FEATURE ESSAY 2.2?Thermogenesis

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Figure 1 Joe Wiskich (Botany Dept, University of Adelaide) examines spadices of *Arum italicum* (left) and *Dracunculus vulgar* (right) collected from the Adelaide Botanic Garden. Both species heat their spadices via 'futile cycles' of respiratory metabolism to volatilise aromatic compounds and attract pollinating insects.

Respiration is a combustive process and about 75% of its free energy is used to phosphorylate ADP to ATP. Cells tend to maintain very high ATP/ADP ratios and this places a limitation on the rate of respiration via allosteric inhibition of glycolysis and via respiratory control applied to the mitochondrial
electron transfer chain.

Heat generation is a useful phenomenon in plants, and is used to melt snow or volatilise aromatic compounds to attract insects. To generate heat it is necessary to have inefficient ATP-utilising processes and a rapid rate of respiration. This can be achieved by ‘futile cycles’ whereby ATP and a kinase phosphorylate a metabolite and a phosphatase regenerates it. The net result of this process is the hydrolysis of ATP to ADP and P$_i$, producing both heat and ADP which allows for rapid respiration. Bumblebees use such a cycle in cold weather to warm up their muscles before ‘take off’. If we are cold, we tend to shiver which produces the same effect.

Another way of achieving heat production is not to make ATP in the first place. Hibernating animals have brown adipose tissue whose mitochondria contain ‘thermogenin’ (a proton-translocating protein) in their inner membranes. When this protein is functional it prevents the establishment of a protonmotive force across the inner mitochondrial membrane and the free energy of respiration is liberated as heat. We are born with some of this brown adipose tissue but lose it early in life.

Plants also produce heat, as was observed by Lamarck in 1788. The family Araceae has more than 100 genera and about 3000 species. In many aroids the fertile flowers are clustered around a central spadix which is full of respiratory fuel. This can be mainly carbohydrate (as in *Arum*) or a mixture of carbohydrate and fat (as in *Philodendron*). In these plants the spadix can increase its respiratory rate 100-fold and raise its temperature above 40°C. The actual increase can be 20–30°C depending on the ambient temperature. For example, the inflorescence of the skunk cabbage (*Symplocarpus foetidus*) melts the snow as it grows up through it. Generally, the aroids generate heat to volatilise aromatic compounds which attract insects to facilitate cross-pollination. The process plants use for this purpose differs from the ‘thermogenin’ protein of brown adipose tissue but achieves the same result. The mitochondria of thermogenic plants contain an ‘alternative oxidase’ which branches from the mitochondrial electron transfer chain at ubiquinone. This is the alternative path and differs from the cytochrome path in that it does not conserve energy (to produce ATP) and is insensitive to cyanide and other inhibitors of cytochrome oxidase.

The aroid spadices with their heat production, rapid respiration rates and complete insensitivity to cyanide were considered to be the ‘type-specimens’ for cyanide-insensitive respiration. However, other plants showed only partial sensitivity to cyanide and these were regarded as ‘cyanide resistant’. Even those tissues completely sensitive to cyanide could be ‘induced’ to become cyanide resistant, for example fresh potato tuber slices are sensitive but become resistant on ageing. Thus, the alternative path is a feature potentially available to all higher plant mitochondria and at some stage during the development of any tissue is likely to become important. Inducing the alternative path really means inducing or activating the terminal ‘alternative oxidase’.

In 1987 Pierre Rustin questioned the nature of the alternative oxidase; was it a ubiquinol oxidase? Were we measuring quinol auto-oxidation? Could it be a lipoxygenase or was it a ‘free radical’ mechanism? Some of the problems we had at that stage were: ?rst, the reactivity of the alternative oxidase to some substrates was different in mitochondria from thermogenic compared to non-thermogenic tissues; second, the activity could only be solubilised from thermogenic mitochondria; third, attempts to purify it were only partially successful; and, ?nally, some results were being interpreted in terms of different ubiquinone pools existing within the membranes so that the NADH from some matrix enzymes (NAD-malic enzyme) had better access to the alternative oxidase than others (NAD-malic dehydrogenase). Since then a number of signi?cant events have occurred.

My laboratory was investigating photorespiratory metabolism in pea leaf mitochondria and we had dif?culty interpreting some of our results. We could explain our data if we assumed either a mixed population of mitochondria or a differential access of NADH generated by the oxidation of glycine to
the electron transport chain compared to the NADH generated by malate oxidation. We eliminated the first possibility with some immunogold-labelling studies, and it was to test the second that Tony Moore (University of Sussex, UK) came to my laboratory with a so-called Q electrode. This measured the redox state of the inner-membrane pool of ubiquinone-10, using a more water soluble quinone analogue as a mediator.

David Day (Australian National University) also visited at this time and brought with him some soybean mitochondria; these have a reasonable degree of alternative path activity. So for the first time we were able to measure simultaneously the rate of alternative oxidase activity and the redox state of its substrate. The initial results were quite clear — the alternative oxidase showed little activity until the ubiquinone pool was about 50% reduced and increased quite markedly above that. The cytochrome path became active as soon as some ubiquinone was reduced and reached apparent saturation at 20–30% reduction. These results appeared to validate, in general — but not precise — terms, the Bahr and Bonner hypothesis that the cytochrome path had to be saturated before any flux through the alternative path could be observed.

However, we still had problems. In soybean mitochondria succinate and NADH reduced the ubiquinone pool to the same extent, yet the alternative path oxidised succinate much more rapidly. Reduced quinone analogues were also poor substrates. It was Harvey Millar in David Day’s laboratory who noticed that malate oxidation via the alternative oxidase was much faster if pyruvate, rather than glutamate, was used to remove the oxaloacetate. It was soon established that pyruvate and other 2 oxo-acids such as glyoxylate, oxaloacetate and 2-oxoglutarate activated the alternative oxidase. In the presence of pyruvate, soybean mitochondria oxidised NADH and quinol analogues via the alternative oxidase. So the real problem was the availability of pyruvate — substrates which could produce pyruvate and activate the alternative oxidase were more readily oxidised than those that didn’t. This solution to one problem highlighted another. When we repeated our analyses on the relationship between the redox state of the ubiquinone pool and rates of O₂ uptake it became obvious that the alternative oxidase was very active at a relatively low level of ubiquinone reduction. This meant that the alternative path was now competing with the cytochrome path and electron flow could ‘switch’ from one pathway to the other. A wealth of data on estimating the contribution of the alternative oxidase to tissue respiration (so-called ‘engagement’ or ‘rho’ determinations) now had to be treated with great caution. This is because the technique used inhibitors and assumed electron flow could not switch from the alternative to the cytochrome path. Further, any inhibition of the electron transfer chain in tissues could lead to an increase in the concentration of pyruvate.

We believe that pyruvate activation eliminates the problems associated with preferential oxidation of substrates via the alternative oxidase. There are still differences in kinetics of the alternative oxidase with respect to the redox state of ubiquinone among mitochondria from different tissues. However, I feel that these should be considered in terms of the balance between input and output from the ubiquinone pool. When Peter Rich (Glynn Research Institute, Bodmin, UK) visited my laboratory he brought some very potent alternative oxidase inhibitors with him. Using these we estimated that Arum and soybean mitochondria contain 720 and 58 pmol alternative oxidase mg⁻¹ mitochondrial protein. Clearly a mitochondrion with a high amount of the enzyme will maintain faster rates of O₂ uptake at lower levels of reduced ubiquinone.

Meanwhile, advances were being made in North America. Tom Elthon, in Lee McIntosh’s laboratory (Michigan State University,) had produced an antibody to alternative oxidase which cross-reacted with proteins from a wide range of plants. The alternative oxidase, when reduced, was detectable on electrophoresis gels in the range of 32 to 37 kDa. There appears to be three separate protein forms which exist in varying combinations in different tissues. Subsequently, David Rhoads and Lee McIntosh isolated the genes from both thermogenic and non-thermogenic tissues. Thus, the alternative oxidase finally reached the status of being a real protein, a nuclear-encoded enzyme. From Jim Siedow’s
laboratory (Duke University, North Carolina), Ann Umbach reported that the enzyme could exist as an inactive oxidised dimer bringing attention to regulation by its redox state, involving sulphydryl–disulphide interactions. This has physiological implications. David Day and I received an Australian government DITAC Collaborative Research Award to visit Lee McIntosh, who had produced transgenic tobacco plants over- and under-expressed in alternative oxidase protein. Although mitochondria isolated from the leaves of over expressed plants contained more alternative oxidase protein than did wild type, their activity was the same. However, full activity was elicited by reducing the enzyme, which could be achieved by adding citrate. We suggest that NADP-isocitrate dehydrogenase produces NADPH which reduces the enzyme, most probably via a thioredoxin-type process.

We now have a feed-forward system to activate the alternative oxidase. It depends on an increase in the supply of mitochondrial substrate which can both reduce the alternative oxidase and activate it. In normal metabolism pyruvate appears to be important and this is usually considered to arise from pyruvate kinase but it must be remembered that plant mitochondria contain NAD-malic enzyme and can produce their own pyruvate from any Kreb’s cycle intermediate. During photorespiration in C₃ leaves the supply of glycine to mitochondria is very rapid. Assuming the photorespiratory rate to be about 25% of net CO₂ fixation the generation of reducing power within the mitochondria would be two to three times that of dark respiration. The fate of this reducing power is problematical — some finding its way to the peroxisomes for hydroxypyruvate reduction and some being oxidised by the electron transport chain. If there are problems in eliminating the reducing power, glyoxylate concentrations would rise and activate the alternative oxidase.

The role of the alternative path in thermogenic aroids has been mentioned. In other plants, its gene expression is induced by cold, so it may have a general role in warming plant tissues. However, it is present in all plants and can be induced by heat, drought, nutrient deficiency, insect and fungal attack, treatment with poisons — in fact any stressful situation — so it must have a more general role as well.

I recall some scientists considering the alternative path to be ‘wasteful’ respiration and who grew plants in the presence of alternative oxidase inhibitors. Presumably, they expected to get bigger and better plants; another great idea ruined by an ugly fact — the plants died. It seems to me that the alternative oxidase is present perhaps to generate some heat (certainly among some plant scientists if not plants), but also to maintain the redox state of the cell at some maximum level of reduction. What causes the tissues to become over-reduced is secondary and of little consequence. Once the tissue reaches a critical value of reduction the alternative oxidase swings into action and if there is insufficient enzyme the gene is signalled to produce more. Over-reduction can lead to the production of deleterious superoxides. The alternative path allows the respiratory pathways to produce intermediates without being subjected to severe adenylate control.

**Further reading**


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