

# The role of thermogenin in non-shivering thermogenesis

“Several unusual types of cells use the respiratory chain for thermogenesis, the generation of heat, rather than the synthesis of adenosine tri-phosphate.”<sup>1</sup> Newborn mammals (including humans), hibernating mammals and some cold-acclimated adult mammals do this in a specialised brown adipose tissue.

Brown adipose tissue is multilocular in appearance and its colour is due largely to the cytochromes in the tissue's abundant surrounding mitochondria. The tissue is found between the shoulder blades, in the axilla (armpit) and in a few other places.

In adults shivering is the most significant involuntary form of thermogenesis. Shivering thermogenesis occurs as a consequence of muscular contraction and depends on the production of ATP. Although facilitated by catecholamines it depends predominantly on the somatic motor system (Thompson 1977).

Non-shivering thermogenesis is most significant in the newborn and in small cold-adapted mammals. It occurs when the acceleratory action of metabolic processes is not associated with muscle contraction and is controlled by the sympathetic nervous system. This is exemplified in the fact that drugs blocking the  $\beta$ -adrenergic receptors inhibit non-shivering heat production. The other responses of the sympathetic nervous system to environmental cold are increased cardiac output and redistribution of blood flow and substrate mobilisation.

The participation of other viscera in non-shivering thermogenesis has long been suspected, although overshadowed by the more important brown adipose tissue (Foster and Frydman, 1978). Involvement of splanchnic viscera seems minimal while “skeletal muscle by virtue of its large mass has attracted the greatest interest as a potentially important alternative site of non-shivering thermogenesis.”<sup>2</sup>

“Obesity may in part be related to a defect in thermogenesis.”<sup>3</sup> Although obesity has a strong genetic component, one popular theory states that predisposition to obesity may result from a breakdown in thermogenesis. This defect may be due to a reduced response of the sympathetic nervous system or a problem with uncoupling proteins. Some individuals with a predisposition to obesity appear to have a partial genetic defect in diet-induced thermogenesis. Diet-induced thermogenesis is where surplus calories are converted to heat instead of being stored as fat. This may in part be influenced by exercise and high-carbohydrate diets that favour increased thermogenesis.

Two additional aspects of thermogenesis involve the provision of substrates as a source of fuel for thermogenesis and the delivery of oxygen and substrates to metabolising tissues.

“In order to utilise mitochondria for deliberate thermogenesis it is necessary to either exploit or circumvent the normal control.”<sup>4</sup> The uncoupling of oxidative phosphorylation is therefore biologically useful, as it gives rise to thermogenesis. “The free energy released from the electron transfer reactions is lost as heat rather than being captured in ATP.”<sup>5</sup> However, before uncoupling of the system is examined, the coupling of electron transport and oxidative phosphorylation must first be understood.

Initial mechanistic interest focused on a chemical coupling mechanism while an alternative model was the conformational coupling hypothesis. Today there is widespread acceptance of a third model, chemiosmotic coupling, proposed in 1961 by British biochemist Peter Mitchell. Although initial resistance to this model existed, overwhelming evidence has confirmed Mitchell's model and his achievements were recognised in 1978 with a Nobel Prize.

“Chemiosmotic coupling refers to the establishment of a transmembrane proton gradient to drive endergonic processes.”<sup>6</sup> Mitchell proposed that the enzymes catalysing the dehydrogenations were arranged asymmetrically across the inner mitochondrial membrane, resulting in protons being pumped from the matrix to the intermembrane space. This action generates an electrochemical gradient, resulting in the matrix having a lower pH than the intermembrane space. Protons in the intermembrane space under thermodynamic forces to restore the electrochemical gradient and equalise the pH, flow back into the matrix. As this occurs free energy is dissipated to maintain the proton gradient.

The energy released when the protons are pumped back into the matrix can be ‘coupled’ to the oxidative phosphorylation of ADP to ATP, with no isolatable intermediates being formed. This process involves the

$F_0F_1$  complex (complex V). The  $F_0$  portion of the complex traverses the inner mitochondrial membrane and contains a specific proton channel. The free energy released as the protons return to the matrix through this channel is somehow harnessed to drive the synthesis of ATP, catalysed by the  $F_1$  component of the complex. The respiratory chain has three distinct coupling sites for ATP synthesis; complex I (between NADH and ubiquinone), complex III and complex IV.

“An uncoupler is a molecule that inhibits ATP synthesis without inhibiting electron transport.”<sup>7</sup> Uncouplers of oxidative phosphorylation include; 2,4-Dinitrophenol (DNP), carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), thermogenin and some other weak acidic aromatic compounds.

Their lipophilic character allows uncouplers to diffuse relatively freely through the phospholipid layer allowing them to carry protons across the inner mitochondrial membrane and thus have an effect on the electron transport chain. (Respiring mitochondria take up lipophilic cations and they extrude lipophilic anions.) In the presence of uncouplers, electron transport between NADH and oxygen occurs normally permitting respiration to continue. However, the proton-motive force across the inner mitochondrial membrane is dissipated, resulting in no ATP synthesis because the return route of the protons via ATP synthase is bypassed. This loss of respiratory control leads to increased oxygen consumption and oxidation of NADH. (If allowed to, oxygen depletion will result as “respiration proceeds more rapidly than would be possible with the constraints of normal cellular ATP turnover.”<sup>8</sup>)

Thermogenin is a dimer of 33-kd that is similar in configuration to ATP–ADP translocase (an antiporter) and is synthesised without any higher molecular mass precursors. The quantity of the protein varies in response to physiological conditions and in animals that subsist at low temperatures it can account for 15% of the protein in the inner mitochondrial membrane. Proton conductance by thermogenin is activated by  $10^{-6} - 10^{-5}$  molecular weight free fatty acids.

Thermogenin forms a dissipative pathway between the matrix and the cytosol of the mitochondria, allowing the free flow of protons between the two. It acts as a channel for anions and permits the rapid passage of  $\text{OH}^-$  or  $\text{Cl}^-$  ions across the inner mitochondrial membrane. It acts as an uncoupler since the movement of  $\text{OH}^-$  ions from the matrix to the cytosol has the same consequences as movement of  $\text{H}^+$  in the opposite direction. “The free energy released in the electron-transfer reactions is stored transiently as an electrochemical gradient for protons but then is degraded largely to heat.”<sup>9</sup> Therefore, thermogenin is important in the role of non-shivering thermogenesis.

Since thermogenesis is controlled by the sympathetic nervous system, the uncoupling of electron transport from oxidative phosphorylation is under hormonal control. A hormone is a substance that is synthesised in a specialised cell and transmitted to remote target cells via the systemic circulation.

In thermogenesis it is thought the pathway is activated by fatty acids liberated from triacylglycerols. The response to hormonal signals from the nervous system is the synthesis of a second messenger via a signal transduction system, which controls metabolic reactions. This system is modular and consists of three protein components; a receptor, a transducer and an effector.

Thermoregulatory centres in the hypothalamus receive signals from recently activated cold receptors causing signals to be sent via sympathetic neurones to brown adipose tissue. This stimulates the neurotransmitter nor-adrenaline to be released into intracellular spaces. The transmitter binds to the 64-kd  $\beta$ -adrenergic receptor protein that spans the plasma membrane of target cells.

The serpentine receptor contains seven transmembrane helices due to its hydrophobicity profile and is a member of the rhodopsin family. The amino-terminal end contains N-linked oligosaccharides that lie on the extracellular side of the membrane. The carboxy-terminus end contains serine and threonine residues that may be reversibly phosphorylated and these lie on the cytosolic side of the membrane. The hormone binds to the receptor by sitting in a pocket formed by the transmembrane helices.

Martin Rodbell found that GTP in addition to the hormone is necessary for activation to occur and furthermore that the hormone stimulates GTP hydrolysis. These findings led to the discovery that a guanyl nucleotide-binding protein is an intermediary in the activation process. (Lubert Stryer) The activated receptor stimulates an intermediary product, the signal-coupling  $G_s$  protein, which carries the activation signal to adenylate cyclase, as it is not directly stimulated by the hormone-receptor. For that reason the hormone is only the first messenger.

Alfred Gilman purified the  $G_s$  protein and found it consisted of a 45-kd  $\alpha$  subunit, a 35-kd  $\beta$  subunit and a 7-kd  $\gamma$  subunit. (Lubert Stryer) The protein has two conformational states, GTP and GDP. In the absence of hormone the majority is found in the inactivated GDP form and adenylate cyclase remains deactivated. In the presence of hormone the hormone-receptor complex binds to the  $G_s$  protein dispersing the bound GDP, allowing GTP to bind to the complex. The  $\alpha$  subunit containing the GTP dissociates from the other two subunits and activates adenylate cyclase by binding to it. For any single bound hormone, multiple copies of the  $G_s$  proteins are formed, providing an example of biological amplification. Adenylate Cyclase can also be activated by glucagon, while insulin inhibits the enzyme.

The GTP bound to the  $\alpha$  subunit of the  $G_s$  protein is hydrolysed by GTPase in minutes. This is a necessary though not sufficient step in the deactivation of adenylate cyclase. In order to return to the original status of the cell, the hormone-receptor complex must also be deactivated. This occurs through phosphorylation of multiple serine and threonine residues in the carboxy-terminal end of the hormone-receptor complex (but not an unoccupied receptor) brought about by the enzyme  $\beta$ -adrenergic receptor kinase. Following this,  $\beta$ -arrestin caps the phosphorylated receptor to further diminish its capacity to trigger adenylate cyclase.

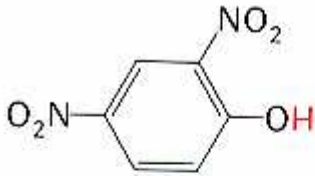
Adenylate cyclase a 120-kd integral membrane protein with multiple transmembrane segments, catalyses the intramolecular reaction of synthesis of cyclic-adenosine mono phosphate (cAMP), through the cyclisation of ATP. cAMP acts as a secondary messenger and switches on the protein kinase cascade resulting in the activation of triglyceride lipase.

Protein kinase A phosphorylates two enzymes in glycogen metabolism giving rise to glycogenolysis and inhibiting further synthesis of glycogen and phosphorylates a transcriptional activator called cAMP-response element binding protein.

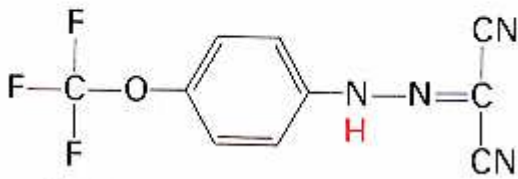
“Reducing equivalents supplied to the respiratory chain by  $\beta$ -oxidation of fatty acids, oxidative decarboxylation of pyruvate and oxidation of acetyl groups in Kreb’s cycle are transferred to oxygen, the process being coupled to  $\Delta\mu H$  generation.”<sup>10</sup> This is mediated by the fatty acid-thermogenin system and this final event results in non-shivering thermogenesis.

## Appendix

1. Structural formulae of DNP and C-*p*-tmph. pp553 Lubert Stryer. W.H. Freeman and Company New York. Biochemistry 4<sup>th</sup> Edition

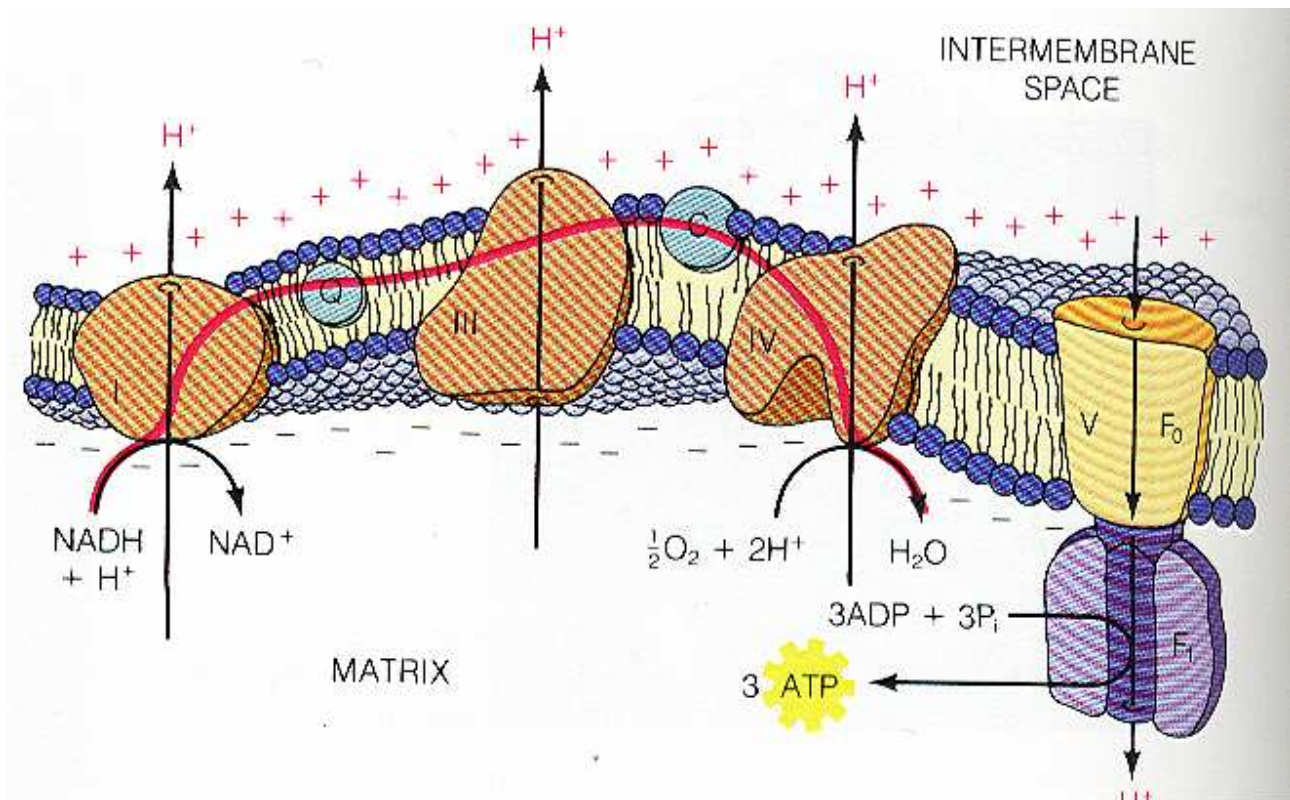


**2,4-Dinitrophenol  
(DNP)**

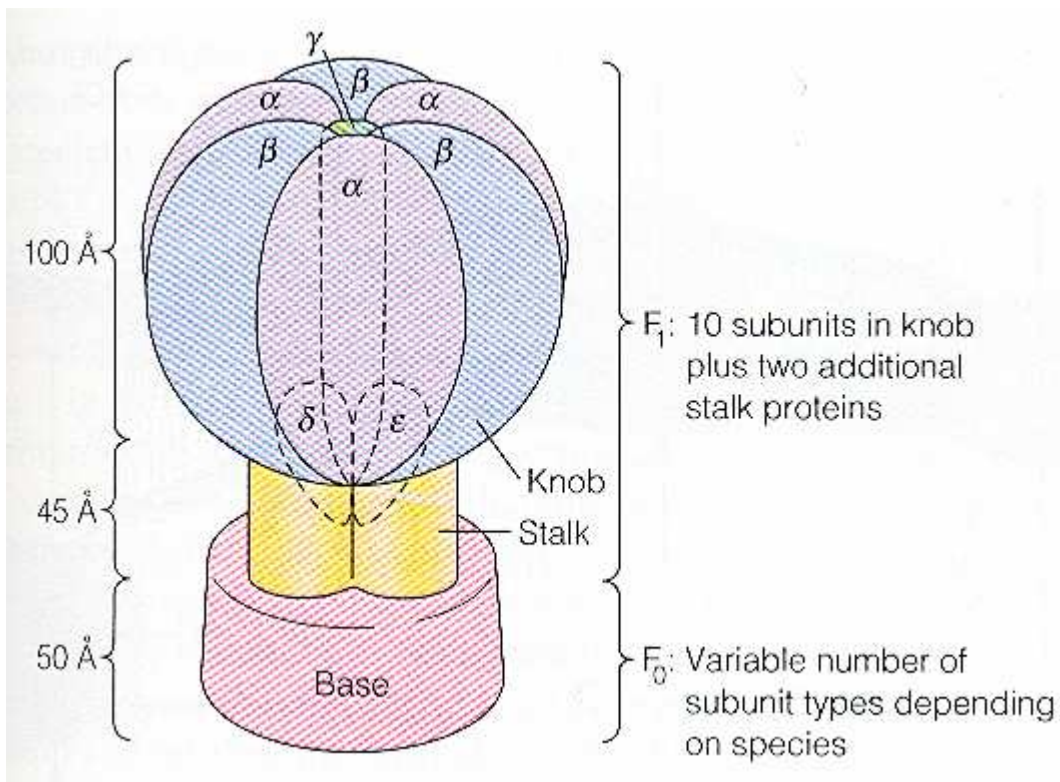


**Carbonylcyanide-*p*-trifluoro-  
methoxyphenylhydrazone**

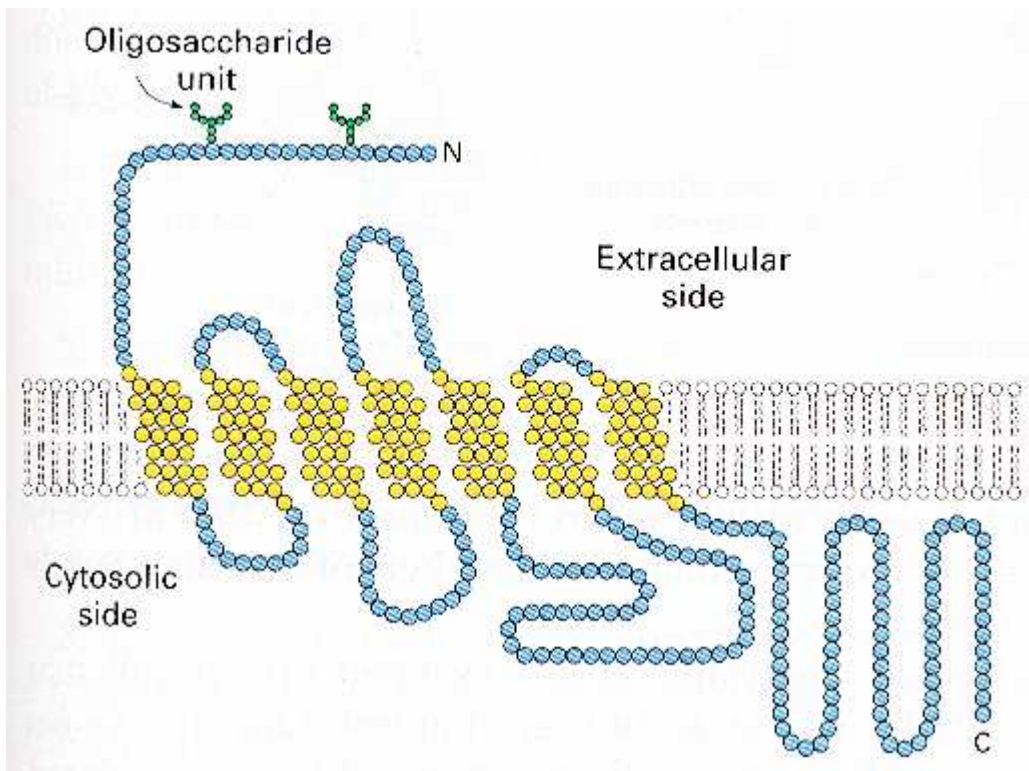
2. Vertical transport of protons by complexes in the respiratory chain. pp540 Matthews, van Holde. The Benjamin/Cummings Publishing Company, Inc. Biochemistry 2<sup>nd</sup> Edition



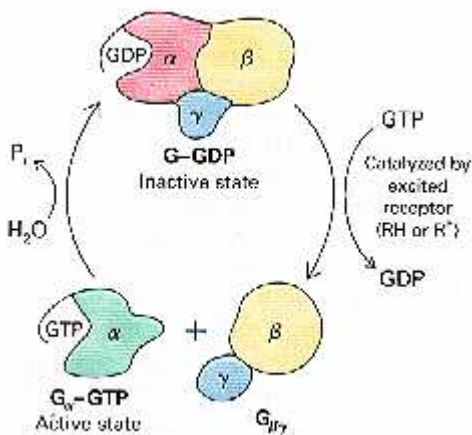
3. Structure of the  $F_0F_1$  complex. pp539 Matthews, van Holde. The Benjamin/Cummings Publishing Company, Inc. Biochemistry 2<sup>nd</sup> Edition



4. Seven-helix motif of the  $\beta$ -adrenergic receptor. pp341 Lubert Stryer. W.H. Freeman and Company New York. Biochemistry 4<sup>th</sup> Edition



5. Figure showing how the  $G_s$  proteins interconvert between their active and inactive forms. pp341 Lubert Stryer. W.H. Freeman and Company New York. Biochemistry 4<sup>th</sup> Edition



6. Enzyme-catalysed synthesis and degradation of cyclic AMP. pp340 Lubert Stryer. W.H. Freeman and Company New York. Biochemistry 4<sup>th</sup> Edition

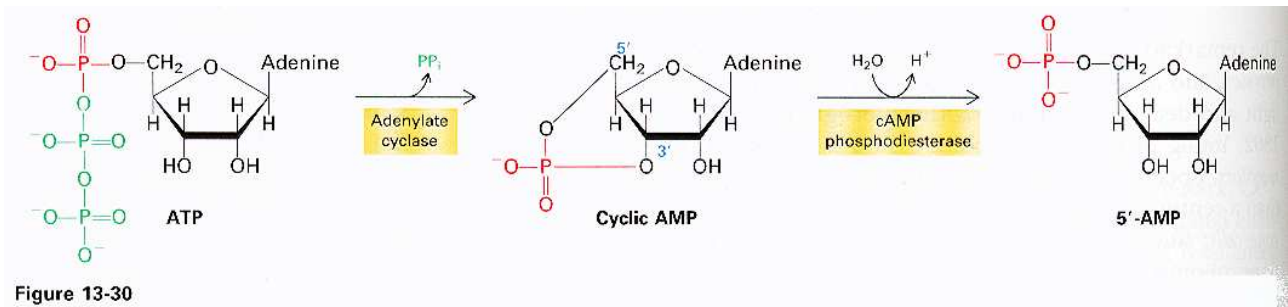


Figure 13-30

## Bibliography

- Zubay, Parson, Vance. Wm. C. Brown Publishers. Principles of Biochemistry pp317-318, 580-582
- Geoffrey Zubay. Columbia University. Wm. C. Brown Publishers. Zubay 4<sup>th</sup> Edition Biochemistry pp358-360, 365
- Matthews, van Holde. The Benjamin/Cummings Publishing Company, Inc. Biochemistry 2<sup>nd</sup> Edition pp431-432, 541
- Thomas M. Delvin. Wiley Liss. Textbook of Biochemistry With Clinical Revelance 3<sup>rd</sup> Edition pp849-852, 1101
- Lubert Stryer. W.H. Freeman and Company New York. Biochemistry 4<sup>th</sup> Edition pp340, 342-347, 514, 553, 594-595, 913-918
- Edited by Robert F. Schmidt and Gerhard Thews. Springer-Verlag Berlin Heidelberg. New York. Human Physiology pp531-532, 544-545, 539

## References

- <sup>1</sup> Geoffrey Zubay. Columbia University. Wm. C. Brown Publishers. Zubay 4<sup>th</sup> Edition Biochemistry
- <sup>2</sup> London New York. Chapman and Hall. Edited by Lucien Girardier and Michael J. Stock. Mammalian Thermogenesis
- <sup>3</sup> Thomas M. Delvin. Wiley Liss. Textbook of Biochemistry With Clinical Relevance 3<sup>rd</sup> Edition pp849-852, 1101
- <sup>4</sup> London New York. Chapman and Hall. Edited by Lucien Girardier and Michael J. Stock. Mammalian Thermogenesis
- <sup>5</sup> Lubert Stryer. W.H. Freeman and Company New York. Biochemistry 4<sup>th</sup> Edition
- <sup>6</sup> Matthews, van Holde. The Benjamin/Cummings Publishing Company, Inc. Biochemistry 2<sup>nd</sup> Edition pp431-432, 541
- <sup>7</sup> Geoffrey Zubay. Columbia University. Wm. C. Brown Publishers. Zubay 4<sup>th</sup> Edition Biochemistry
- <sup>8</sup> London New York. Chapman and Hall. Edited by Lucien Girardier and Michael J. Stock. Mammalian Thermogenesis
- <sup>9</sup> Geoffrey Zubay. Columbia University. Wm. C. Brown Publishers. Zubay 4<sup>th</sup> Edition Biochemistry
- <sup>10</sup> Citation unavailable at time of going to press.