Copper chaperones in bacteria: association with copper-transporting ATPases

The recent *TIBS* article by Harrison *et al.*[1] discussed the cellular role of copper (Cu) chaperones in eukaryotes and briefly mentioned the presence of a Cu chaperone, CopZ, in *Enterococcus hirae* and related mercury-binding proteins in other bacteria. The importance of Cu chaperones in delivering Cu to Cu-dependent enzymes, while protecting the intracellular milieu from Cu toxicity[2,3], suggests that they should be universally present in microbial cells. To check whether this is the case, we examined the distribution of CopZ-like heavy-metal-associated (HMA) domains[4] among the organisms with completely sequenced genomes. Indeed, a number of microbial genomes were found to encode stand-alone HMA domains (Cu chaperones) (Fig. 1). Remarkably, in a number of distantly related microorganisms, *copZ* genes were located adjacent to the genes encoding corresponding metal-transporting ATPases, which contain HMA domains on their N termini, presumably forming operons (Fig. 1). The diversity of the operon organization, as well as the lack of sequence similarity of each particular Cu chaperone to the adjacent HMA domains of Cu-transporting ATPases, suggests that Cu chaperones evolved independently from the Cu-transporting ATPases. It is known that operons have relatively short evolutionary half-lives, and few operons are conserved in phylogenetically distant organisms[5]. Thus, the presence of intact *copZ*–Cu-transporting ATPase operons in such a diverse set of microorganisms suggests strong selective pressure to maintain co-regulation of Cu chaperones and Cu-transporting ATPases, probably to ensure the presence of protective Cu chaperones in the cytoplasm, even before the ATPase can start importing metal ions. It should be noted that we also found some organisms (*Synechocystis* sp., *Neisseria meningitidis*, *Aeropyrum pernix*) in which these genes were unlinked (Fig. 1). In these cases, *copZ* genes can be easily overlooked in genome annotation (e.g. in *Aquifex aeolicus*). Certain microorganisms with smaller genomes, such as *Borrelia burgdorferi*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Mycoplasma genitalium*, *Mycoplasma pneumoniae* and *Rickettsia prowazekii*, do not encode either Cu chaperones or Cu-transporting ATPases, which is probably due to their parasitic lifestyle. Finally, some genomes (*Escherichia coli*, *Mycobacterium tuberculosis*) appear to encode Cu-transporting ATPases but not Cu chaperones. In the *E. coli* case, this puzzle has been resolved by the observation[6] that these cells still contain substantial amounts of the 6.5 kD N-terminal fragment of the Cu-transporting ATPase YbaR (ATCU_ECOLI), corresponding to its HMA domain. Assuming the genomic sequence is correct, formation of the Cu chaperone in *E. coli* must be achieved by a specific proteolysis of ATCU_ECOLI. Similar mechanisms of Cu chaperone formation by proteolytic separation of the N-terminal HMA domains can be imagined for other organisms that do not appear to have specific chaperone-encoding genes.

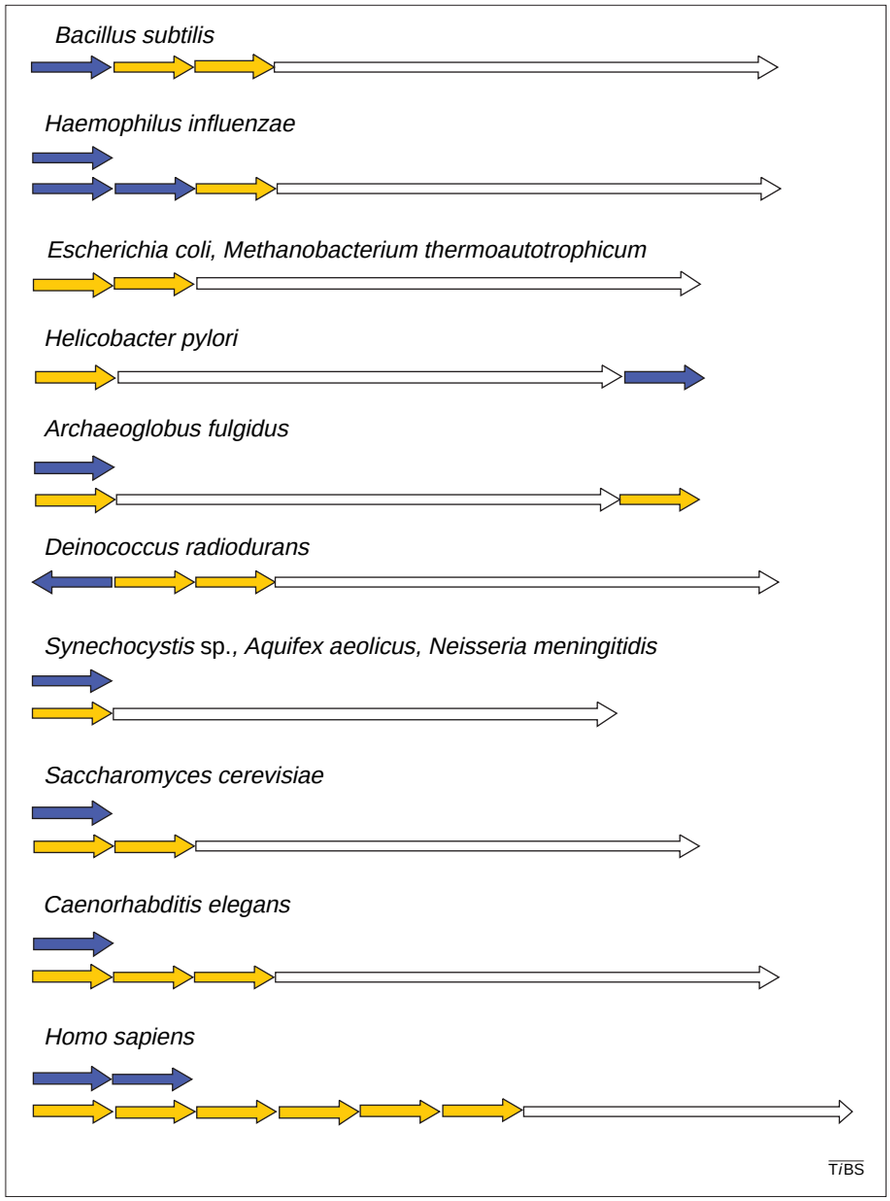

Figure 1

Association of genes encoding copper chaperones and copper-transporting ATPases. Stand-alone heavy-metal-associated (HMA) domains (copper chaperones) are indicated by short blue arrows, HMA domains of copper-transporting ATPases are indicated by yellow arrows; the remaining portions of these ATPases (not drawn to scale) are unshaded. Copper chaperone genes that are not linked to the ATPase genes are shown on separate lines. The following chaperones are shown: *Bacillus subtilis*, YvgY; *Haemophilus influenzae*, HI1050, HI0292 and HI0291; *Helicobacter pylori*, HP1073; *Archaeoglobus fulgidus*, AF0346; *Deinococcus radiodurans*, DR2452; *Aquifex aeolicus*, aq_1840a; *Synechocystis* sp., ssr2857; *Neisseria meningitidis*, NMB1271; *Saccharomyces cerevisiae*, ATX1; *Caenorhabditis elegans*, CUC1. In humans, copper chaperone CCS contains two HMA domains, and the copper-transporting ATPases (Wilson and Menkes proteins) contain six HMA domains each.

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Domains in gene silencing and cell differentiation proteins: the novel PAZ domain and redefinition of the Piwi domain

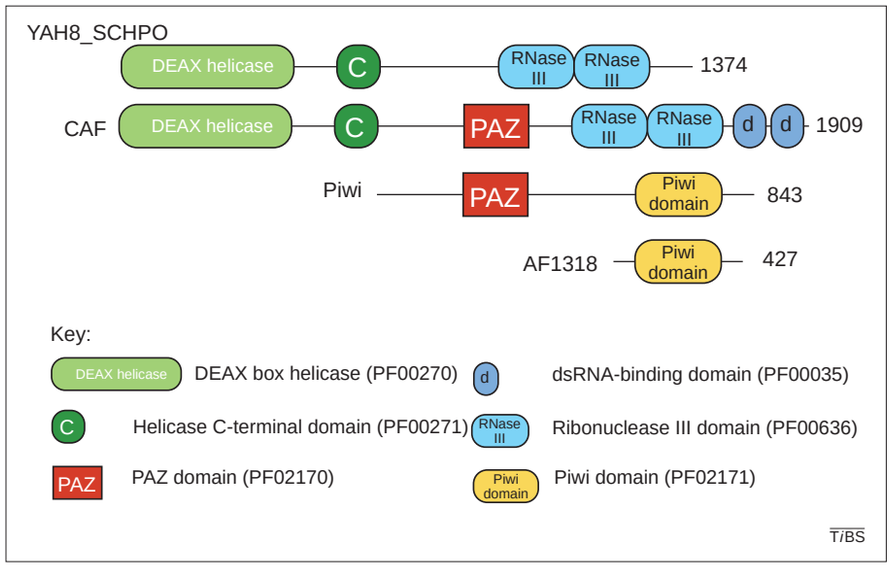

Figure 1
The domain organization of the CAF and PIWI protein family. The Pfam accession number of each domain is given in the key (http://www.sanger.ac.uk/Software/Pfam/).

RNA interference (RNAi) refers to the sequence-specific silencing of a gene by its homologous double-stranded RNA (dsRNA). This phenomenon has been observed in a wide range of eukaryotic organisms, including protozoa, *C. elegans*, *Drosophila* and vertebrates[1,2]. Although RNAi is widely used as a technique to inactivate genes[3], little is known about its biological mechanism.

RNAi could represent an ancient antiviral defence mechanism and a possible system to inhibit transposon mobility by maintaining low levels of transposon transcripts[4–6]. Permanent modification of the gene or disruption of its transcription has been experimentally excluded for RNAi[7], and it is now generally accepted that silencing results from the degradation of the mRNA.

In *N. crassa*, the introduction of transgenes can also induce reversible gene silencing without modification of the genomic DNA by a phenomenon called quelling[8]. Similarly, post-transcriptional gene silencing (PTGS) in plants induces gene silencing in the presence of transgenes and in response to viral RNA[4,9]. Genomic DNA methylation has frequently been correlated with the occurrence of PTGS and depends on the prior accumulation of ~25-nt antisense RNA[10,11].

Isolation of mutants from *N. crassa*, *D. melanogaster* and *C. elegans* has shed some light on the possible molecular mechanisms involved in RNAi/PTGS/quelling. The *N. crassa qde-1* gene encodes an RNA-dependent RNA polymerase (RdRP) essential for quelling[12]. RNAi and PTGS both produce small ~25-nt RNAs, and it has been suggested that the RdRP amplifies these small RNAs[11,13,14]. Possible QDE-1 homologues have been found in *Arabidopsis*, *S. pombe* and *C. elegans*, and, recently, a *C. elegans* RdRP, called EGO-1, has been implicated in RNAi and the development of germline cells[15].

The QDE-2 protein of *N. crassa* and its homologue in *C. elegans* (RDE-1) are essential for quelling[5,8]. They belong to an evolutionary conserved family of proteins implicated in stem-cell maintenance, suggesting, together with the dual role of *C. elegans* EGO-1, that cell-fate determination and RNAi might be achieved by similar mechanisms[15,16]. This family comprises *Drosophila* PIWI and STING, human HIWI, *Arabidopsis* Argonaute 1 and Zwille/Pinhead, and *C. elegans* PRG-1 and PRG-2[17]. Cox and collaborators showed that the central and C-terminal region of these proteins are well conserved and defined an ~40-amino-acid conserved C-terminal region, called the Piwi box[18].

We have found that the Piwi box is part of a larger 300-amino-acid domain (Figs 1,2a), which we called the Piwi domain. Analysis of this region suggested that prokaryote homologues exist, including AF1318 from the archaeabacteria *Archaeoglobus fulgidus*. To verify this, we used PSI-BLAST[19] at the NCBI with AF1318 as query protein (with $E = 0.01$ inclusion threshold). We identified two bacterial proteins containing a Piwi domain in the first round: HYP from *Aquifex aeolicus* and SC3D9.08c from *Streptomyces coelicolor*. In the second round we found another archaeal protein, MJ1321 from *Methanococcus jannaschii*, as well as the *Drosophila* PIWI protein and its eukaryote homologues (Figs 1,2a). This demonstrates that the Piwi domain is not restricted to eukaryotes, but is also found in prokaryotes. The role of prokaryotic proteins containing the Piwi domain is unknown, but it will be of great interest to address the question experimentally if they have a role similar to the PTGS observed in eukaryotes.

A protein containing the Piwi domain (Fig. 2a) and a RdRP protein[12] are found in *S. pombe*, suggesting that a PTGS mechanism might be present in this organism. Interestingly, neither RdRPs nor proteins containing the Piwi domain are found in the *S. cerevisiae* genome, indicating that these proteins are absent or that they have highly diverged.

By running a PSI-BLAST (with $E = 0.01$ inclusion threshold) with the central region of the *Drosophila* PIWI protein (residues 200–400), which is common to all members of the PIWI family, we identified a region of similarity in the carpel factory (CAF) protein from *A. thaliana* by round three. CAF appears to suppress cell division in floral meristems, but it seems to be expressed ubiquitously throughout the shoot tissues, indicating a more general role of the protein[20]. We termed

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