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cytotoxicity of MNNG. Shown in Figure 1B is a schematic incorporating the *DCD1*-related findings reported here and those from a previous report [2].

The MMR-dependent response to S_n1-type methylating agents most likely involves additional proteins besides those normally associated with MMR-dependent spellchecking (for a review, see [1]). Our study has uncovered one such protein, Dcd1, which modulates dCTP:dTTP pool levels and therefore influences sensitivity to agents that induce formation of $O^{6met}G$. Several studies with cultured rodent cells suggested that Dctd deficiency increased the dCTP:dTTP pool ratio [7–9]. However, we are not aware of any isogenic pairs of proficient/deficient cell lines to test rigorously the role of Dctd in the response to methylation damage. Of interest, however, Meuth [7] showed that elevated dTTP pools increased sensitivity to MNNG in Chinese hamster ovary cells and speculated that misincorporation of thymine opposite $O^{6met}G$ was the basis for the observed toxicity.

Finally, our findings may also have relevance to cancer chemotherapy. For example, reduced DCTD levels in a tumor might compromise the clinical response to the S_n1-type methylation agent temozolomide or the purine analog 6-mercatopurine, used in the treatment of glioblastoma multiforme [10] and certain hematological malignancies [11], respectively.

Supplemental data

Supplemental data are available at http://www.current-biology.com/cgi/content/full/17/17/R755/DC1

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Horizontal gene transfer of two cytoskeletal elements from a eukaryote to a cyanobacterium

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The concept of horizontal gene transfer (HGT) as a potent evolutionary force has prompted the re-evaluation of prokaryotic genome shaping and speciation. Horizontal gene transfer enables prokaryotes to rearrange their genomes dynamically, facilitating responses to changing environmental conditions and invasions of new ecological niches. Here we report that the genome of the cyanobacterium *Microcystis aeruginosa* contains a genomic island encoding proteins with extensive amino-acid sequence identity to two components of the eukaryotic actin cytoskeleton: actin itself; and profilin, an actin binding protein hitherto only known in eukaryotes. Our data indicate that a rare eukaryote-to-prokaryote HGT has introduced both sequences into the *Microcystis* lineage. We found both genes to be actively expressed and propose a unique role in *Microcystis* cell stabilization for actin, differing substantially from what is observed for bacterial actin homologs. Because we detected both eukaryote-like genes only in one strain in culture and in recent samples collected from its original habitat we suggest that both proteins may contribute to the adaptation of this strain to its specific ecological niche.

Eukaryotic actin and profilin from *M. aeruginosa* (ActM and PfnM, respectively) are encoded ~280 nucleotides apart from each other. Top-scoring BLASTp hits both for

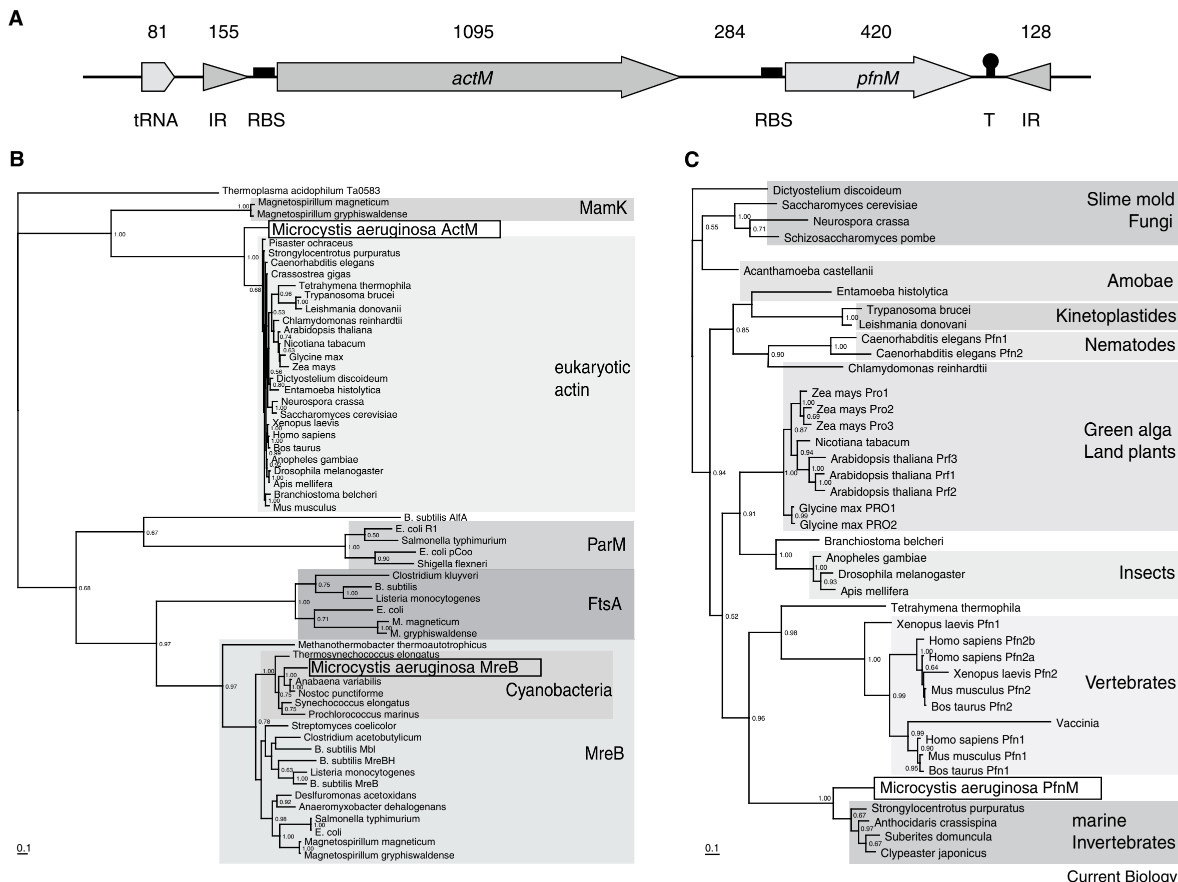

Figure 1. Overview of actin and profilin sequences.

(A) Schematic representation of the genomic context of *actM* and *pfnM*. Ribosome binding sites (RBS), inverted repeats (IR), the tRNA gene (tRNA) and a transcription terminator (T) are indicated. Numbers refer to nucleotide lengths. Phylogenetic trees of actin (B) and profilin (C). Posterior probability values above 0.5 are given. Phylogenetically related sequences are shaded and labeled. *Microcystis aeruginosa* is highlighted with framed boxes.

ActM and PfnM are eukaryotic proteins whose amino-acid sequence identities amount to 65% and 57%, respectively (see Table S2 in the Supplemental data available on-line with this issue). According to proposed methods for detecting HGT, these values indicate that both proteins have not arisen in *Microcystis* but were transferred from a eukaryote [1]. These values are significantly higher than corresponding percent identities between tubulin and its homologs BtubA/B (~35%) which are believed to be the result of an HGT from a eukaryote into the bacterial genus *Prosthecobacter* [2]. The *actM–pfnM* region is flanked by short inverted repeats and is closely associated with a leucine tRNA encoded directly upstream (Figure 1A). This organization is reminiscent of genomic islands, bacterial mobile genetic elements known to drive the horizontal spread of advantageous clusters of functionally related genes across species barriers [3] and is a further indication of the involvement of this region in an HGT. Notably, the bacterial actin homolog MamK also is encoded on a genomic island involved in HGTs, conferring the trait of magnetotaxis [4]. Because genomic islands rapidly spread through bacterial communities, *Microcystis* was not necessarily the primary recipient of the ancestral eukaryotic sequences and might have acquired the *actM-pfnM* genomic island from some other bacterium. But this does not refute the view that both sequences ultimately are of eukaryotic origin.

To elucidate the ancestry of ActM and PfnM we constructed phylogenetic trees with actins and profilins from a range of organisms (see Supplemental experimental procedures). The actin tree reflects the widely accepted classification of actin homolog subfamilies and shows a common ancestry for ActM and eukaryotic actins (Figure 1B). Because of their close evolutionary distances we could not single out one distinct eukaryotic actin sequence as the nearest relative of ActM, but phylogenetic analyses of ActM and a broad range of eukaryotic actins suggest a shared ancestry for ActM and marine invertebrate actins (Figure S1). This finding is consistent with results from the profilin phylogenetic analysis where PfnM also clusters with profilins from marine invertebrates (Figure 1C), suggesting that representative(s) of this taxon can be regarded as the donor(s) of *actM* and *pfnM*.

Screening a selection of *Microcystis* strains for *actM* and *pfnM* by PCR and DNA–DNA hybridization analyses, we found

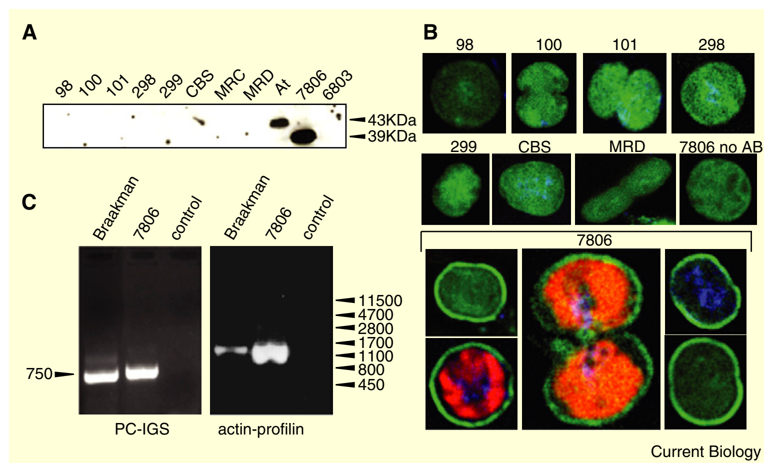

Figure 2. Presence and *in situ* localization of actin in strains of *Microcystis aeruginosa*.
(A) Immunodetection of eukaryotic actin. *Arabidopsis thaliana* ('At') and *Synechocystis* sp. PCC 6803 ('6803') were used as controls. Strains are indicated above images. (B) Immunofluorescence micrographs of *Microcystis* strains. Actin was stained using an anti-actin primary antibody and a green fluorescent FITC coupled secondary antibody. DNA is stained blue, red and green autofluorescence of *Microcystis* is also displayed. Cells denoted '7806 no AB' emit green autofluorescence only. Strains are indicated above respective images. (C) PCR of field samples from the original habitat of *Microcystis aeruginosa* PCC 7806 ('Braakman'). PCC 7806 and water controls were prepared in parallel. Specific primers show the presence of cyanobacterial DNA ('PC-IGS' [10]). *ActM–pfnM* specific primers yielded the expected fragment ('actin–profilin'). A base-pair size marker is shown.

that these sequences were only present in the strain PCC 7806 (Figures S2,S3). Accordingly, immunodetection with an antibody against eukaryotic actin confirmed the presence of actin in that strain only (Figure 2A). Immunofluorescence microscopy confirmed these results and revealed an *in situ* distribution of ActM towards the cell's periphery in a ring-like manner, suggesting that ActM forms a shell-like structure in PCC 7806 (Figure 2B). Intriguingly, PCR analyses performed with cyanobacterial field samples from the habitat from which PCC 7806 had been isolated originally [5] yielded appropriately sized PCR amplicons using primers spanning the *actM–pfnM* genomic region (Figure 2C). These findings suggest that *actM–pfnM* has been introduced into the *Microcystis* lineage after the separation of extant strains and show that these sequences are able to persist in a natural environment and have therefore not arisen as a consequence of laboratory culturing. *ActM* and *pfnM* apparently confer some advantage restricted to the original habitat which has a history of repeated changes in water salinity. We therefore speculate that this habitat calls for adaptations to elevated osmotic stress in the otherwise freshwater preferring *Microcystis*. The shell-like structure formed by ActM would be suitable to adopt a cell-supporting function since it differs significantly from the cellular distribution of prokaryotic actins in other bacteria [6–9]. Apparently the bacterial actin MreB does not co-localize with ActM in PCC 7806 (Figure S4). An interaction of ActM with its cognate eukaryotic binding partner PfnM (Figure S5) might enable the formation of a "shell". Since bacterial actins do not bind profilin, MreB and ActM may have distinct and/or complementary functions in PCC 7806.

Supplemental data
Supplemental data are available at http://www.current-biology.com/cgi/content/full/17/17/R757/DC1

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