Bacterial photosynthesis genes in a virus

A bacteriophage may protect itself and its host against a deadly effect of bright sunlight.

Cyanobacteria contribute to the overall photosynthetic production of oxygen in the oceans, but they are susceptible to infection by viruses and also to photoinhibition when sunlight is too intense. Here we show that the genomic sequence of one such virus, a bacteriophage known as S-PM2, encodes the D1 and D2 proteins that are key components of one of the photosynthetic reaction centres (photosystem II, PSII), which are crucial sites of damage in photo-inhibition. The presence of this virus, and others like it, in the ocean may ensure that photo-inhibition is prevented in infected cells, allowing photosynthesis to continue and therefore provide the energy needed by the virus for its replication.

Primary production in the oligotrophic regions of the oceans is dominated by the cyanobacterial components of the picophytoplankton organisms *Synechococcus* and *Prochlorococcus*[1]. The amounts of visible and ultraviolet solar radiation in oceanic ecosystems may be sufficient, particularly in surface waters, to cause photo-inhibition[2]. The primary cause of photo-inhibition in chloroplasts and cyanobacteria is damage to PSII, a large protein–pigment complex that catalyses the light-dependent oxidation of water to molecular oxygen.

At the core of PSII is a dimer of two related proteins, D1 and D2, which binds the pigments and co-factors necessary for the complex's primary photochemistry. During photosynthesis, D1 and, to a lesser extent, D2 turn over rapidly as a result of light-induced damage and are replaced by newly synthesized polypeptides in a repair cycle. When the rate of photo-inactivation and damage of D1 exceeds the capacity for repair, photo-inhibition occurs, resulting in a reduction in the maximum efficiency of PSII photochemistry[3].

Viruses in general, and bacteriophages (viruses that infect bacteria) in particular, are abundant in marine ecosystems and are thought to exert major biogeochemical and ecological effects on the marine environment[4]. We analysed the genome sequence of S-PM2 (Fig. 1), a bacteriophage that infects marine *Synechococcus* strains[5] and is about 194 kilobases long[6], and found that it encodes the D1 and D2 proteins of PSII.

The genome contains a region of about 3.8 kilobases (GenBank accession no. AY329638) that extends from just over 100 base pairs upstream of the D1 gene (*psbA*), through two genes that are unrelated to photosynthesis, to a point some 100 base pairs downstream of the carboxy terminus of the D2 gene (*psbD*). The two intervening genes encode a homologue of the bacteriophage T4 gp49

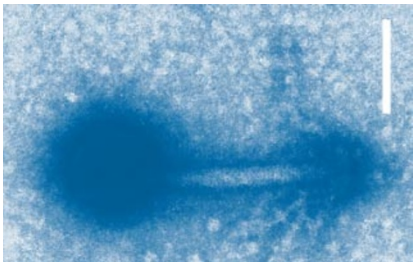

Figure 1 The bacteriophage S-PM2 (here artificially coloured blue), which infects marine cyanobacteria. Scale bar, 100 nm.

(also known as recombination endonuclease VII) and a protein that has some similarity to a putative membrane protein present in the bacterium *Escherichia coli*.

The *psbA* gene appears to be interrupted, as the translated product of the gene aligns with other cyanobacterial and plant D1 proteins up to residue 276 (see supplementary information). There is then a region of 212 base pairs that encodes an amino-acid sequence without any similarity to known D1 proteins; this is followed by a region that encodes the remaining 25 amino acids of D1. This suggests that the *psbA* gene in S-PM2 contains a self-splicing intron. Introns occur in the *psbA* genes of the protozoan *Chlamydamonas*, and there is evidence for light/redox-regulated splicing of *psbA* precursor-messenger RNAs[7]. We detected copies of *psbA* genes after amplification by the polymerase chain reaction in five out of eight other *Synechococcus* viruses, although these genes seem to lack the putative intron.

The complete D1 protein of S-PM2 is similar to the D1 proteins of the marine *Synechococcus* sp. WH8102 (see supplementary information). There is homology in the DNA sequences, indicating that S-PM2 might have acquired the gene horizontally from its *Synechococcus* host. Presumably, *psbD* was acquired independently, given the presence of two unrelated intervening genes.

The expression of virus-encoded D1 and D2 proteins in infected cells would allow a repair cycle to operate in PSII after the host's protein synthesis had been shut down, thereby maintaining the cells' photosynthetic activity and the concomitant evolution of oxygen, and ensuring the provision of energy for the continued replication of the virus. This survival strategy resembles one used by a virus that infects the green alga *Chlorella*, which enhances the mechanism used by the host cell to rid itself of surplus light energy to avoid photo-inhibition[8].

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1. Partensky, F., Blanchot, J. & Vaulot, D. in *Marine Cyanobacteria* (eds Charpy, L. & Larkum, A. W. D.) Vol. 19, 457–475 (Institut Océanographique, Monaco, 1999).
2. Piazena, H., Perez-Rodrigues, E., Häder, D.-P. & Lopez-Figueroa, F. *Deep Sea Res. II* **49,** 3513–3528 (2002).
3. Aro, E. M., Virgin, I. & Andersson, B. *Biochim. Biophys. Acta* **1143,** 113–134 (1993).
4. Fuhrman, J. A. *Nature* **399,** 541–548 (1999).
5. Wilson, W. H., Joint, I. R., Carr, N. G. & Mann, N. H. *Appl. Environ. Microbiol.* **59,** 3736–3743 (1993).
6. Hambly, E. *et al. Proc. Natl Acad. Sci. USA* **98,** 11411–11416 (2001).
7. Deshpande, N. N., Bao, Y. & Herrin, D. L. *RNA* **3,** 37–48 (1997).
8. Seaton, G. G. R., Hurry, V. M. & Rohozinsky, J. *FEBS Lett.* **389,** 319–323 (1996).

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COMMUNICATIONS ARISING

Cell signalling

Cell survival and a Gadd45-factor deficiency

Tumour-necrosis factor-α (TNF-α) is an important protein that regulates inflammation, immunity, cell survival and cell death in response to infection or chronic stress[1–5]. De Smaele *et al.* report that the gene encoding an inducible cellular factor, Gadd45β (for 'growth arrest and DNA damage'), is essential for promoting TNF-α-mediated cell survival[6]. However, we show here that neither TNF-α signalling nor cell survival is affected in mice lacking *gadd45β*, a fact which demonstrates that genes other than *gadd45β* (refs 5, 7) might regulate cell survival in response to TNF-α.

TNF-α concomitantly activates two signalling pathways — a cell-survival pathway regulated by the transcription factor NF-κB and a cell-death pathway associated with the c-Jun N-terminal kinase (JNK) cascade[1–3,5]. Understanding the molecular basis for the cell's choice between life and death in response to TNF-α is a topic of intense investigation[4,5]. The fate of a cell exposed to TNF-α is determined by cross-talk between these two signalling pathways[4–7]: NF-κB activation switches on genes that blunt the pro-cell-death activity of the JNK pathway[4,6,7].

De Smaele *et al.* propose that one such NF-κB target that is essential in TNF-α-mediated cell survival is the *gadd45β* gene[6]. *Gadd45β* (also termed *MyD118*) encodes

one of the family of Gadd45 proteins, which are implicated in growth arrest, DNA-damage repair and programmed cell death[8,9]. De Smaele *et al.* showed that ectopic overexpression of Gadd45β in mouse-embryo fibroblasts (MEFs) and in NF-κB-deficient cell lines antagonizes TNF-α-induced cell death and increases cell survival. In addition, ectopic expression of antisense *gadd45β* messenger RNA, which presumably blocks Gadd45β expression, was found to decrease cell survival and prolong JNK activity; this is similar to the response in cells lacking NF-κB. The authors conclude[6] that TNF-α-mediated activation of NF-κB induces Gadd45β, which inhibits TNF-α-mediated cell death and JNK signalling and promotes cell survival; however, this conclusion contradicts an earlier study[10] that implicates Gadd45β as an activator of JNK.

We used *gadd45β*-null mice, in which the *gadd45β* gene is ablated (D.L. and A.F., unpublished results), to assess further the effect of *gadd45β* deficiency on TNF-α-mediated cellular responses, including cell survival and JNK signalling. We found that TNF-α induced *gadd45β* expression in wild-type but not in *gadd45β*-deficient MEFs (Fig. 1a). Like wild-type MEFs, *gadd45β*-deficient (*gadd45β*$^{-/-}$) MEFs were not susceptible to TNF-α-mediated cell death (Fig. 1b). However, in the presence of the protein-synthesis inhibitor cycloheximide, which prevents the expression of the pro-survival genes induced by NF-κB, both *gadd45β*$^{-/-}$ and wild-type MEFs were equally susceptible to TNF-α-mediated cell death.

Our findings indicate that *gadd45β* expression is not essential for the NF-κB pro-survival function. Furthermore, the kinetics of downregulation of JNK activity were similarly rapid in *gadd45β*$^{-/-}$ MEFs and in wild-type cells (Fig.1c), as well as in another cell type, splenic lymphocytes (data not shown).

Our results indicate that other NF-κB target genes[5,7] are more likely than *gadd45β* to be primary mediators of the survival function of NF-κB. The discrepancy between our observations and those of De Smaele *et al.*[6] might reflect limitations in their experimental approach — for example, ectopic overexpression of *gadd45β* or of its antisense RNA in cells stimulated with TNF-α might have affected cell survival and JNK activity in some indirect or nonspecific way. Further work is needed to assess what role, if any, Gadd45β has in the cell's response to TNF-α.

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1. Baud, V. & Karin, M. *Trends Cell Biol.* **11,** 372–377 (2001).
2. Locksley, R. M., Killeen, N. & Lenardo, M. J. *Cell* **104,** 487–501 (2001).
3. Chen, G. & Goeddel, D.V. *Science* **296,** 1634–1635 (2002).
4. Kyriakis, J. M. *Nature* **414,** 265–266 (2001).
5. Karin, M. & Lin, A. *Nature Immunol.* **3,** 221–227 (2002).
6. De Smaele, E. *et al. Nature* **414,** 308–313 (2001).
7. Tang, G. *et al. Nature* **414,** 313–317 (2001).
8. Liebermann, D. A. & Hoffman, B. *Leukemia* **16,** 527–541 (2002).
9. Fornace, A. J. Jr, Jackman, J., Hollander, M. C., Hoffman-Liebermann, B. & Liebermann, D. A. *Ann. NY Acad. Sci.* **663,** 139–153 (1992).
10. Takekawa, M. & Saito, H. *Cell* **95,** 521–530 (1998).

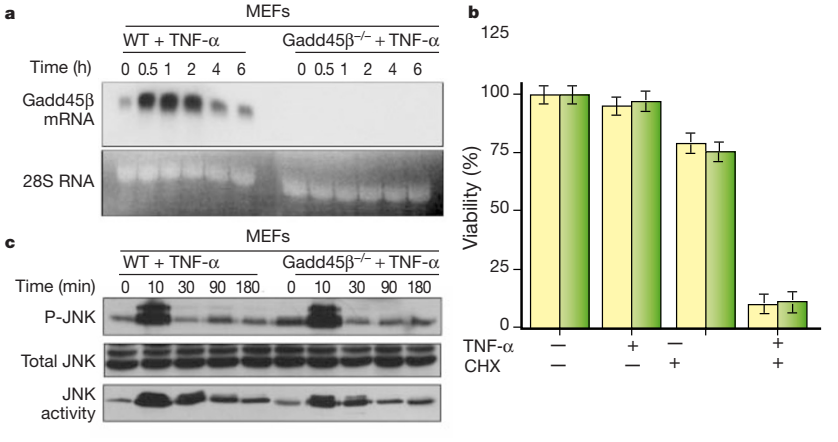

Figure 1 Gadd45β deficiency does not alter TNF-α-mediated cell survival or JNK signalling. **a,** TNF-α induces expression of *gadd45β* in wild-type (WT) but not in *gadd45β*$^{-/-}$ mouse-embryo fibroblasts (MEFs). RNA from cells collected at different times after treatment with TNF-α (1,000 U ml^{-1}) was resolved on a 2% agarose–formaldehyde gel and analysed by northern blotting using a ^{32}P-labelled *gadd45β* probe. 28S RNA, loading control. **b,** *gadd45β*$^{-/-}$ cells are not susceptible to TNF-α-mediated cell death in the absence of cycloheximide (CHX). Viability of WT (left bars) and *gadd45β*$^{-/-}$ MEFs (right bars) was determined by trypan-blue staining after 14-h treatment with TNF-α (1,000 U ml^{-1}), CHX (1 mg ml^{-1}) or both. **c,** Gadd45β is not required for rapid downregulation of JNK after TNF-α treatment. MEFs from WT and *gadd45β*$^{-/-}$ mice were treated with TNF-α (1,000 U ml^{-1}) and, at the indicated times, cell lysates were resolved by SDS–PAGE and western blotted (antibody probes: antiphospho-JNK, anti-JNK; Cell Signaling, Inc). A fusion protein of glutathione-*S*-transferase and c-Jun was used as substrate in the JNK assay (bottom).

De Smaele et al. *reply* — We and others have shown that the control of TNF-α-induced apoptosis by NF-κB/Rel transcription factors involves suppression of the JNK enzyme cascade[1–3], and we have proposed that this suppression is mediated in part by Gadd45β/Myd118 (refs 1,4). Amanullah *et al.* suggest that the ablation of *gadd45β* has no effect on JNK activation and apoptosis by TNF-α and argue that the protective effects of NF-κB are mediated by factors other than Gadd45β.

However, caution is needed in drawing inferences from these provocative findings about the role of Gadd45β in the cell. Under the conditions used by Amanullah *et al.*, knockout mutation of any of the NF-κB targets identified so far[5,6] — including those of the putative JNK inhibitor XIAP (ref. 7) and of NF-κB/RelA itself[8] (our unpublished observations) — would not have affected TNF-α-induced killing. This is because cytokine treatment of fibroblasts was far too short and was performed in the absence of low doses of cycloheximide (about 0.1 μg ml^{-1}; ref. 1) , which is needed to downregulate functionally redundant factors.

Our antisense experiments[1] indicate that in certain cells, such as lymphoid cell lines, downregulation of *gadd45β* leads to exaggerated JNK signalling and apoptosis in response to TNF-α. It is likely that the pro-survival programme that is activated by NF-κB depends on tissue-specific elements[5,6], so the relevance of Gadd45β to this protective activity of NF-κB might be more marked in certain cell types. As the analysis of Amanullah *et al.* is limited to the fibroblastoid lineage, it might not be appropriate to generalize conclusions about the effects of Gadd45β on the JNK pathway and apoptosis to other cell types. We agree with Amanullah *et al.* that further investigation is needed to define the precise contribution of this factor and of other targets to the anti-apoptotic function of NF-κB.

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1. De Smaele, E. *et al. Nature* **414,** 308–313 (2001).
2. Tang, G. *et al. Nature* **414,** 313–317 (2001).
3. Javelaud, D. & Besancon, F. *Oncogene* **20,** 4365–4372 (2001).
4. Franzoso, G., Zazzeroni, F. & Papa, S. *Cell Death Diff.* **10,** 13–15 (2003).
5. Baldwin, A. S. *J. Clin. Invest.* **107,** 241–246 (2001).
6. Karin, M. & Lin, A. *Nature Immunol.* **3,** 221–227 (2002).
7. Harlin, H., Reffey, S. B., Duckett, C. S., Lindsten, T. & Thompson, C. B. *Mol. Cell. Biol.* **21,** 3604–3608 (2001).
8. Beg, A. A. & Baltimore, D. *Science* **274,** 782–784 (1996).