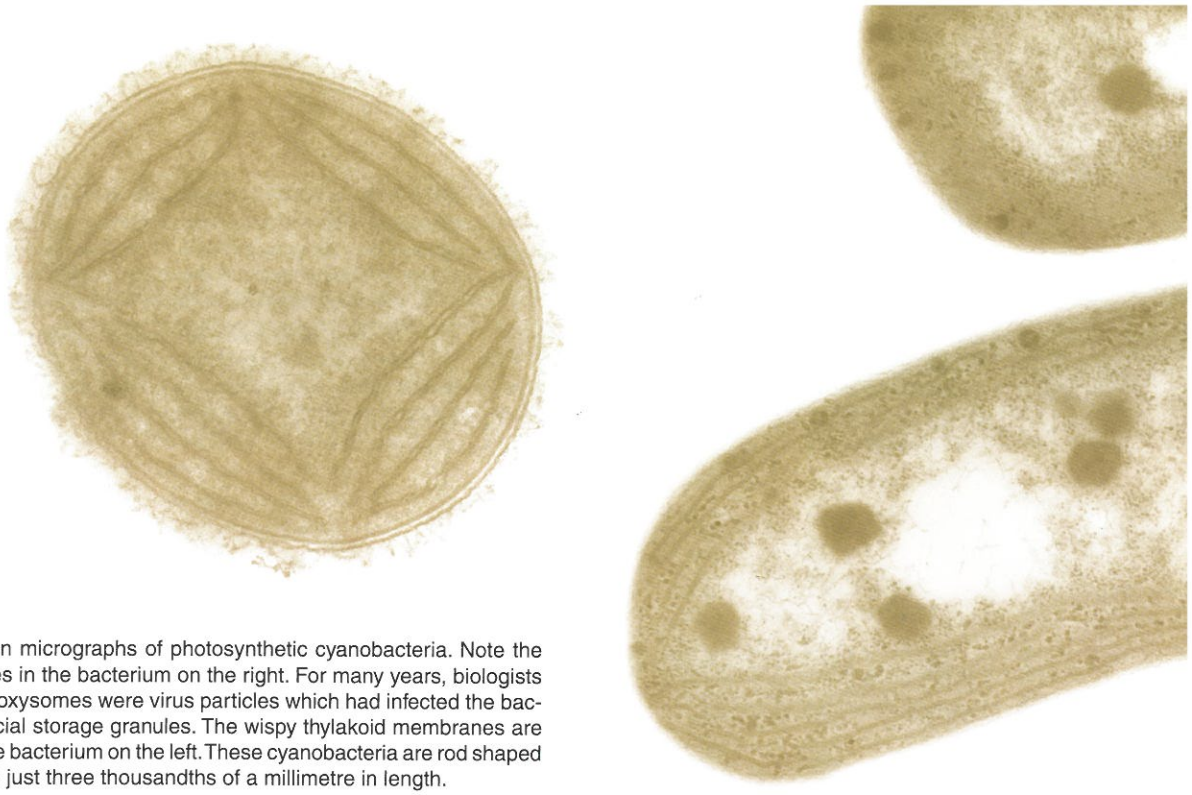


Figure 1. Electron micrographs of photosynthetic cyanobacteria. Note the dark carboxysomes in the bacterium on the right. For many years, biologists assumed the carboxysomes were virus particles which had infected the bacteria, or were special storage granules. The wispy thylakoid membranes are clearly visible in the bacterium on the left. These cyanobacteria are rod shaped cells that measure just three thousandths of a millimetre in length.

dioxide around Rubisco, which has the dual effect of increasing the rate of carbon fixed by Rubisco and reducing photorespiration (see: *And the winner is...* Biologic, Summer 1994).

C_4 plants have greatest advantage in hot, dry climates such as Australia's, since these are conditions under which Rubisco performs particularly badly. At high temperatures, Rubisco is even less able to distinguish between carbon dioxide and oxygen, increasing photorespiratory losses. C_4 plants also use water more efficiently. All plants lose water when they take in carbon dioxide through their leaves (see *Getting more wheat for your water*, page 10), and since C_4 plants utilise their carbon dioxide more efficiently, they can afford to open their stomata less often in dry conditions leading to reduced water consumption.

Lessons from cyanobacteria

Dr Murray Badger and Dr Dean Price, of the RSBS Department of Molecular Plant Physiology, lead a research team looking at a photosynthetic bacterium that has evolved a much more simple way to address the problem of Rubisco's limitations and photorespiration. Aquatic cyanobacteria are some of the most primitive photosynthetic organisms, conducting photosynthesis within the confines of a single cell. Although they are responsible for around a third of total global photosynthetic activity, relatively little is known about these organisms. Like C_4 plants, cyanobacteria minimise photorespiration by concentrating carbon dioxide around the Rubisco enzyme.

"Cyanobacteria possess a carbon dioxide concentrating mechanism (CCM) which gives them similar advantages to C_4 plants," Dr Price explains. "It obviously has a different mechanistic basis to C_4 photosynthesis because cyanobacteria can actually achieve this CCM within a single cell."

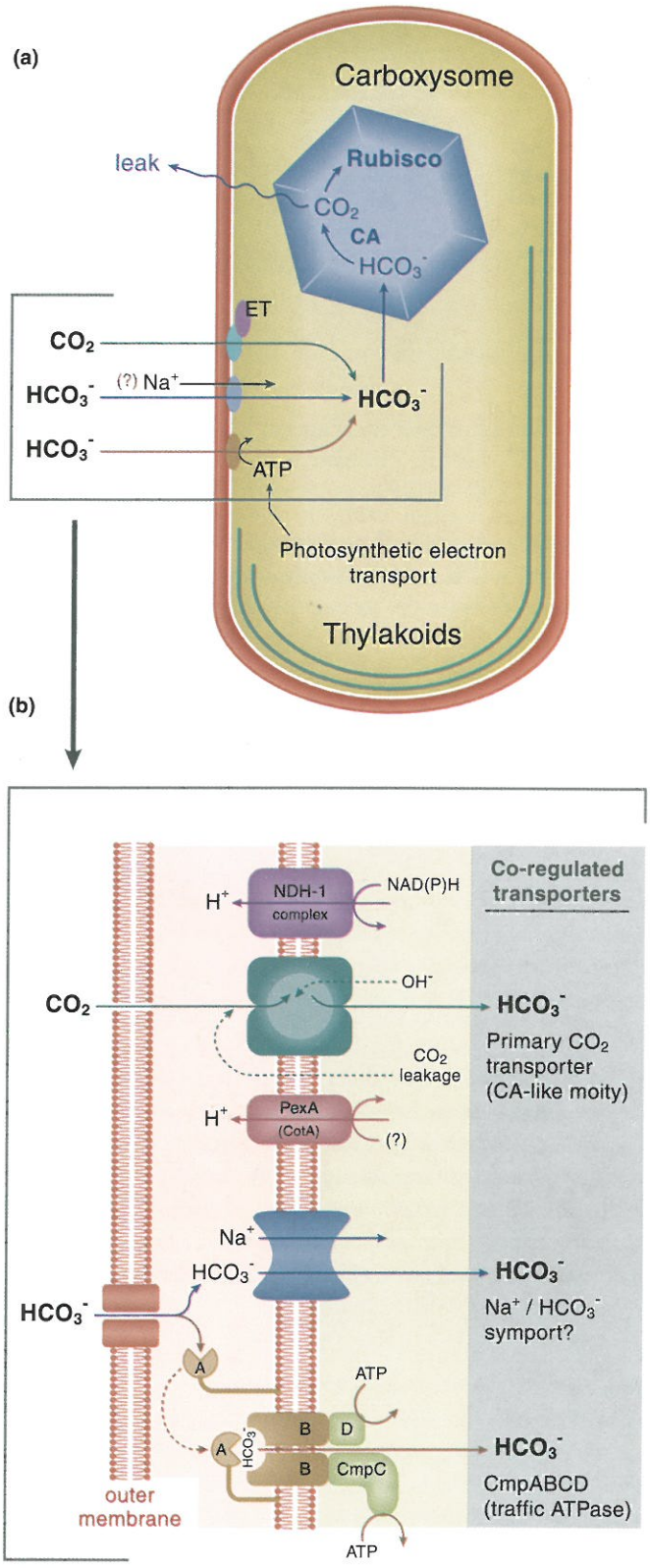
Understanding just how the cyanobacterial photosynthetic system works could have important agronomic applications. Because the mechanism appears to be relatively simple, Murray Badger and Dean Price hope components of the system might one day be incorporated into crop plants like wheat and rice to improve their photosynthetic efficiency. The benefits of such an improvement would be immense. Crops would use water and nitrogen more efficiently and be less dependant on other nutrients and fertilisers – benefits especially relevant to an arid country such as Australia whose soils are impoverished in valuable nutrients. Crops with improved photosynthetic efficiency are likely to grow faster and have higher yields, especially in marginal growth conditions.

The bounding plasma membrane of the cyanobacteria cell is studded with protein pumps that scavenge carbon dioxide from the water in which the bacteria live. These pumps work in a manner analogous to C_4 plants to elevate carbon dioxide inside the bacterium in the vicinity of the Rubisco enzyme. Perhaps this explains why the bluegreen algae can become such a problem in our waterways under some conditions.

Dr Badger explained that the cyanobacteria have at least two types of pumps: those that pump carbon dioxide gas (CO_2) and those that pump the ionic form of the gas (bicarbonate; HCO_3^-). Together, these pumps increase the concentration of bicarbonate within the cell before it is converted to CO_2 in specialised structures called carboxysomes that house the Rubisco enzyme.

Figure 2. Scavenging for inorganic carbon: (a) Carbon dioxide (CO_2) and bicarbonate (HCO_3^-) are pumped into the cyanobacterial cell across its dual bounding membranes, where it accumulates out of chemical equilibrium as HCO_3^-. This pool of internal HCO_3^- then diffuses into the carboxysome to be fixed by the photosynthetic enzyme, *Rubisco.* The carboxysome contains a small amount of the enzyme, carbonic anhydrase (CA) to speed the conversion of HCO_3^- to CO_2. The operation of the CCM depends on a feature of the carboxysome that effectively limits the leakage of CO_2 from the Rubisco-containing structure.

(b) Three types of inorganic carbon transporters have been detected at the physiological level in cyanobacteria. These are a primary CO_2 transporter and two distinct HCO_3^- transporters. Recently it has been established at the molecular and physiological level that one of the HCO_3^- transporters is a high affinity traffic ATPase.



The appeal of this mechanism to scientists like Badger and Price is its simplicity. If a single-celled bacterium has carbon dioxide pumps in its outer membrane, why couldn't the same pumps be used to concentrate carbon dioxide around Rubisco in plants such as wheat or rice? Dean Price argues that once the genes encoding these proteins have been identified, the transporters might be engineered into the the chloroplast of plants where the Rubisco enzyme is located.

"Ultimately," says Dr Price, "we would like to understand enough about the cyanobacterial CCM to ask the question 'could it be extended to a higher plant chloroplast?'. If the answer is 'yes' then we would definitely think of incorporating those genes into higher plants."

Of course, such a plan is still a way off. No one knows how many genes make up the functional cyanobacterial CCM. "We're at the stage at the moment where we know some of the genes," Dr Price says. Several laboratories around the world are working to identify its components. But part of the problem is that no one has ever identified a carbon-dioxide transporter before and there is no precedent for this type of protein. No one knows what it will look like or how it might work, making it difficult to know where to start looking.

"Most people in the field see discovering the carbon dioxide transporter as perhaps the most exciting challenge," says Dr Badger. "Mind you, we may all be disappointed when we find out what it is – it may not be as simplistic or novel as we thought. But then again, you never know."

DNA online

The search for the all-important carbon dioxide transporter was recently given a boost when a research team in Japan completely sequenced the DNA encoding the cyanobacterium *Synechocystis* (sp. strain PCC 6803). The 3.6 million bases, encoding all the genes found in the entire organism, were

posted on the internet last year, giving scientists around the world free access to the cyanobacteria's genetic information.

For Murray Badger and Dean Price, the availability of the new database (called Cyanobase) means many of the time consuming sequencing steps needed to identify the transporter gene can be bypassed. The use of the internet as a research tool is something Dr Badger believes will not only become more common, but will lead to more meaningful research in genetics and molecular biology. "Databases like Cyanobase take the focus away from just discovering genes and sequencing them," he says. "It means you have to get back to the primary, and perhaps hardest part of the system, which is discovering what those genes actually do."

An intriguing evolutionary twist

The idea of using cyanobacterial genes to improve plant photosynthesis has an ironic evolutionary twist: The chloroplast, which carries out photosynthesis in higher plants, is believed to have actually originated from a cyanobacterial ancestor in the first place. According to current evolutionary theory, the ability of plants to photosynthesise arose when the first plant cell engulfed a photosynthetic bacterium around 400 million years ago. Over time, the photosynthetic bacteria evolved into the modern-day chloroplast, but the chloroplast still retains many vestiges of its bacterial heritage.

What is not known is whether the original ancestor of the chloroplast had its own carbon-dioxide pump. "That is an unknown question," says Dr Price. "With regard to the higher plants, the intriguing question is why higher plants don't have a remnant of the CCM like the one in cyanobacteria."

According to Dr Badger, there are two potential possibilities: Either plants had the option to keep the cyanobacterial CCM, but discarded it during evolutionary selection. Or alternatively, the original ancestors of the chloroplast didn't have a CCM at all.

Improving on nature?

If Price and Badger's work succeeds, scientists in the future may be able to substantially increase the productivity of the world's most important plant crops by fixing a glitch of evolution. It all seems a bit too good to be true. Indeed, many scientists have argued that attempts to improve photosynthesis are destined to fail. If increasing plant productivity is so easy, they argue, why wouldn't nature have done it millions of years ago?

Nobody knows for sure, but perhaps the strongest argument suggesting photosynthetic improvments are possible comes from evidence of changes to the composition of the earth's atmosphere. The levels of carbon dioxide in the atmosphere have changed considerably over time. Atmospheric carbon dioxide levels have steadily declined since photosynthesis first evolved around 3.5 billion years ago, reaching an all time low about 45 to 40 million years ago. This was the time when C_4 plants are thought to have first evolved, and a period of history when there was maximum pressure on plants to develop some sort of CCM. "For the first few hundred million years of plant evolution, there probably wouldn't have been a need for a CCM," says Dr Price. "Carbon dioxide levels in the atmosphere were very much higher and oxygen levels low." Given how recent, in evolutionary terms at least, the need for a CCM in plants has been, perhaps there has simply not been enough time for one to have evolved, Dr Price argues.

Badger and Price depend on these important assumptions for their work to succeed. However the agronomic applications of their research are not their sole motivation. "It is the fundamental biology that most interests us," Dr Price confesses. "Simply understanding how this system works. And from what we understand now, improvements in the yields of crop plants seem possible to achieve."

If you want to know more ...

Dr Dean Price

- The CO_2 concentrating mechanism in cyanobacteria and microalgae (mini review).

  Badger MR and Price GD. Physiologia Plantarum, 84, 606-615 (1992)

- The functioning of the CO_2 cencentrating mechanism in several cyanobacterial strains: a review of general physiological characteristics, genes, proteins and recent advances.

  Price GD, Sültemeyer D, Klughammer B, Ludwig M and Badger MR. Canadian Journal of Botany (in press).

- You are welcome to contact Dr Price on (02) 6279 8423, or via email: dean.price@anu.edu.au

Corals yield clues to the

Figure 1. Colonies of *Acropora millepora*, the species under study (photo, Ken Anthony).

Figure 2. The beach at Nelly Bay, Magnetic Island, off Townsville, Queensland with the tanks and tubs into which the corals will be placed in the foreground (photo, Dr Julian Catmull).

It is late October, the first week after the full moon. An odd group of people is gathered on a beach on Magnetic Island, Queensland. Plastic baby pools and bins line the shore but there are no sounds of children at play. Scientists from all over the world have gathered and locals watch their activities with a mixture of curiosity and amusement. It is time for the Great Barrier Reef coral spawn.

In the late afternoon light, scientists lift coral heads into buckets and carry them by boat to the baby pools. The sounds of laughter and speculation carry through the air – will this be the night? The environmental cues that trigger the mass coral spawn remain mysterious, but if the scientists' guess is right, the majority of the Great Barrier Reef's corals will spawn simultaneously on this night, sometime between sunset and midnight. If they're wrong, the researchers will return the coral heads to the sea and collect them again the next day.

As night starts to obscure the scene on the beach, a carnival atmosphere and sense of expectancy develop. Suddenly, illuminated by the glow of the waning moon, the surfaces of the pools become covered with egg and sperm bundles. With whoops and cheers the entire assembly sets to work. In one night, many of these scientists collect enough material to study for an entire year. Some researchers fix material for electron microscopy. Some freeze embryos at different stages in liquid nitrogen for later preparation of RNA, DNA, and protein. Others perform experiments to see if cross-fertilisation between coral species is occuring.

Drs Eldon Ball and David Hayward of the Research School of Biological Sciences, Drs David Miller and Julian Catmull of James Cook University, and Dr Peter Harrison of Southern Cross University have united their efforts: Their aim is to follow the development of embryos from the coral *Acropora millepora*. As the corals spawn, the embryos are collected, placed in containers open to the sea via a fine mesh at both ends and left bobbing on buoys in the bay. Over the next five or six days the containers are collected at intervals and the microscopic coral embryos are chemically preserved or frozen for later study.

Coral Biology

The mass coral spawning phenomenom was first documented in 1982 when stumbled upon by researchers from James Cook University. Before then, corals were believed to spawn throughout the year,

Figure 3. *(left)* Waiting for corals to spawn, Magnetic Island (photo, Dr Bette Willis). *(right)* John Reece-Hoyes, a collaborative PhD scholar in the Miller and Ball labs, collects developing coral embryos from the surface of a bucket (photo, Dr Julian Catmull).

releasing only fully developed larvae. "As a biologist, I'm fascinated by this whole process," Ball explains. "It is exciting that something so fundamental has remained unknown for so long. When I was a student people never had seen corals spawning, so they assumed that it must be a rare event and that coral colonies must be very old. But how do coral embryos divide? How do they gastrulate? This is all basic information that we're gathering along the way."

As investigations of coral biology intensified, it became clear that the dominant reproductive pattern for corals worldwide is the release of eggs and sperm into the sea for external fertilisation and development, a process by which sperm and eggs from parents in different locations have a chance to find each other and settle, all within an environment of continuously moving water. It is still unclear why the strikingly beautiful phenomenon of synchronous interspecies coral spawning evolved.

Corals are members of the class Anthozoa, which also includes sea anemones. Anthozoa belongs to the larger phylum, Cnidaria (coelenterates), which contains hydroids and jellyfish. *Acropora* are stony corals; reef builders nourished both by symbiotic algae and the capture of planktonic prey. They reproduce asexually and sexually by the production of planula larvae from fertilized eggs. The mature larvae are about 1.5 millimeters long and each is capable of settling on the ocean floor, forming a polyp, and beginning a new colony.

Eldon Ball and his collaborators are interested in *Acropora* because cnidarians are the simplest animals with true tissues and a nervous system. Corals, like other cnidarians, have only two tissue types, ectoderm and endoderm (more complex animals have a third tissue layer, mesoderm). Historically, hydras have been the cnidarian model animal of choice but for Ball's purposes hydras produce too few embryos at unpredictable times. Corals release massive quantities of eggs and sperm in unison during the coral spawn, allowing large amounts of embryonic material to be collected which is all at the same stage of development. Mass spawns like the one at the Great Barrier Reef are rare and scientists in Europe or America have little access to such events.

Embryonic Development

After fertilisation, *Acropora* eggs begin to develop. First they divide but no growth occurs. Within hours each embryo forms a flattish structure that looks like a miniature prawn-chip. This then grows into a hollow ball of cells referred to as a blastula. Later, the embryo folds in on itself, forming an internal sac of endoderm with an external covering of ectoderm in a process referred to as gastrulation. In the mature coral, the endoderm will form the lining of the gut and the ectoderm will form the epidermis and nervous system. Cilia develop and the embryos float about as part of the plankton, gradually changing from spherical to spindle shaped before becoming planulae. By this stage each embryo has a nerve net and nematocysts (stinging cells).

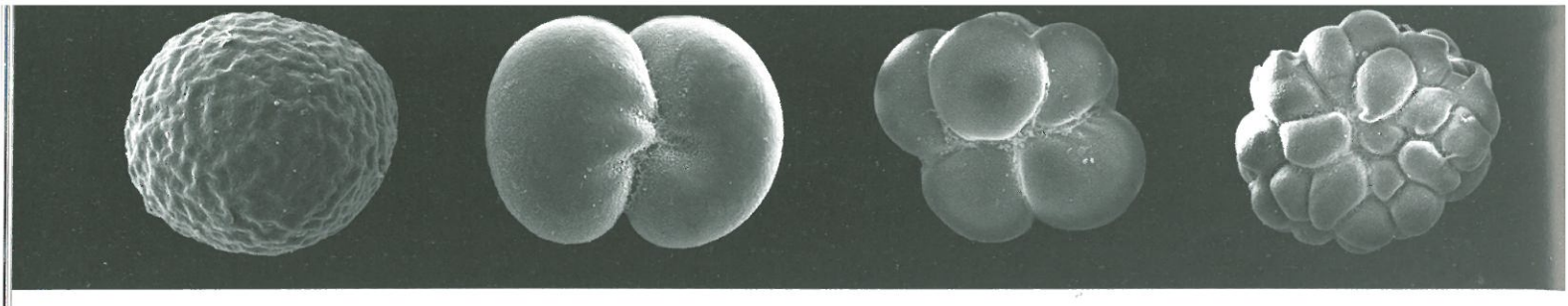


Figure 4. Scanning electron microscopy reveals major stages in the embyonic development of the coral, *Acropora millepora.* The fertilised egg (far left) begins to divide and initially forms a flattened sphere of cells. Cell division then continues, predominantly in a single plane, to form a stage informally known as a "prawn chip". The prawn chip then rounds up to form a sphere in a process known as gastrulation, thus

During development every embryonic cell becomes specalised for a distinct job. Multicellular animals are composed of cells with identical genetic information, but different cells express different genes. The genes expressed early in development control each cell's ultimate developmental fate. Eldon Ball and his collaborators are trying to figure out how genes control this highly regulated specialisation.

Developmental Control Genes

Throughout evolution, organisms have increased in complexity but have retained the genes that oversee the first, fundamental stages of development. These genes are called developmental control genes, and are often nearly identical in organisms as distantly related as humans and corals.

One important group of developmental control genes common to all animals is the Hox gene family. The apparent function of Hox genes is to assign each cell a position along the anterior-posterior axis of the embryo; for example, in insects the combination of Hox genes expressed in different cells determines where legs and wings develop. Hox genes code for DNA-binding proteins called transcription factors. By binding to DNA these proteins switch other genes on or off. In this way Hox proteins work like master switches, controlling the synthesis of proteins that help shape the function of each cell and its lineage. "These genes do multiple jobs," Eldon Ball explains. "Complex animals may have taken a gene that was formerly doing one job and adapted it to do another. That's the sort of evolutionary story that we hope will come out of this. Where do developmental control genes come from and how have their functions changed during evolution?"

Gene functions shared by simple and complex animals are likely to be primitive, while dissimilar functions are likely to have evolved later. By determining how *Acropora* Hox genes are arranged on chromosomes, Eldon Ball and his collaborators hope to gain an understanding of when the genes first appeared, when and how the original Hox genes gave rise to others, and when they became organised into clusters on the chromosomes. They are also studying developing embryos to see when Hox genes become active, how they are expressed, and how their expression relates to the changing shape of the embryo.

Pooling Coral Research

Ball, Miller, and Harrison often convene and share scholars amongst their labs. Miller's group cloned the first coral developmental control genes in 1993. To identify these first genes and the remaining complement of developmental control genes of *Acropora*, members of the Ball and Miller labs have used a polymerase chain reaction (PCR)-based approach (see: *PCR*. Biologic, February 1996)). They used published DNA sequences of Hox genes from animals such as mice and fruitflies to clone three

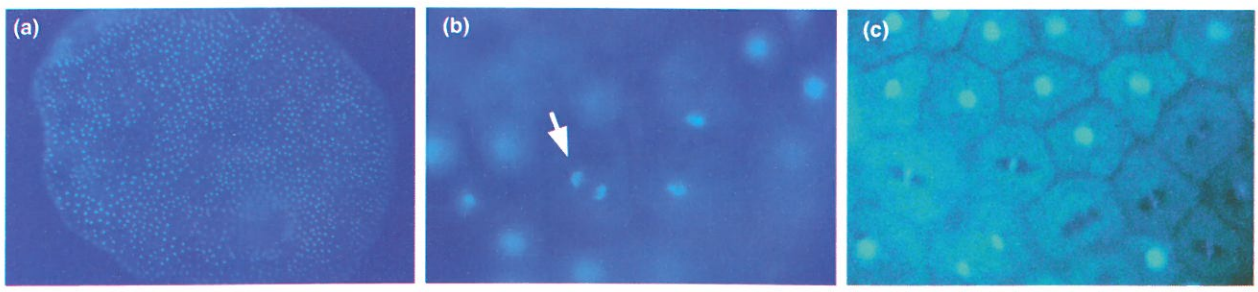
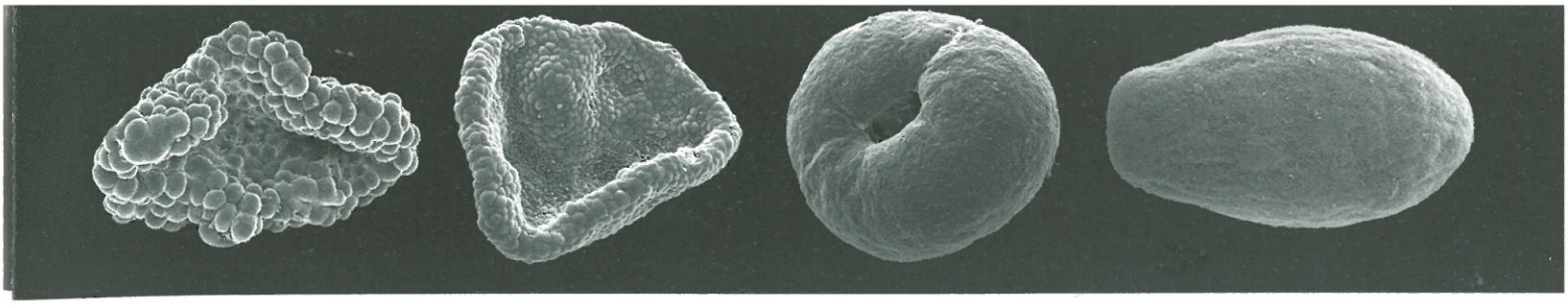


Figure 5. *Acropora* embryos stained to reveal the DNA in the cell nuclei. (a) A whole embryo at the "prawn chip" stage, the embryo consists of a sheet of cells resembling a prawn chip. (b) A closer view showing patterns of cell division. The nucleus of the arrowed cell has separated into two, while to its right are two cells further along in the process of cell division. (c) Here the mitotic spindles of dividing cells have been stained black with an antibody to the tubulin of which they are composed.



forming the two body layers: the outer ectoderm and the inner ectoderm. This stage can move, driven by cilia on the outer surface. Over the next few days additional cell types develop and the embryo elongates to form a planula larva (far right). The planula will settle to found a coral colony whenever it encounters favourable conditions, but if such conditions are not found it may go on swimming for weeks or months.

coral genes with similarity to Hox genes (antpC, cnox1, and cnox2) and the related genes eveC and ampaxB, the latter in collaboration with Walter Gehring of the University of Basle, Switzerland. Investigations into the role and organisation of these genes continue.

To determine when and where developmental control genes are turned on and how this affects the growing embryo, the researchers are looking at embryos at different stages of development. The collaborators are using a technique called northern analysis on RNA isolated from embryos collected at different stages (originally harvested from the containers bobbing on buoys at Magnetic Island) to determine when these genes are turned on in coral embryos. So far, these experiments have shown there are two distinct phases of cnox2 expression in embryogenesis. First, the gene is expressed at a low level during gastrulation. Its level of transcription then declines until about 48 hours into development, after which its expression picks up when the embryos become spindle shaped.

One of the clones, ampaxB, is especially interesting. In more complex organisms, homologues of this gene control aspects of eye formation. Corals have no known photoreceptors, so understanding the expression of this gene may help reveal the gene's primitive function. Another area of particular interest is how the regulatory regions of the gene have changed during evolution. This will be studied by comparing the nucleotide sequences of the genes' regulatory regions and perhaps by introducing the coral gene into a fruitfly to see whether it can produce an eye there. Although the animal kingdom contains a diversity of eye types, it is now clear they are all related in the sense that their formation is initiated by the same set of genes.

Eldon Ball's group is also working to establish where cnox2 and the other genes are being switched on in developing embryos. To create a frame of reference for these molecular studies, Ball and Harrison are investigating the cell biology of coral development using various types of microscopy, immunocytochemistry, and DNA staining.

Bringing the Mysteries of Development to Light

The early cells of an embryo are like the framework of a building; if not assembled correctly, the final structure will not be sound. But unlike a building, animal embryos build themselves, a creative miracle no matter how you look at it. Scientists like Ball and his collaborators face great challenges when attempting to unravel the complex process of embryonic development. Nevertheless, the discoveries of his group and others have illuminated the unity of life at a level previously unimagined. Since the same genes unite developmental pathways of all animals, early embryos from vastly different creatures (like humans and fish) often look similar. The more closely related animals are, the more developmental control genes they share and the longer they remain nearly indistinguishable during development. In this way, all living things share a genetic framework that has been built upon since the emergence of life on earth.

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Getting more wheat for your water

The earth's climate is changing and some of this may be caused by human activity. World-wide concerns regarding atmospheric change were discussed recently at the Kyoto Convention on Climate Change held in Japan, where Professor Farquhar from RSBS was a delegate and science adviser. He has concluded that the increase in atmospheric carbon dioxide concentration will have some benefits, as well as possible perils. One benefit includes the possible improvment of vegetation growth in dry areas. Nevertheless, occasional drought is still likely to remain a problem for Australian agriculture in the foreseeable future.

In harsh conditions, even small variations in crop yields can determine whether farmers survive or go to the wall. A group of Canberra scientists, including Professor Farquhar are now working on ways to soften the blow, by breeding plants that grow better in dry conditions. Using wheat, the researchers have been able to improve yields for grain grown in arid conditions by up to 20 per cent.

For a nation with an average annual wheat crop of around $40 billion, the find could not only protect farmers from the most severe ravages of drought, but earn hundreds of millions of dollars in export revenue.

The breeding project – run jointly by researchers in RSBS, the CSIRO Division of Plant Industry and the Cooperative Research Centre for Plant Science – aims to breed more water-efficient crops which are better suited to Australia. Unlike conventional breeding programs, however, which select new crop varieties more-or-less on a trial and error basis, the team has approached the problem starting with the basic physiology of how a wheat plant works.

"We've taken a very targeted approach, a very risky approach," explains Dr Richard Richards, of the CSIRO Division of Plant Industry. "Few people have ever taken gains in the physiology of a plant and applied them to a breeding program. In the past, breeding has always been somewhat haphazard. But

we're reaching a stage in crop production now where gains in yield are becoming very difficult to make unless we understand more about the underlying processes that govern how plants grow."

More wheat for your water

One of the main factors limiting yields of Australian crops is water, Dr Richards said. The researchers reasoned that by breeding plants that wasted less water, the plants would be more suited to Australia's notoriously harsh climate and would have improved yields.

Wheat varieties currently used by Australian farmers are not well adapted to the climate, Dr Richards said. Most stem from Mexican plant lines selected under high water and nutrient growth conditions – quite opposite to the conditions in most of Australia.

Yet the current wheat lines do have other excellent features such as good grain quality.

"We wanted to maintain the favourable characteristics of the traditional varieties but enhance them by adding improved water use efficiency – to adapt the varieties very precisely to our local conditions," Dr Richards said.

Heavy carbon and wasted water

An important clue suggesting how plants could be made to use water more carefully came from fundamental research carried out by Professor Graham Farquhar of RSBS back in the early 1980's. At the time, Professor Farquhar was examining the peculiar way that plants discriminate between isotopes of carbon (see: *Plants and CO_2 swap isotopic visiting cards*. Biologic, Summer 1994).

Most carbon in the atmosphere exists as the common form of carbon, carbon-12, but a small proportion of it (about one per cent) contains the slightly heavier isotope, carbon-13. During photosynthesis, the primary photosynthetic enzyme of the plant, Rubisco (see: *It's not easy being green*, page 6), combines preferentially with the lighter form of carbon dioxide. As a consequence, the plant tends to preferentially accumulate carbon-12.

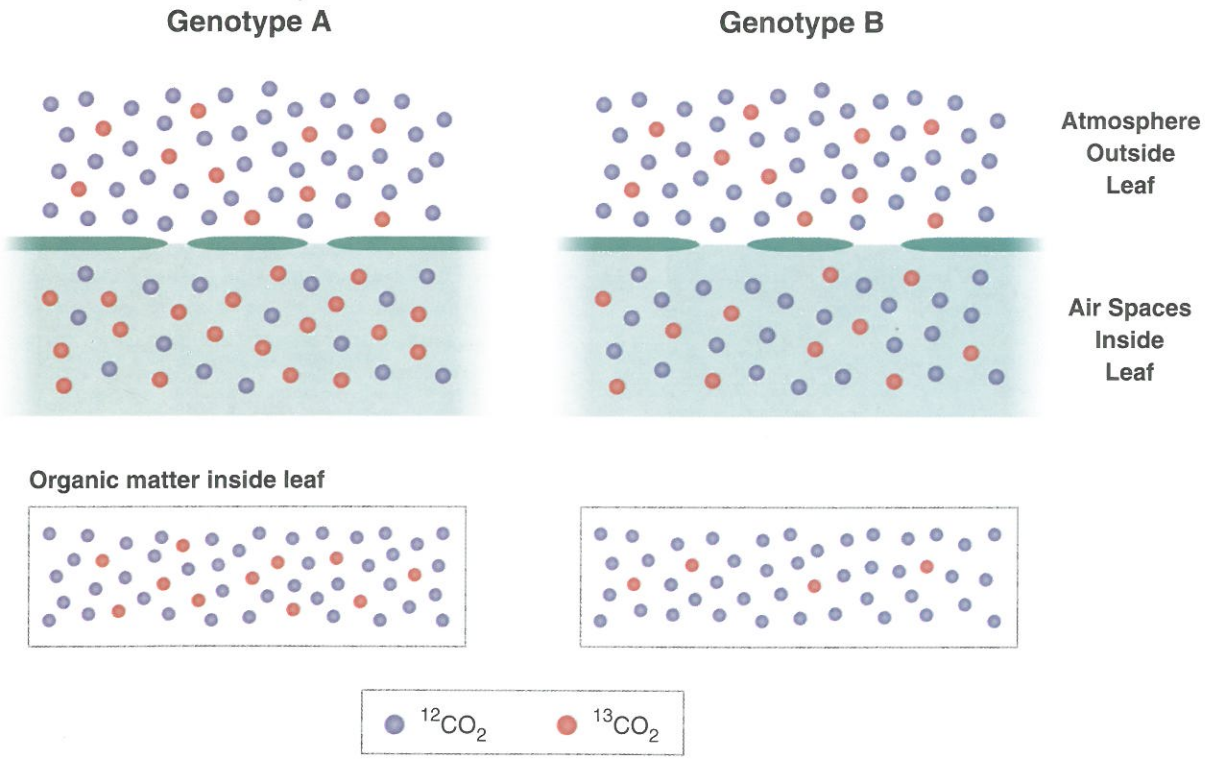

Figure 1. The *Rubisco* enzyme inside the plant leaf uses light $^{12}CO_2$ (blue) preferentially. Consequently, the heavier $^{13}CO_2$ (red) accumulate within the air spaces inside the leaf. Plants with better water-use efficiency (Genotype A) incorporate more $^{13}CO_2$ (red) into their leaf organic matter, because it cannot escape as easily through the smaller stomata. This allows Genotype A to be selected for better water-efficiency in plants.

Figure 2. *(left)* Mr Bernie Mickelson of CSIRO Division of Plant Industry, spraying one of the wheat lines.

(right) From left to right, Dr Richard Richards, Professor Graham Farquhar, and Dr Greg Rebetzke and Dr Tony Condon of CSIRO Division of Plant Industry, examining some of the selected wheat lines.

The extent of the discrimination depends on the plant and, fortuitously, also relates to the plant's water efficiency.

Plants absorb carbon dioxide through pores in their leaves called stomata. When demand increases, the stomata open wider letting carbon dioxide flood in but also letting water escape. When the stomata are shut, heavy carbon-13 builds up inside the leaves and is eventually incorporated by the plant. What results is a useful marker – plants that grow with somewhat closed stomata accumulate more carbon-13, while those that grow with open stomata contain less (Figure 1).

Figure 3. Ms Vikki Fischer of CSIRO Division of Plant Industry, measuring stomatal openings on wheat genotypes.

Cautious crops

What the research team wanted was the best of both worlds: to breed plants that used water more conservatively in dry conditions but did not have reduced yields when it was wetter.

First, they identified plants with low carbon discrimination (which they guessed corresponded to good water-use efficiency) by measuring the relative amounts of carbon-12 and carbon-13 in their leaves.

Obtaining accurate measurements proved difficult. Dr Tony Condon spent years refining the isotope sampling procedure in the glasshouse and in the field, first as a PhD student with Dr Richards and Professor Farquhar and then as a CSIRO scientist. "Sampling time is very important," Dr Richards explained, "and it has taken us years to know when and what plant parts to sample for the breeding work. The stomata respond to all sorts of things like clouds moving over, or humidity, and the isotope ratio of the plant changes hugely over its life."

Once low carbon-discriminating plants were identified, their improved water-use characteristics were bred into conventional wheat varieties, using carbon discrimination as a marker. After a series of crosses and back-crosses, several wheat lines were identified which retained approximately 90 per cent of the genes from the conventional wheat strain, but which also had low carbon discrimination.

Field trials of the water-efficient wheat lines in 1996 were spectacular, with yields up three to twenty per cent on conventional varieties. Best results were obtained at the most arid trial site near Condobolin, NSW, where overall grain yields were up 10 per cent

and individual lines grew as much as 20 per cent more grain than standard crops. Less arid sites in Wagga Wagga and Narrabri (NSW), and Merredin and Wongan Hills (WA) had yield increases of between three and ten per cent. More recently (1997 season), the water-use effecient lines topped trials held at six sites in Queensland.

Even if wheat production was improved by as little as one or two per cent, it would still pay the costs of the whole research program several times over in a single year, Professor Farquhar pointed out. A lucrative return, he said, for an investment in fundamental research.

The next step, he said, is to use the procedure to make other crops more drought resistant. In theory, carbon discrimination testing could improve yields of a range of crops including cotton, sunflowers, peanuts and many pasture species, all of which are water-intensive to grow. "Globally, this technique is very important," Professor Farquhar said. "We just happen to be more advanced with the wheat work, but it has huge potential worldwide."

Harvesting the fruits of research

Despite the undoubted commercial value of the new wheat variety, breeders have been slow to embrace the work. For Professor Farquhar, the experience has opened his eyes to the difficulties of merging scientific discoveries with commerce and industry.

"All of us working as researchers have this view that new discoveries are accepted with open arms by breeders, but that is not so," he said. "If scientific advances in research are to be applied usefully in the field, researchers must be prepared to sell their ideas."

Yet fundamental science will play an increasingly important role in breeding programs as it becomes more and more difficult to improve crop varieties, he predicted.

"We have got to start thinking about the underlying physiology that will improve yield potential," Dr Richards said. "The breeders do not know if they can get another 10 per cent yield out of their plants. They hope they can, but we are starting to reach the limits of conventional breeding techniques."

Research in plant physiology is the best way to reverse Australia's lagging crop productivity, Professor Farquhar said, and he sees the new wheat lines as an example of the direction future research should go.

"These are probably the first wheat lines whose breeding has been driven by physiology," he said. "It may be that conventional breeding methods are starting to hit a ceiling and a more directed approach is needed to increase crop yields. For me, it is an act of faith that in the long run we will eventually have to go that way."

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Prof Graham Farquhar

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Alternative oxidase

evolutionary mistake or essential safety valve?

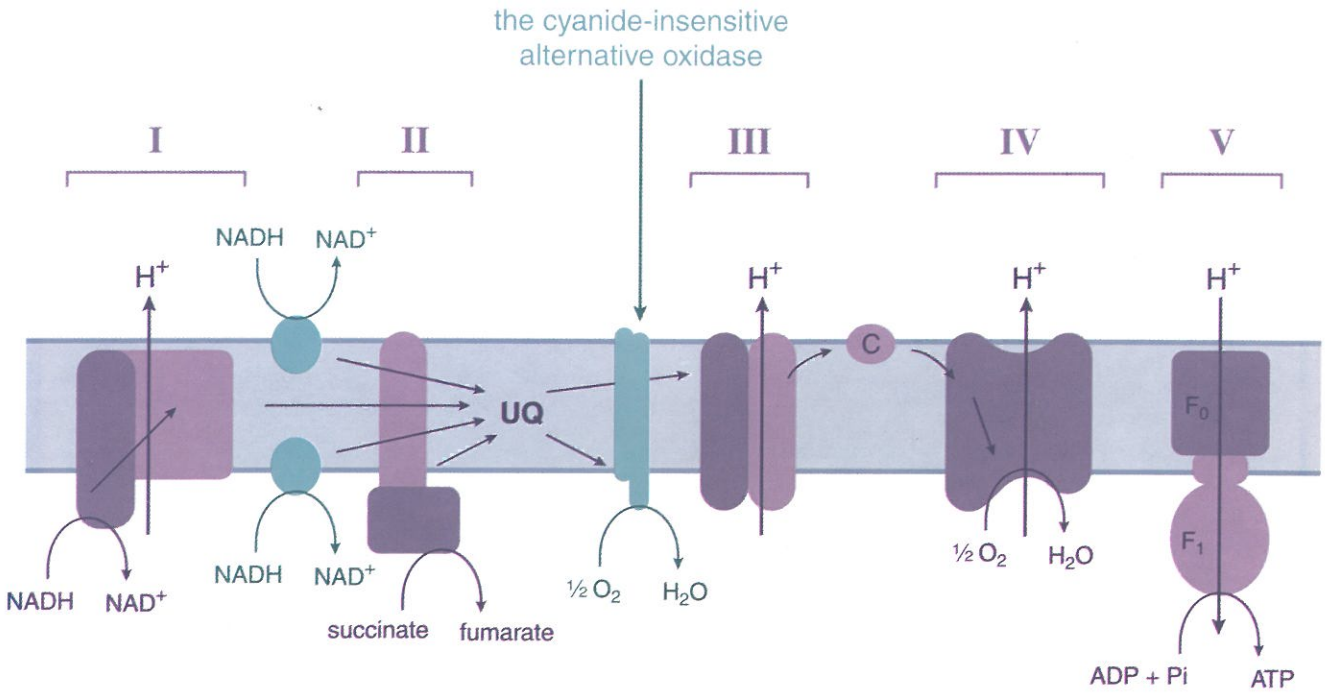

Figure 1. An illustration depicting the electron transport chain of plant mitochondria. Electrons from NADH and succinate, some of the end-products of sugar breakdown in the cell, are passed from protein to protein imbedded in the inner mitochondrial membrane, ultimately reducing oxygen to water. The roman numerals identify the IV major enzyme complexes of the ubiquitous respiratory chain found in all mitochondria. As electrons move through these complexes, protons (H^+) are translocated across the inner membrane to form a protonmotive force; movement of these protons back through the complex V, the ATP synthase complex, drives the synthesis of the high energy compound, ATP, which is used to do work elsewhere in the cell. In this way, the energy stored in NADH and succinate is conserved and exported to the rest of the cell. In addition to complex IV, the terminal oxidase (called cytochrome oxidase) of the electron transport chain, plant mitochondria possess an alternative oxidase which still reduces oxygen to water but does not pump protons. Passage of electrons through the alternative oxidase bypasses complexes III and IV, and the energy is not conserved but rather lost as heat. The alternative oxidase, in particular, has been the subject of intense study recently and is thought to play a role in coping with environmental stress that plants encounter.

Figure 2. A thermogenic lily, a smaller cousin of the huge Indonesian *Arum titanum*.

One of the world's more exotic plants is the giant aroid, *Arum titanum*, a native to the tropical rainforests of Sumatra. Every seven years, for a few fleeting weeks, the plant grows a single monstrous flower towering three metres above the surrounding forest floor. Over several days, the bloom warms itself up several degrees above the cool forest temperature and floods the heavy air with the stench of rotting meat. An army of insects, drawn by the olfactory promise of decomposing flesh, flock to the plant, carting away pollen on their spindly legs and bodies as they leave.

The effort of such an extravagent pollination technique extracts a heavy toll from the plant. Within a week, the orchid exhausts its energy reserves, carefully stockpiled over many months, and the massive flower wilts and dies leaving behind the seeds of the next generation.

The thermogenic abilities of plants like *Arum titanum* have baffled scientists for years. However the mystery of heat production in plants is now beginning to unfold. Central to the process is an intriguing protein called alternative oxidase which operates somewhat like a biochemical trip-switch to direct energy away from normal plant activity and into heat production.

But the alternative oxidase story is complex. Recent research suggests the protein has other roles in more regular plants and, if one group of ANU-based scientists are correct, it may play a crucial role in helping plants deal with stress.

The Breath of Life

The biological power stations that generate energy in plant and animal cells are mitochondria – microscopic kidneybean-shaped structures huddled inside each cell can be described crudely as working like hydroelectric plants. Fuel stored in the cell, such as sugars or reserves of carbohydrate or fat, are broken-down within mitochondria in a series of chemical reactions, releasing energy in the process. This energy in turn drives molecular pumps lodged in the mitochondrial membrane that move protons from inside the mitochondria out into the rest of the cell. The effect is to build up a gradient across the inner membrane of the mitochondria, similar to water pressure in a dam. In the same way that water pressure is used to generate electricity, the proton gradient across the mitochondrial membrane drives the production of a fuel molecule called ATP.The energy derived from letting protons back into the mitochondria is used to make ATP. The Nobel prize for chemistry in 1997 was awarded for discoveries about the mechanism of ATPase enzymes.

This ancient process, known broadly as respiration, has gone on essentially unchanged for billions of years, converting energy from photosynthesis or digested food into ATP. Respiration does the same job for the large and minuscule alike – driving the metabolism of a speck of mould growing on a discarded piece of cheese, maintaining growth in a geranium leaf basking in sunshine on a mantelpiece, or generating energy to power the movements and thoughts of the world's most influential decision-makers.

So when the mitochondrial power-houses malfunction, the results are invariably debilitating – Parkinson's Disease and Alzheimer's are just two examples of diseases associated with mitochondrial malfunction, and declining mitochondrial efficiency has been implicated as the most significant cause of aging.

In order for an animal or a plant to produce heat, this elegant respiratory process must be short-circuited. Fuel must be burnt without generating ATP – somewhat like revving the metabolic engine while it's not in gear. In animals, this is done in the brown adipose tissues by collapsing the proton gradient across the mitochondrial membrane. Channels in the membrane are opened up in response to hormones, allowing protons to flow freely across the mitochondrial membrane. As a consequence, fuel is burnt-off faster, creating the heat needed to maintain body temperature.

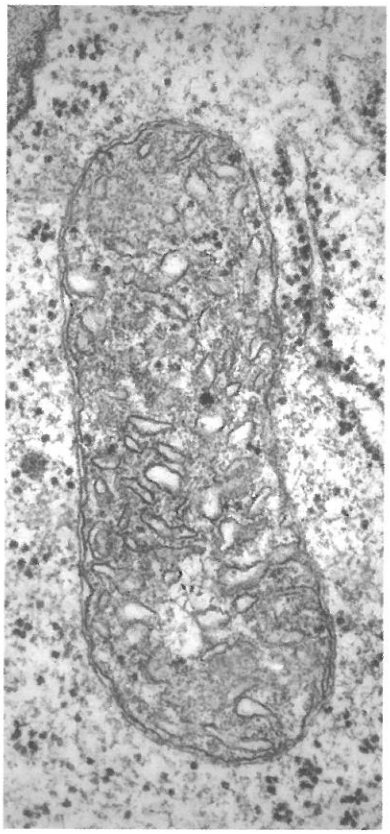

Figure 3. A kidney-shaped mitochondrion – one of the cell's power stations – as seen in an electron microscope. Alternative oxidase proteins sitting in the inner membrane of the mitochondria may protect the cell from harmful free-radicals.

Thermogenic plants like the *Arum titanum*, on the other hand, generate heat in an entirely different way. Instead of diffusing the mitochondrial proton gradient, they rely on alternative oxidase to bypass the proton pumps altogether (see Figure 1). Instead of the energy going into normal respiration and proton pumping, the alternative oxidase simply dissipates it as heat. For the *Arum titanum*, the enzyme allows it to rapidly burn-off of its carefully accrued reserves of oil and starch, elevating its temperature and creating an almighty stench in the Indonesian jungle.

Frosty leaves

However, this is not where the alternative oxidase story ends. To the surprise of almost everyone involved, when scientists started looking at alternative oxidase in more detail, they found it not just in exotic tropical flowers, but in all plants. Clearly it was not just used to generate heat. So what is alternative oxidase doing in ordinary plants? The question intrigues Professor David Day from the ANU Division of Biochemistry and Molecular Biology, who heads a research team trying to unravel the mystery of alternative oxidase.

"Thermogenic tissues are very cute," he says, "they provide wonderful slides at the beginning of talks and some of them are very exotic. But what most people working on respiration in plants want to know is, what is the alternative oxidase doing in a regular plant?"

An important clue is revealed by looking at plants under stress. Tobacco leaves, for example, contain hardly any alternative oxidase. But if the leaves are chilled to six degrees for a few days, alternative oxidase production skyrockets. The same thing happens in tomatoes. And interestingly, once alternative oxidase activity picks up, the plant copes better with cold temperatures – a process referred to as cold hardening. Although the connection has yet to be proven, it looks like alternative oxidase somehow protects the plant from frost damage.

Alternative oxidase is not the only protein whose activity jumps with cold hardening. There are at least two others, SOD (superoxide dismutase) and CAT (mitochondrial specific catalase) and together these proteins form part of a large family of stress proteins produced by plants in extreme conditions. But how these proteins work, and what they are protecting the plant from, are still matters of intense speculation.

Nature's Safety Valve

The most likely explanation of alternative oxidase's role, David Day says, is that it works something like a safety valve on a pressure cooker. He theorises that under times of stress such as at low temperatures, the plant's respiratory system becomes sluggish and can not keep up with the energy input. When this happens, fuel arriving at the mitochondria from photosynthesis can overload the respiratory machinery to a point where the whole system collapses.

If the mitochondria are overloaded this way, the cell can sustain serious damage. The greatest danger is that electrons, which are shunted along the mitochondrial membrane as part of the proton pumping process, jump off the membrane and combine with oxygen in the cell to form highly reactive "free-radicals". These wreak havoc inside the cell, destroying anything they collide with – damaging mitochondria, punching holes in membranes or mutating the cell's DNA. The partner enzymes of alternative oxidase, CAT and SOD, both work to neutralise oxygen free-radicals, minimising the impact these dangerous by-products have within the cell.

"The way we look at it now, although hard evidence is still to come, is that anything which damages or overloads the respiratory chain forms reactive oxygen species," says Professor Day. "The problem with a plant is that it can not escape its environment. If a plant goes from shade to bright light, for example, it is going to have dramatic changes in the flow of carbon into respiration. But the plant needs to avoid electrons bleeding-off to oxygen radicals, so it diverts them through the alternative oxidase."

The alternative oxidase sits in the mitochondrial membrane and provides an alternate route for electrons being shuffled down the membrane for proton pumping, siphoning-off electrons and directing them away from the normal respiratory system. So if electrons start to bank-up along the mitochondrial membrane, Professor Day reasons, alternative oxidase steps in and dissipates the problem before free-radicals have a chance to form.

This role of the alternative oxidase is supported by the recent work of Dr Harvey Millar, a former PhD student in Day's group. Millar has shown that the oxidase is insensitive to a signal molecule nitric oxide (NO), which inhibits normal respiration in animals and plants. In animal cells, NO normally acts as a neurological and immunological signal. But under stress conditions, it is over-produced and shuts down the respiratory pathway causing oxygen radicals to form. NO itself then combines with these oxygen radicals to form even more destructive chemicals. In plants, the alternative oxidase is not bothered by NO and can continue to act as a sink for excess electrons, preventing this sort of oxidative damage from occurring.

"One of the early aims of getting hold of the alternative oxidase gene was to eliminate it from plants to prevent so-called wasteful respiration and to improve plant growth," Professor Day says. "But I don't think plants do wasteful things without some good reason. In most plants we think the enzyme is regulated in such a way to prevent energy wastage. Thermogenesis could be looked at as a wasteful process, but in reality it is a deliberate strategy of the plant to have profligate sex!"

Professor Day and his collaborators are now trying to understand how the alternative oxidase is regulated – how it manages to juggle the competing needs of protecting the plant but, at the same time, not funnelling off too much of the plants precious energy to be burnt-off as heat. From what they have learnt, the activity of the protein is tightly governed by several different checks and stops, making the alternative oxidase an extremely sensitive energy overflow system. "Most people tend to think of plants as being pretty bland and dull," Professor Day says. "But this is an example of plants sensing the environment and responding to it very quickly. So on this cellular level, plants are actually very animate."

If you want to know more ...

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DIRECT FROM THE DIRECTOR

Taking Life for Granted...

Some will have noticed that this year, the Nobel Prize in Chemistry was awarded for research in bioenergetics, for molecular mechanical understanding of the ATPsynthase system. Walker (UK), Boyer (USA) and Skou (Denmark) have been leaders in a broad field of biological research that explains how the chemical energy needed to sustain life is made by rotational catalysis, driven by proton pumping, in the ATP synthase complex of chloroplasts, mitochondria and cell membranes.

That the Nobel Prize was finally awarded in this field is no surprise. It has been evident for some time that the molecular-rotary engine of life would one day attract the attention of the Nobel Committee. A brief paper in *Nature* from Japanese researchers a little over a year ago may have been the proof the Committee was waiting for. These scientists tagged the rotating head of the molecular machine with long, fluorescently labelled tubulin molecules and actually filmed the spinning propeller or windmill (depending on whether the tubulin was linked at the end or the centre) in action when substrates were added.

This is but one, perhaps one of the more important, breakthroughs in understanding biomolecular structure and function that are of weekly, almost daily, occurrence. Rapid progress, facilitated by techniques of molecular biology, information technology and especially by the networking of creative individuals, is now possible only because of past decades of often less spectacular research.

A large part of the molecular and mutational analysis upon which the model is based was done in the ANU some 15 years ago by Professors Graeme Cox and Frank Gibson in the John Curtin School of Medical Research. In *Biologic* (two issues back), Professor Des Clark-Walker from RSBS described his research in yeast that has to do with critical amino acids making up the bearings in the sleeve of the complex molecular machine.

What is the scale of the chemical industry that these molecular machines routinely undertake in the course of our daily lives? In a word, massive. Adult humans, in the course of a day's hard physical work, may process a tonne of ATP in their mitochondria. The solar-driven, chloroplast ATPase, of a spruce forest may process ten tonnes, and a maize field a hundred tonnes per hectare, per day, all to gain a few hundred kilos of dry matter growth. If we scale up the electrical potentials sustained by proton pumping in bioenergetic membranes to the size of a car battery, it would deliver not twelve, but about a million volts. To top this, plants then find ways to short circuit it all when the need arises, as described by Professor David Day in the last article in this issue. All of this is taken for granted, possibly because we rarely express life in these terms.

So much for the gee whizz of progress in biological research. I have often argued that it is more important to understand the process of research than its progress. Proper attention to the process will ensure progress. The process begins with nurturing the innate curiosity of childhood and learning to accept the uncertainty that accompanies the excitement of inquiry into the unknown. These natural enthusiasms need to be focussed and directed in our schools if we are to build a more scientifically literate community. Then we need to sustain the highest quality of tertiary education and the infrastructure for research.

The West Report into tertiary education seems to presume that information technology will replace face to face contact at all levels. Teachers and parents already know that this is not likely to be so. The report treats university teachers as workers in the education industry, and undervalues research in sustaining the most effective teaching at the tertiary level. The West Report's failure to recognise that research is one of the things Australians do best, a major contribution to human knowledge, welfare and the quality of life, is a great disappointment.

Our research infrastructure and international connections are not being sustained at anything like the level required to keep up with our competitors. Having provided many insights, and much evidence that has led to breakthroughs today, Australian researchers are now in real danger of being bypassed, e-mail and the Internet not withstanding.

Wouldn't it be wonderful if a significant proportion of the Federation Fund were to be invested in science? We should plan now to celebrate the achievements of 20th century Australian science in the anniversary year, 2001. Endowment of Federation Fellowships for our very best younger researchers could provide them with overseas experience, and then secure their career paths in Australia in the national interest. The Federation Fund could address the big problems of the next century, of which global change is one of the most pressing, with a commitment to the infrastructure needed to sustain research in Australia's interest.

This is my last diatribe for the back page of *Biologic* as Director of RSBS. My successor from June 1998, Professor John Hearn, is likewise anxious to strengthen our links with science teachers, to discover and nurture the creative intellects of the future. Thank you for your past support of *Biologic*, which, incidentally will not become Web only, just yet, and all good wishes for future endeavours.