IUBMB *Life*, 61(2): 184–188, February 2009

Is There an Answer?

The "Icefish Paradox." Which Is the Task of Neuroglobin in Antarctic Hemoglobin-Less Icefish?

C.-H. Christina Cheng[1], Guido di Prisco[2] and Cinzia Verde[2]
[1]*Department of Animal Biology, University of Illinois, Urbana, IL, USA*
[2]*Institute of Protein Biochemistry, CNR, Via Pietro Castellino 111, Napoli, Italy*

Is There an Answer? is intended to serve as a forum in which readers to *IUBMB Life* may pose questions of the type that intrigue biochemists but for which there may be no obvious answer or one may be available but not widely known or easily accessible. Readers are invited to e-mail ascenzi@uniroma3.it if they have questions to contribute or if they can provide answers to questions that are provided here from time to time. In the latter case, instructions will be sent to interested readers. Answers should be, whenever possible, evidence-based and provide relevant references.
Paolo Ascenzi

The Southern Ocean surrounding Antarctica offers a uniquely stable thermal environment where cold adaptation of fishes has occurred, obviating the need to retain the functional plasticity required in more variable ecosystems. Notothenioidei, the dominant Antarctic fish suborder, offers opportunities for identification of the biochemical characters or the physiological traits responsible for thermal adaptation. In the process of cold adaptation, the evolutionary trend of notothenioids has shaped unique specialisations, including modification of hematological characteristics, *e.g.*, decreased amounts and multiplicity of hemoglobins. The Antarctic family Channichthyidae (the notothenioid crown group) is devoid of hemoglobin. Our recent discovery of neuroglobin in the brain of three species of red-blooded notothenioids and in at least 13 of the 16 channichthyid species, as well as the identification of a single α-globin gene in the brain of a red-blooded species, has potential implications in our understanding of the function of this protein and suggests future avenues of investigation.

Received 8 August 2008; accepted 8 August 2008
Address correspondence to: Cinzia Verde, Institute of Protein Biochemistry, CNR, Via Pietro Castellino 111, 80131 Napoli, Italy. Tel/Fax: +39-081-6132710. E-mail: c.verde@ibp.cnr.it

ISSN 1521-6543 print/ISSN 1521-6551 online
DOI: 10.1002/iub.138

Elucidating molecular mechanisms of protein cold adaptation is one of the main goals in evolutionary biology, although many questions remain unanswered as yet, because of the difficulty to mechanistically link the structure and function of proteins and genes to species fitness (*1*). Currently, there is a growing interest in polar marine organisms and how they have evolved at constantly cold temperatures. More important, life sciences are not the only area to gain key insights from studying biological communities inhabiting the polar environments. In fact, understanding how polar ecosystems respond to climate change has global significance (*2, 3*).

Fishes thriving in polar habitats offer many opportunities for comparative approaches aimed at understanding thermal adaptations and their ability to counteract ongoing climate changes. Historically, studies on the molecular mechanisms underlying fish biodiversity and thermal adaptations in extreme cold environments had found their natural scenario in the most extreme marine habitat on earth, the Antarctic Ocean. The variety of adaptations underlying the ability of modern Antarctic fish to survive at the freezing temperatures represents the extreme of low-temperature adaptations found among vertebrates (*4*). In the Southern Ocean, Notothenioids underwent impressive diversification that led them to fill the numerous ecological niches left empty because of the establishment of colder conditions and the effective isolation of the Southern Ocean by the Antarctic Circumpolar Current (ACC) (*4, 5*). In the Arctic, there has been no comparable adaptive radiation of a fish group probably because tectonic events never isolated the northern land masses and their continental shelves, and there are no major oceanographic barriers limiting species distribution and gene flow (*4, 5*). Therefore, the conditions that favoured evolution in isolation as it occurs in the Southern Ocean are not met in the Arctic (*4*).

Notothenioidei, mostly confined within Antarctic and sub-Antarctic waters, are the dominant component of the Southern Ocean fauna (Fig. 1). The availability of notothenioid taxa living in a wide range of latitudes (Antarctic, sub-Antarctic, and temperate

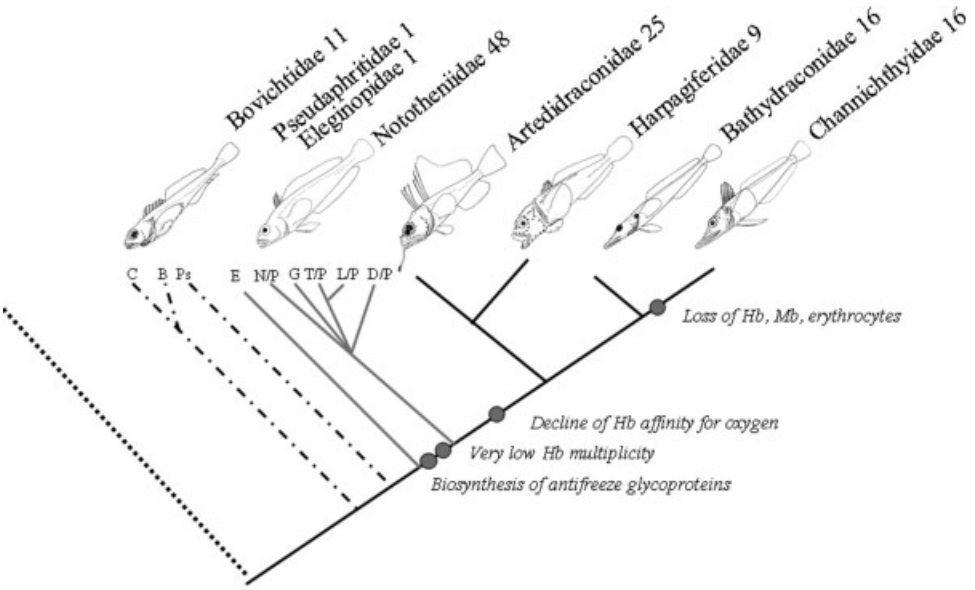

Figure 1. Phylogenetic tree depicting interrelationships among notothenioid families, based on morphological and molecular data. Some physiological innovations are indicated (grey-filled circles). Numbers indicate the number of species in each family. C, *Cottoperca gobio*; B, *Bovichtus*; Ps, *Pseudaphritis urvillii*; E, *Eleginops maclovinus*; N/P, *Notothenia*/*Paranotothenia* group; G, *Gobionotothen*; T/P, *Trematomus*/*Pagothenia* group; L/P, *Lepidonotothen*/*Patagonotothen* group; D/P, *Dissostichus*/*Pleuragramma* group ("pelagic group"). Modified from (*6*).

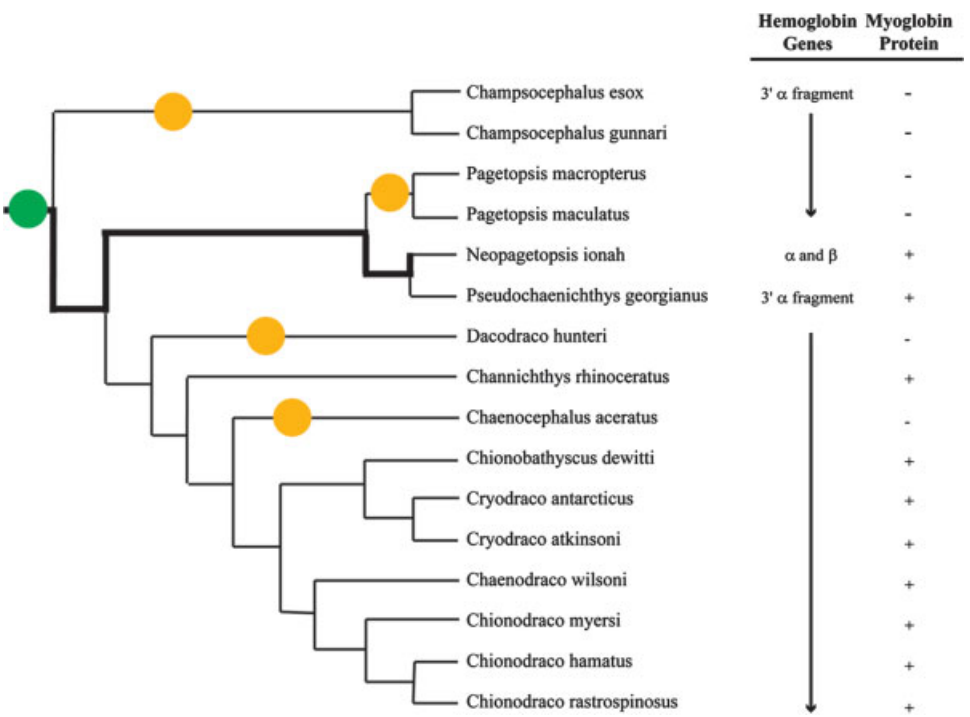

Figure 2. Hemoprotein loss in the icefish family. The loss of globin genes and the expression of Mb are mapped on a consensus phylogeny of Channichthyidae (*16*). The green-filled circle represents the loss of the ability to express Hb, which probably occurred in the ancestral channichthyid. The thick black line traces the retention of adult α- and β-globin genes by *N. ionah*. The yellow-filled circles indicate the four independent mutational events that explain the loss of Mb expression. Modified from (*17*). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

regions) offers a remarkable opportunity to study the physiological and biochemical characters gained and, conversely, lost in response to cold and to reconstruct the likely evolutionary events leading to the ability to carry oxygen in cold habitats (*7*).

Hemoglobin (Hb) has generally been regarded as *a sine qua non* factor for oxygen transport in vertebrates (*8–10*). What a surprise it must have been to physiologists when icefishes (family Channichthyidae, the most derived family of the suborder Notothenioidei) have been reported as "vertebrates without erythrocytes and blood pigment" (*11*). All extant icefish species lack Hb (*12–14*) and many have lost myoglobin (Mb) expression (*15*) (Fig. 2). Oxygen delivery to tissues occurs by transport of the gas physically dissolved in the plasma (*18, 19*). Investigations on icefish as yet elicit questions such as "How were these conditions developed?"

Although in humans and most vertebrates, mutations in the α- or β-globin genes often cause severe genetic diseases (*8–10*), in icefish they are correlated to large increases in cellular mitochondrial density, blood volume, and heart size. The homeostatic activity of nitric oxide (NO), a key modulator of angiogenesis and mitochondrial biogenesis, probably facilitates the evolution of these compensatory characters (*20*). Sidell and O'Brien (*17*) have suggested that the evolution of the cardiovascular adaptations was "jump-started" by homeostatic NO response. Note that NO stimulates mitochondrial biogenesis, so that expansion of tissue capillary density, enlargement of the heart, and increased mitochondrial density in heart and other aerobic tissues may occur in organisms subjected to chronic elevation of NO (*21, 22*). As pointed out by Sidell and O'Brien (*17*) being the icefishes natural knockouts, they offer remarkable advantages to answer intriguing questions more than the experimentally produced knockouts for Mb expression in mice (*23, 24*). However, Mb deletion in mice leaves the cardiac function uncompromised, probably because of the development of multiple compensatory mechanisms (*23, 24*). These adaptation mechanisms strongly support the crucial role of Mb in facilitating oxygen delivery to cardiomyocites (*25, 26*). All these physiological responses match the icefish phenotype (*20*).

However, the development of the compensatory physiological and circulatory adaptations in icefishes argues that the loss of Hb and erythrocytes was probably maladaptive under conditions of physiological stress (*17*). The evolutionary development of an alternative physiology based on the hemoprotein-free blood may adequately work in the cold for notothenioids, and in general loosing globin genes may not be lethal in thermostable environments (*27*). The benefits due to these losses include reduced costs for protein synthesis (*28*). Because the deletion of Mb in mice leads to enhanced sensitivity to NO (*29*), the question "How may icefishes cope with NO despite the lack of Hb and Mb?" is timely.

Novel globins, such as neuroglobin (Ngb) and cytoglobin, have been recently described in many vertebrates (*30, 31*). Ngb is a monomeric globin displaying the classical vertebrate folding 3/3 (*32, 33*). The protein is able to bind oxygen and other ligands and it is transcriptionally induced by hypoxia and ischemia (*34*). Ngb is mainly expressed in retinal neurons and fibroblast-like cells and plays a neuroprotective role during hypoxic stress (*34*). Although many other roles have been suggested, including scavenging of reactive nitrogen and oxygen species (*35*), signal transduction (*36*) and regulation of apoptotic pathways (*37*), the Ngb physiological function is still unknown.

Our recent discovery of Ngb in the brain of red-blooded notothenioids (*Dissostichus mawsoni, Gymnodraco acuticeps*, and *Bovichtus variegatus*) and in at least 13 of the 16 channichthyid species, as well as the identification of a single α-globin gene in the brain of *D. mawsoni* (*38*), open the question "what is the role of Ngb in fishes lacking Hb and Mb?".

The finding that icefish retains the Ngb gene, despite having lost Hb and Mb in most species, is very intriguing. Whether these globin genes are expressed is the next important question, to be followed by others, such as "if brain does express α globin, why would it do so?". Although the functions of these monomeric globins in the brain are not well understood as yet, this discovery may have important implications in the physiology and pathology of the brain. To our knowledge, the expression of a single globin gene in non-erythroid cells has been reported in two cases only, *i.e.*, in activated macrophages from adult mice and lens cells (*39*) and in alveolar epithelial cells (*40*).

Although these results have yet to be extended to other notothenioid species, the finding that a single α-globin gene is present in non-erythroid tissues opens new perspectives on the roles played by these monomeric vertebrate globins, including gas exchange, NO metabolism, and protection against oxidative and nitrosative stress. Moreover, a monomeric globin may function by mimicking the role of Mb in oxygen storage. The protection against oxidative stress is very likely because at low temperature the increased gas-solubility increases the production rate of reactive oxygen species (*38*). These results, if confirmed in other species, raise important questions in genetics, physiology, development, hematology and, more in general, evolution.

Polar fish are a suitable model to learn more about the function of globins in the brain, and especially about their role in species devoid of Hb and Mb. In particular, modern Notothenioidei appear to be the end result of an extraordinary natural experiment, as they possess the exceptional physiological features (both adaptive and non-adaptive) engineered by organisms that live at permanently cold temperatures.

ACKNOWLEDGEMENTS

This study is financially supported by the Italian National Programme for Antarctic Research (PNRA) and by the U.S. National Science Foundation, Office of Polar Programs, Grant OPP-0636696. It is in the framework of the SCAR programme Evolution and Biodiversity in the Antarctic (EBA) and of the project CAREX (Coordination Action for Research Activities on Life in Extreme Environments), European Commission FP7 call ENV.2007.2.2.1.6. The authors thank Paolo Ascenzi as his

comments and suggestions have significantly improved the quality of the manuscript. The nucleotide sequence data have been deposited in EMBL Nucleotide Sequence Database.

REFERENCES

1. Verde, C., Vergara, A., Mazzarella, L., and di Prisco, G. (2008) The hemoglobins of fishes living at polar latitudes—current knowledge on structural adaptations in a changing environment. *Curr. Protein. Pept. Sci*., in press.
2. di Prisco, G. and Verde, C. (2006) Predicting the impacts of climate change on the evolutionary adaptations of polar fish. *Rev. Environ. Sci. Biotechnol.* **5**, 309–321.
3. Clarke, A., Murphy, E. J., Meredith, M. P., King, J. C., Peck, L. S., Barnes, D. K. A., and Smith, R. C. (2007) Climate change and the marine ecosystem of the western Antarctic Peninsula. *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* **362**, 149–166.
4. Eastman, J. T. (1993) *Antarctic Fish Biology. Evolution in a Unique Environment*. 322 pp, Academic Press, San Diego.
5. Eastman, J. T. (2005) The nature of the diversity of Antarctic fishes. *Polar Biol.* **28**, 93–107.
6. Verde, C., Lecointre, G., di Prisco, G. (2007) The phylogeny of polar fishes and the structure, function and molecular evolution of haemoglobin. *Polar Biol.* **30**, 523–539.
7. di Prisco, G., Eastman, J. T., Giordano, D., Parisi, E., and Verde, C. (2007) Biogeography and adaptation of Notothenioid fish: hemoglobin function and globin-gene evolution. *Gene* **398**, 143–155.
8. Forget, B. G. and Pearson, H. A. (1995) Hemoglobin synthesis and the thalassemias. In *BLOOD: Principles and Practice of Hematology*. (Handin, R. I., Lux, S. E., Stossel, T. P., eds.). pp. 1525–1590, J. B. Lippincott Company, Philadelphia.
9. Nagel, R. L. (1995) Disorders of hemoglobin function and stability. In *BLOOD: Principles and Practice of Hematology*. (Handin, R. I.,Lux, S. E., Stossel, T. P., eds.). pp. 1591–1644, J. B. Lippincott Company, Philadelphia.
10. Platt, O. S. (1995) The sickle syndromes. In *BLOOD: Principles and Practice of Hematology*. (Handin, R. I., Lux, S. E., Stossel, T. P., eds.). pp. 1645–1700, J. B. Lippincott Company, Philadelphia.
11. Ruud, J. T. (1954) Vertebrates without erythrocytes and blood pigment. *Nature* **173**, 848–850.
12. Cocca, E., Ratnayake-Lecamwasam, M., Parker, S. K., Camardella, L., Ciaramella, M., di Prisco, G., and Detrich, H. W., III. (1995) Genomic remnants of α-globin genes in the hemoglobinless Antarctic icefishes. *Proc. Natl. Acad. Sci. USA* **92**, 1817–1821.
13. Zhao, Y., Ratnayake-Lecamwasam, M., Parker, S. K., Cocca, E., Camardella, L., di Prisco, G., and Detrich, H. W., III. (1998) The major adult α-globin gene of Antarctic teleosts and its remnants in the hemoglobinless icefishes. Calibration of the mutational clock for nuclear genes. *J. Biol. Chem.* **273**, 14745–14752.
14. di Prisco, G., Cocca, E., Parker, S. K., and Detrich, H. W. (2002) Tracking the evolutionary loss of hemoglobin expression by the white-blooded Antarctic icefishes. *Gene* **295**, 185–191.
15. Sidell, B. D., Vayda, M. E., Small, D. J., Moylan, T. J., Londraville, R. L., Yuan, M. L., Rodnick, K. J., Eppley, Z. A., and Costello, L. (1997) Variable expression of myoglobin among the hemoglobinless Antarctic icefishes. *Proc. Natl. Acad. Sci. USA* **94**, 3420–3424.
16. Near, T. J., Pesavento, J. J., and Cheng, C. H. (2003) Mitochondrial DNA, morphology, and the phylogenetic relationships of Antarctic icefishes (Notothenioidei: Channichthyidae). *Mol. Phylogenet. Evol.* **28**, 87–98.
17. Sidell, B. D. and O'Brien, K. M. (2006) When bad thing happen to good fish: the loss of hemoglobin and myoglobin expression in Antarctic icefishes. *J. Exp. Biol.* **209**, 1791–1802.
18. Hemmingsen, E. A. (1991) Respiratory and cardiovascular adaptations in hemoglobin-free fish: resolved and unresolved problems. In *Biology of Antarctic Fish*. (di Prisco, G., Maresca, B., Tota, B., eds.). pp. 191–203, Springer-Verlag, Berlin.
19. Hemmingsen, E. A. and Douglas, E. L. (1977) Respiratory and circulatory adaptations to the absence of hemoglobin in Chaenichthyid fishes. In *Adaptations Within Antarctic Ecosystems*. (Llano, G. A., ed.). pp. 479–487, Smithsonian Institution, Washington, DC.
20. Cheng, C.-H. C. and Detrich, H. W., III. (2007) Molecular ecophysiology of Antarctic notothenioid fishes. *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* **362**, 2215–2232.
21. Nisoli, E., Clementi, E., Paolucci, C., Cozzi, V., Tonello, C., Sciorati, C., Bracale, R., Valerio, A., Francolini, M., Moncada, S., and Carruba, M. O. (2003) Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* **299**, 896–899.
22. Nisoli, E., Falcone, S., Tonello, C., Cozzi, V., Palomba, L., Fiorani, M., Pisconti, A., Brunelli, S., Cardile, A., Francolini, M., Cantoni, O., Carruba, M. O., Moncada, S., and Clementi, E. (2004) Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals. *Proc. Natl. Acad. Sci. USA* **101**, 16507–16512.
23. Garry, D. J., Ordway, G. A., Lorenz, J. N., Radford, N. B., Chin, E. R., Grange, R. W., Bassel-Duby, R., and Williams, R. S. (1998) Mice without myoglobin. *Nature* **395**, 905–908.
24. Gödecke, A., Flögel, U., Zanger, K., Ding, Z., Hirchenhain, J., Decking, U. K. M., and Schrader, J. (1999) Disruption of myoglobin in mice induces multiple compensatory mechanisms. *Proc. Natl. Acad. Sci. USA* **96**, 10495–10500.
25. Wittenberg, B. A. and Wittenberg, J. B. (1987) Myoglobin-mediated oxygen delivery to mitochondria of isolated cardiac myocytes. *Proc. Natl. Acad. Sci. USA* **84**, 7503–7507.
26. Wittenberg, J. B. and Wittenberg, B. A. (2003) Myoglobin function reassessed. *J. Exp. Biol.* **206**, 2011–2020.
27. Verde, C., Balestrieri, M., de Pascale, D., Pagnozzi, D., Lecointre, G., and di Prisco, G. (2006) The oxygen-transport system in three species of the boreal fish family Gadidae. Molecular phylogeny of haemoglobin. *J. Biol. Chem.* **281**, 22073–22084.
28. Pörtner, H. O., Peck, L., and Somero, G. (2007) Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* **362**, 2233–2258.
29. Flögel, U., Merx, M. W., Gödecke, A., Kecking, U. K. J., and Schrader, J. (2001) Myoglobin: a scavenger of bioactive NO. *Proc. Natl. Acad. Sci. USA* **98**, 735–740.
30. Burmester, T., Weich, B., Reinhardt, S., and Hankeln, T. (2000) A vertebrate globin expressed in the brain. *Nature* **407**, 520–523.
31. Burmester, T., Ebner, B., Weich, B., and Hankeln, T. (2002) Cytoglobin: a novel globin type ubiquitously expressed in vertebrate tissues. *Mol. Biol. Evol.* **19**, 416–421.
32. Pesce, A., Dewilde, S., Nardini, M., Moens, L., Ascenzi, P., Hankeln, T., Burmester, T., and Bolognesi, M. (2003) Human brain neuroglobin structure reveals a distinct mode of controlling oxygen affinity. *Structure* **11**, 1087–1095.
33. Vallone, B., Nienhaus, K., Matthes, A., Brunori M., and Nienhaus, G. U. (2004) The structure of carbonmonoxy neuroglobin reveals a heme-sliding mechanism for control of ligand affinity. *Proc. Natl. Acad. Sci. USA* **101**, 17351–17356.
34. Brunori, M. and Vallone, B. (2007) Neuroglobin, seven years after. *Cell. Mol. Life Sci.* **64**, 1259–1268.
35. Brunori, M., Giuffrè, A., Nienhaus, K., Nienhaus, G. U., Scandurra, F. M., and Vallone, B. (2005) Neuroglobin, nitric oxide, and oxygen: functional pathways and conformational changes. *Proc. Natl. Acad. Sci. USA* **102**, 8483–8488.
36. Wakasugi, K., Nakano, T., and Morishima, I. (2003) Oxidized human neuroglobin acts as a heterotrimeric Gα protein guanine nucleotide dissociation inhibitor. *J. Biol. Chem.* **278**, 36505–36512.

37. Khan, A. A., Mao, X. O., Banwait, S., Jin, K., and Greenberg, D. A. (2007) Neuroglobin attenuates β-amyloid neurotoxicity *in vitro* and transgenic Alzheimer phenotype *in vivo*. *Proc. Natl. Acad. Sci. USA* **104**, 19114–19119.
38. Cheng, C. H. C., di Prisco, G., and Verde, C. (2008) Cold-adapted Antarctic fish: the discovery of neuroglobin in red-blooded and hemoglobinless Notothenioidei. *Mar Genom*, Submitted.
39. Liu, L., Zeng, M., and Stamler, J. S. (1999) Hemoglobin induction in mouse macrophages. *Proc. Natl. Acad. Sci. USA* **96**, 6643–6647.
40. Newton, D. A., Rao K. M., Dluhy, R. A., and Baatz, J. E. (2006). Hemoglobin is expressed by alveolar epithelial cells. *J. Biol. Chem.* **281**, 5668–5676.

New Question

1. What is the relationship between epigenetic and genetic regulation in tumour progression?