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# Bacterial silver resistance: molecular biology and uses and misuses of silver compounds

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**Abstract**

Resistance to silver compounds as determined by bacterial plasmids and genes has been defined by molecular genetics. Silver resistance conferred by the *Salmonella* plasmid pMGH100 involves nine genes in three transcription units. A sensor/responder (SilRS) two-component transcriptional regulatory system governs synthesis of a periplasmic Ag(I)-binding protein (SilE) and two efflux pumps (a P-type ATPase (SilP) plus a three-protein chemiosmotic RND Ag(I)/$H^+$ exchange system (SilCBA)). The same genes were identified on five of 19 additional IncH incompatibility class plasmids but thus far not on other plasmids. Of 70 random enteric isolates from a local hospital, isolates from catheters and other Ag-exposed sites, and total genomes of enteric bacteria, 10 have recognizable *sil* genes. The centrally located six genes are found and functional in the chromosome of *Escherichia coli* K-12, and also occur on the genome of *E. coli* O157:H7. The use of molecular epidemiological tools will establish the range and diversity of such resistance systems in clinical and non-clinical sources. Silver compounds are used widely as effective antimicrobial agents to combat pathogens (bacteria, viruses and eukaryotic microorganisms) in the clinic and for public health hygiene. Silver cations ($Ag^+$) are microcidal at low concentrations and used to treat burns, wounds and ulcers. Ag is used to coat catheters to retard microbial biofilm development. Ag is used in hygiene products including face creams, 'alternative medicine' health supplements, supermarket products for washing vegetables, and water filtration cartridges. Ag is generally without adverse effects for humans, and argyria (irreversible discoloration of the skin resulting from subepithelial silver deposits) is rare and mostly of cosmetic concern.


*Keywords:* Silver resistance; *sil* gene; Silver-binding protein; Plasmid resistance; Argyria; Silver sulfadiazine; Burn infection

**Contents**



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## 1. Introduction

The mechanisms of resistance to heavy metals that are encoded by various plasmid-based genes have been thoroughly studied [1–3] and are considered in various articles is this issue of *FEMS Microbiol. Rev.* (e.g. [5–8]). The best understood such system is that for resistance to inorganic mercury and organomercurials [3,4,8]. The chemical basis of mercury resistance is enzymatic cleavage of the Hg–C bond of organomercurials by the enzyme organomercurial lyase, to release Hg(II), followed by reduction of Hg(II) to volatile Hg(0) by the flavoprotein mercuric reductase [3,8], for which one structure was solved by X-ray crystallography [9]. This X-ray-derived structure of mercuric reductase plus the crystal structures of three arsenate reductases [10–13], arsenite oxidase [14], a cadmium-responding transcriptional repressor protein [15] and the nuclear magnetic resonance (NMR) solution of a periplasmic mercury-binding protein [16] are the first protein structures for the several dozen different proteins involved in various metal ion resistance systems [1–8]. This is a rapidly growing list. No silver resistance-related protein has a solved structure to date, although it is expected that such structures will increase the understanding of function and metal ion specificity.

Most toxic heavy metal resistances result not from chemical detoxification, but from energy-dependent ion efflux from the cell by membrane proteins that function either as ATPases or as chemiosmotic cation/proton antiporters [1–3,5,17]. Ag(I) resistance has become a new example of such efflux pumping and is the first co-transcribed resistance system that has both classes of efflux pumps [18]. While mechanistic studies of the silver resistance proteins are currently unavailable and we have published preliminary summaries of on-going work [36–38], this is the first in depth review of the molecular genetics of silver resistance. Silver-resistant bacteria have frequently been reported [19–29], but these initial reports have generally not been followed by further work. Silver bioaccumulation by microbes has been occasionally reported [30–35]. The relationship between resistance and accumulation was not clear [32–35]. We have previously summarized preliminary understanding of genetically determined bacterial silver resistance [36–38]. Clement and Jarrett [39] provided a careful review of the antimicrobial actions of silver compounds.

Silver-resistant bacteria have been found repeatedly in environments where silver toxicity might be expected to select for resistance, in particular from burn wards of hospitals where silver salts (silver nitrate but especially silver sulfadiazine) are used as antiseptics to treat burns [19,40–44,125] (see below). The wide variety of other environments where silver is found and/or used has recently been reviewed [38,39]. These include clinical use of solid silver or silver-coated catheters [45–48], silver-coated wound bandages [49–52,126], polluted soil around mines [24,30,31], water catchment associated with photographic film production and processing [23], institutional water distribution systems [53–55] where metal compounds are used for control of infectious agents such as *Legionella*, and as presumedly beneficial components of health food supplements [56–59]. Silver-containing consumer products include silver-coated mints ('Jintan') in Japan, Ag(I)–citrate complexes as health food additives in Florida, domestic water purification cartridges in the USA ('Brita'), and supermarket-available colloidal 'silver-gelatinate' for washing salad vegetables in Mexico ('Microdyn'). The most common human exposure to Ag is with dental amalgams, which contain 35% Ag [60,61]. Since the other major component of amalgams, Hg(0), is slowly released into nearby tissues, the gut and maternal milk [61–63], it seems likely that Ag is also released. It seems probable that Ag will remain more localized and not be 'bio-active' in the gut as is Hg. The released Hg(0) is oxidized to soluble Hg(II), which then selects for Hg-resistant bacteria [61,62]. It seems possible that a similar release of Ag(0) from the amalgams followed by oxidation to Ag(I) occurs, but this has never been measured.

Silver is familiar in laboratory use as a stain for proteins in polyacrylamide gels. There are uncertainties about the details, but the silver in stained gels is reduced polymeric Ag(0) [64]. Initially Ag(I) binds to denatured protein, primarily to histidine residues. This is followed by stabilization of the polymeric Ag center, with multiple Ag reduction events [64].

## 2. Bacterial resistance to silver and silver compounds

Conditions for distinguishing silver-resistant from silver-sensitive bacteria are not well-known and even the existence of silver-resistant bacteria that cause a clinical problem is repeatedly challenged. Halide ions that act as precipitating agents and proteins and other biological Ag(I) ligands profoundly affect the 'bioavailability' of Ag(I). Earlier and more recent experiments [65] suggest three levels of effects: firstly at low halide (usually chloride), especially in the clinic and in external environments, soluble Ag(I) binds tightly to the bacterial cell surface, inhibiting respiration and having other toxic effects [66–68]. Moderate levels of chloride remove the Ag as precipitated AgCl. Paradoxically, higher levels of $Cl^-$ bring the silver back into solution as a 'bioavailable' anion, $AgCl_2^-$, increasing the Ag(I) sensitivity of sensitive bacteria while

making the difference between susceptibility levels for sensitive and for resistant bacteria greater [65]. $Br^-$ has a similar effect to $Cl^-$, but functions at lower concentrations reflecting the lower solubility of AgBr compared with AgCl [65], and $I^-$ basically removes Ag(I) into a non-bioavailable 1:1 precipitate.

Less familiar to most readers is that Ag(I)–halide precipitates come back into solution at higher halide concentrations, by forming water soluble anionic complexes ($AgX_2^-$ and $AgX_3^{2-}$), with relative stabilities $I^- > Br^- > Cl^-$. The water soluble anionic Ag–halide complexes appear to be more bioavailable, and high halide levels increase Ag(I) toxicity to both sensitive and resistant bacteria [65]. A note of warning: colonies of bacteria exposed to Ag(I) show black pigmentation that is likely to be reduced metallic Ag(0); however, silver reduction does not occur during growth but rather after growth is complete. It is thought that post-growth respiratory chain reduction of Ag(I) to Ag(0) is not related to silver resistance.

### 2.1. Molecular genetics of silver resistance

Plasmid pMG101 [19] is a 180-kb IncH1 silver resistance plasmid [69] that also confers resistance to mercury and tellurite, and to several antibiotics. The Ag(I)-resistant *Salmonella* strain from which pMG101 was isolated resulted in the death of several patients and required closing of the burn ward at the Massachusetts General Hospital [19]. Although silver sulfadiazine-resistant bacteria have occasionally been observed elsewhere in burn ward infections, these resistances have not been followed with further research. The region of pMG101 that determines increased resistance to Ag(I) was cloned and sequenced (GenBank accession AF067954) [18]. The gene cluster for silver resistance contains a total of nine genes, seven of which were named and the two less-recognized open reading frames are still called ORFs: in order *silP ORF105 silAB ORF96 silC silSR silE*.

On the right of the silver resistance determinant (Fig. 1A), the first gene *silE* encodes a periplasmic Ag(I)-binding protein (SilE) (Fig. 1B). Two parallel membrane Ag(I) efflux pumps (SilCBA and SilP) are encoded. The central six genes (*silA* through *silS*) produce products that are closely homologous to a gene cluster on the *Escherichia coli* genome (previously called *ybdE*, *ylcD*, *ylcC*, *ylcB*, *ylcA* and *ybcZ*, in order, but renamed [69] *agr* (for Ag(I) resistance) once this phenotype was determined (Fig. 2) [69,70]. SilE is a small periplasmic protein that is 47% identical to PcoE, of the *E. coli* plasmid copper resistance system [18,71]. The SilE polypeptide has its first 20 amino acids removed on movement across the membrane to the periplasm [72] and is synthesized only during growth in the presence of Ag(I) [73]. *silE* and *pcoE* DNAs and transcriptional promoters and regulatory sites (Fig. 1A) are homologous [18,71,73] but the sequences upstream of *silE* and *pcoE* are transcribed separately [73].

Upstream from *silE* are the *silRS* genes for a presumed two-component signal transduction pair, consisting of a membrane kinase sensor SilS and a transcriptional regulatory responder, SilR, homologous to other two-component family pairs [74,75]. The deduced SilRS sequences are most closely related to a sensor/responder pair that was encoded in the final segment of the *E. coli* chromosome sequence to be sequenced [18,69,70]. The copper resistance Pco system includes genes for a two-component regulator, *pcoRS*, upstream of *pcoE* [71,74]. The presence of these three pairs of paralogous genes (and proteins) in silver and copper resistance determinants provides the first suggestion that the *sil* Ag(I) resistance system may have evolved from an earlier existent *pco* copper resistance system.

Upstream of *silRS*, the orientation of the genes and the functions of the gene products of the *sil* silver resistance system are unrelated to those of the *pco* copper resistance system. The six *sil* genes are transcribed divergently from *silRSE* (Fig. 1A). The *silCBA* genes determine a three-polypeptide membrane potential-dependent RND class cation/proton antiporter with homologs in the cadmium, zinc and cobalt resistance system (Czc) of *Ralstonia* and the multi-drug Acr resistance system of *E. coli* [5,79]. The components of this presumed Ag(I) efflux system are (a) the large 1048-amino acid inner membrane proton/cation antiporter, SilA, for which the homologous AcrB protein was recently solved crystallographically [79] and seen to form a trimer in the membrane with a membrane domain and an equally sized domain in the periplasmic region that forms a cavity/pore/funnel pathway for the substrate (here $Ag^+$ cations) from the cytoplasmic region directly to the outer membrane protein, SilC (Fig. 1B) [5,79]. This assures movement across the periplasmic space of Gram-negative bacteria and directly to the outside of the cell [5,79] without release into the periplasmic space. The third protein, SilB, belongs to a paralogous class of 'membrane fusion proteins' that anchor into the inner membrane and connect to the outer membrane protein, SilC. Three-protein membrane potential-driven cation/proton exchangers were first recognized in our laboratory with a bacterial $Cd^{2+}$/$Zn^{2+}$/$Co^{2+}$ system [76–78] and are now called RND systems as they affect resistance, nodulation in *Rhizobium* and cell division in *E. coli* [5]. Between *silC* and *silB*, a small ORF occurs that could determine a polypeptide of 96 amino acids (Fig. 1A), which would be 45% identical to the product of a 110-amino acid long ORF in the *E. coli* chromosomal homolog (Fig. 2) [69,70]. This ORF had been called *agrF* by D.H. Nies for Ag resistance, a term we have retained [69], although Nies [5] has renamed these *E. coli* genes *cus* for Cu Ag resistance, as copper resistance can also be demonstrated in some strains with additional mutations [5,6]. In any case, the small polypeptide may also be periplasmic. This *agrF* or *cusF* gene has not been found in other RND/CBA systems. Another ORF, potentially encoding a protein of 105 amino acids in length,

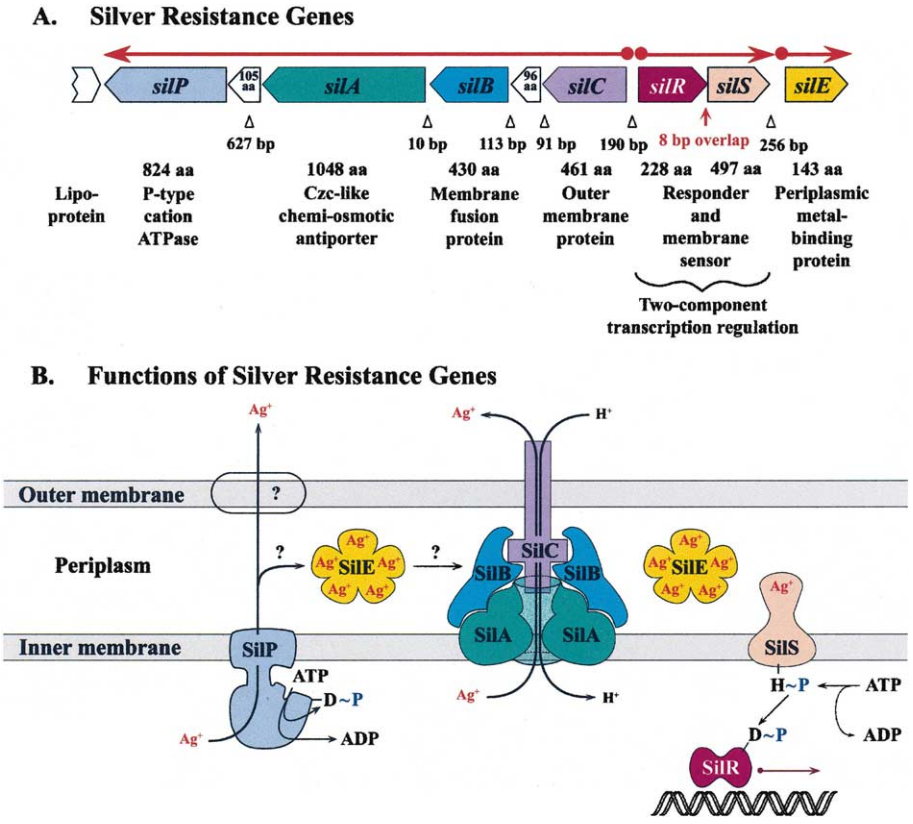


Fig. 1. Silver resistance genes, transcripts and protein products. A: Top line shows the mRNAs. The open boxes indicate different genes or ORFs and their orientations. Nucleotides (nt) between genes and the sizes of gene products in amino acids (aa) are marked. B: The proposed function of each gene product, deduced from homologies to known proteins (modified from [18]).

occurs between *silA* and *silP* (Fig. 1A), but the product of this ORF lacks known homologs [69]. The function of the last silver resistance gene product, SilP, can be recognized by homology to other gene products that have been studied as a membrane P-type ATPase that probably pumps Ag(I) from the cell cytoplasm to the periplasmic space (Fig. 1B) [18,80–83]. How periplasmic Ag(I) is removed is unclear and Fig. 1B shows both the possibility of movement through an un-specified outer membrane protein and the possibility of sequestering by SilE, followed or not by movement across the outer membrane via the SilCBA complex. SilP is most similar to $Cu^{+}$ and $Zn^{2+}$ efflux ATPases [82–84] encoded on the chromosome of *E. coli* [6]. The silver resistance system is the first time when we have seen three different resistance mechanisms (a periplasmic multi-metal-binding protein, a chemiosmotic efflux pump and an ATPase efflux pump) encoded in a single toxic metal cation resistance gene cluster.

The silver resistance system appears to be transcriptionally controlled by the products of two genes, SilS (a histidine-containing membrane ATP kinase 'sensor') and SilR (a cytoplasmic DNA-binding activator 'responder' that contains an aspartate residue that is *trans*-phosphorylated from SilS; Fig. 1B). SilRS are homologous in sequence to members of the large family of two-component sensor/responder transcriptional regulators that respond to extracellular signals [74]. With the five additional IncH plasmid *sil* resistance determinants [69], *silE*, *silP* and *silS* DNA sequences were obtained and compared with those from plasmid pMG101. Each of the three genes in each of the six plasmids seems to have a different history (that is different degrees of sequence relatedness) but to be closely related. For *silE*, plasmid pWR23 has a 4.5% difference in nucleotides (6.3% difference in amino acid positions), whereas the other five plasmids had identical or near-iden-

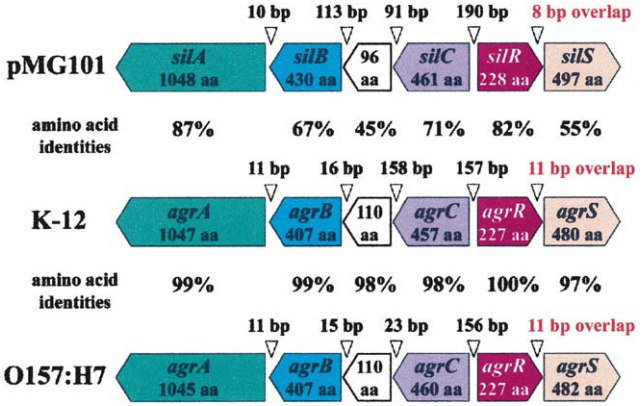


Fig. 2. Relationship between silver resistance genes on bacterial plasmid pMG101 and on the chromosome of *E. coli* strains K-12 and O157:H7 (adapted from [69]).

tical *silE* genes. For the *silP* gene, plasmid R476b was most different from the others with about 4% different nucleotides [69]. And for the *silS* gene, the five additional IncH plasmids showed about 4% nucleotide differences from pMG101 and each appears relatively distinct from one another. This range of 0–4% divergence in DNA sequence for the IncH plasmid *sil* genes is nevertheless less than the 13–55% divergence in sequence between the IncH *sil* genes and the *E. coli* chromosomal *agr* homologs [69] (Fig. 2).

Transcription of the silver resistance determinant was measured by reverse transcriptase-polymerase chain reaction (PCR), Northern blot RNA/DNA hybridization and primer extension analysis [18,73]. Three mRNAs are synthesized, one each for *silE*, *silRS* and *silCBAP*, as indicated in Fig. 1A. Their inducibility (by Ag(I)) and precise start sites were determined [73]. Although oriented in the same direction, *silE* was not co-transcribed with *silRS*, which were transcribed as a single mRNA [73]. A single 9.5-kb transcript included genes *silC* through *silP* [73] (Fig. 1A). Primer extension experiments established the precise mRNA start points immediately upstream of *silE*, *silR* and *silC* [18].

The chromosomes of both *E. coli* strains K-12 and O157:H7 have six gene regions listed in GenBank accession AE000162 (for strain K-12) that are very closely homologous to the plasmid silver resistance *silAB ORF96 silCRS*. We have called these genes *agrAB ORF110 CRS* [69] (Fig. 2) and Nies [5] has renamed these *cus* for Cu and Ag resistance. Disruption of *agrA* leads to hypersensitivity to Ag(I) but not to other cations tested [69,70], indicating a role in Ag(I) resistance. Cu(II) was tested in these growth experiments but not Cu(I) and the experiments were run under aerobic conditions, as usual for *E. coli*. Chromosomal mutations of clinical strains to Ag(I) resistance (of unknown relationship to the *agr* silver resistance determinant) may also cause a problem in infection [85]. Since these chromosomal systems function by Ag(I) efflux [85], they may indeed result from mutations in the *sil*-related *agr* systems (Fig. 2) [69].

Following the availability of the DNA sequence of plasmid pMG101 in Fig. 1, whether additional bacteria from clinical sources (that either had known silver exposure or not) might contain similar Ag(I) resistance determinants and DNA sequences was tested. The results to date indicate that homologous DNA sequences can be identified by Southern blotting (DNA/DNA hybridization; Fig. 3; A. Gupta et al., in preparation) and PCR (in vitro DNA synthesis) analysis (Fig. 4; A. Gupta et al., in preparation) and are found in many hospital isolates of a wide range of enteric bacterial species.

In Southern blotting DNA/DNA hybridization and PCR analysis of clinical isolates with homologous DNA, the central six genes (*silA* through *silS*; Fig. 1) appear always to be present together, but homologs of the outer two genes, *silP* and *silE*, are occasionally missing [69] (Fig. 4; A. Gupta, et al., in preparation). For example for the 10 out of 70 random enteric isolates from our local University of Illinois hospital clinical labs (P.C. Schreckenberger, personal communication) that scored strongly positive for the presence of *silA* by slot blotting (Fig. 3). Strains 30 and 32 both produced PCR products for *silA*, *silS* and *silP*, while strain 30 but not 32 produced a PCR product for *silE* (Fig. 4). Similarly, strains 55, 62, 63, 64 and 66 produced PCR products for *silA* and *silP*, but strain 55 failed to produce a *silE* product and strains 55 and 65 failed to produce *silP* products (Fig. 4). It was consistently found (A. Gupta et al., in preparation) that the central six *sil* genes, *silA* through *silS* (Figs. 1 and 2), were always present or absent together, but that the outer two genes *silP* and *silE* might be absent separately. The physiological and phenotypic effects of these missing genes need to be studied.

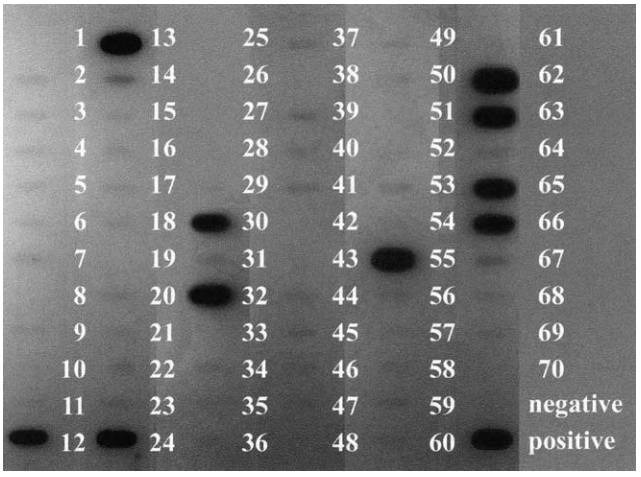


Fig. 3. DNA/DNA hybridization 'slot blotting' with a radioactive *silA* gene probe and total cellular DNA from 70 random enteric bacteria from the University of Illinois Chicago Hospital (from A. Gupta et al., in preparation).

The deduced product of the final gene of the silver resistance determinant, on the left of Fig. 1A, is an 824-amino acid P-type ATPase, SilP. A deletion of DNA in the middle of *silP* results in reduced silver resistance by the bacterial cells [18]. SilP belongs in the family of heavy metal resistance efflux ATPases [3,18,81–83]. The SilP sequence contains all the specific features of this group of P-type ATPases, including (a) the conserved region around the phosphorylated aspartyl residue, (b) the ATP-binding region, (c) the aspartyl-phosphatase determinant, (d) the CysProCys conserved in the predicted sixth transmembrane α-helical region (thought to be part of the cation translocation pathway), and (e) the HisPro between the phosphorylation site and the ATP-binding region in the large aspartyl kinase domain [3,5–7,81]. There is one striking difference between deduced SilP and most earlier described soft metal efflux ATPases. The previously described cadmium [1–3], zinc [82] or copper [3,80,83] efflux ATPases (of animals and bacteria) generally have

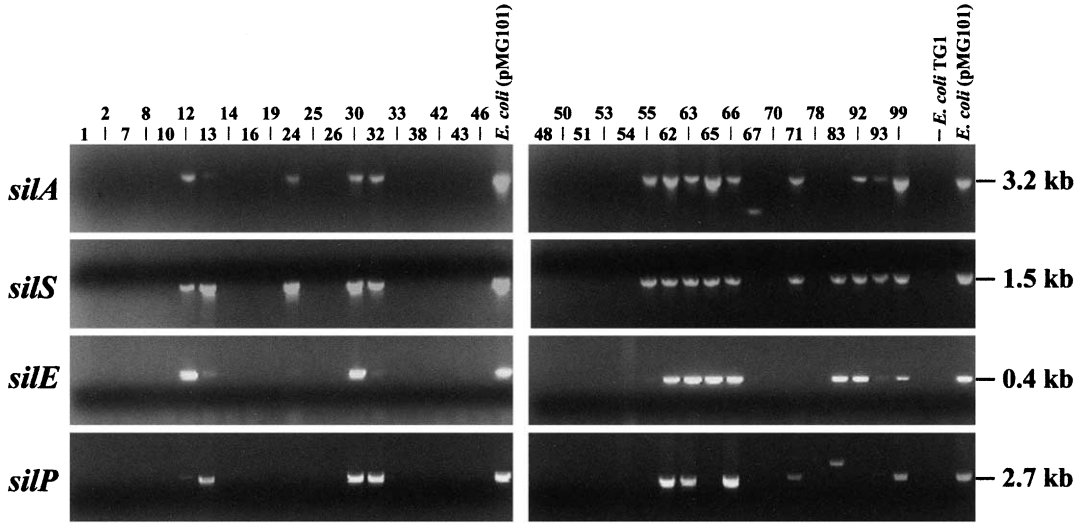


Fig. 4. PCR amplification *silA*, *silS*, *silE* and *silP* gene-specific primers and total cellular DNA from 39 University of Illinois Hospital enteric bacteria used in Fig. 3. PCR-amplified DNA was analyzed by agarose gel electrophoresis, and stained with ethidium bromide and photographed (from A. Gupta et al., in preparation).

sequences including GlyMetXCysXXCys towards the N-terminus and apparently in the cytoplasmic region [1–3,81]. These sequences are thought to provide cation binding for transport or its modulation. SilP lacks this motif, although eight cysteines are found in the N-terminal 200 amino acids of SilP, including two $CysX_{2\text{ or }3}Cys$ vicinal cysteine pairs. There is no direct evidence that these are involved in cation binding. In addition, 17 histidine residues are present in the N-terminal 200 residues of SilP, including a $His_5AspHis_2$, also as potential cation-binding residues. In its N-terminal 200 amino acids, SilP is not homologous to N-terminal sequences of copper or other P-type ATPases. No functional studies with the SilP protein are available as yet. There is no *silP* homolog in the *E. coli* chromosomal *agr* system that is closely similar to *silPABCRSE*.

### 2.2. The periplasmic Ag(I)-binding protein

SilE is a small periplasmic Ag(I)-binding protein that binds Ag(I) ions specifically at the cell surface, presenting the first line of resistance against Ag(I) toxicity (Fig. 1B). The SilE protein was purified to homogeneity from bacterial periplasmic proteins [18], and its sequence confirmed by N-terminal amino acid sequencing [72]. Studies with purified SilE protein using atomic absorption spectroscopy (AAS) and inductive-coupled plasma analysis showed very high specificity for Ag(I) binding. SilE protein that had not been loaded with metal ions contained less than one cation per 100 polypeptide chains [72]. When SilE protein was loaded with Ag(I), Cu(II) or Cd(II) and dialyzed, five Ag(I) cations were retained per polypeptide, but less than one Cu(II) or Cd(II) per 100 polypeptide chains [72]. Cu(II) was tested here and aerobically, since the periplasmic SilE protein is expected to exist in an aerobic environment. However, Cu(I) or anaerobic conditions were not tested and given the similarities in amino acid sequences, it is anticipated that Cu(I) would bind to SilE under anaerobic conditions. The SilE protein contains 10 histidine residues that bind the five Ag(I) cations [72] (Fig. 5). In contrast to other metal-binding proteins such as metallothionein, SilE has no cysteine residues. Binding of Ag(I) to the SilE protein brings about a usually large change in protein folding, as best measured by circular dichroism

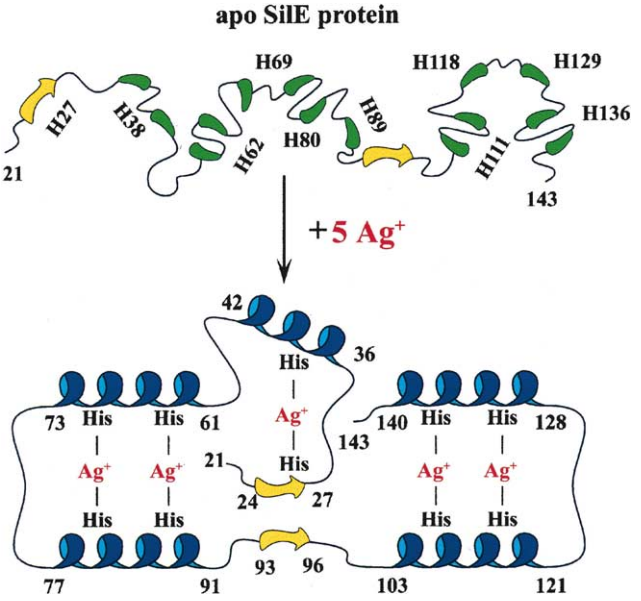


Fig. 5. Model for Ag(I) binding and folding of the periplasmic Ag(I)-binding protein SilE. Top: 122-amino acid processed SilE protein after removal of 20-amino acid leader sequence [18] with positions of the 10 histidine residues noted. Bottom: secondary structure predictions of α-helical (coils) and β-sheet (arrows) regions from standard software and predicted cross-linking of five Ag(I) cations by 10 histidines (modified from [72]).

(CD) [72], from essentially no secondary structure for the protein without bound cation to a predominantly α-helical structure with bound Ag(I) (Fig. 5). Folding from a protein lacking secondary structure to a well-defined structure on binding metal cations also occurs with the poly-cysteine protein metallothionein [86]. In addition to AAS measurements of Ag(I) binding and CD measurements of secondary structure before and after binding Ag(I) [72] (Fig. 5), proton NMR spectroscopy demonstrates the specific binding of Ag(I) to the 10 histidine imidazole N atoms by following perturbation of the proximal C2 and C4 carbon protons (Fig. 6) [72]. NMR analysis of the purified SilE protein showed at least nine different positions for the C2 and C4 imidazole protons of the 10 histidine side groups [72] thought to be the primary binding groups for the five Ag(I) cations (Figs. 5 and 6). As Ag(I) cation was added to SilE in the NMR experiments, the positions of the imidazole ring protons moved indicative of cation binding. Eight of the imidazole proton pairs bound Ag(I) initially, and these are thought to be the eight that occur in four pair $HisX_6His$ motifs in the primary polypeptide sequence, which would place the two histidine imidazoles on the same surface of (highly predicted by protein modeling algorithms) α-helical regions (Fig. 5), two turns of α-helix apart. We suggest that the binding of Ag(I) between the imidazole N positions would be linear and across space as shown in Fig. 5, because molecular modeling does not allow binding between adjacent histidines on the same α-helix without disruption of the secondary structure, contrary to the CD results [72].

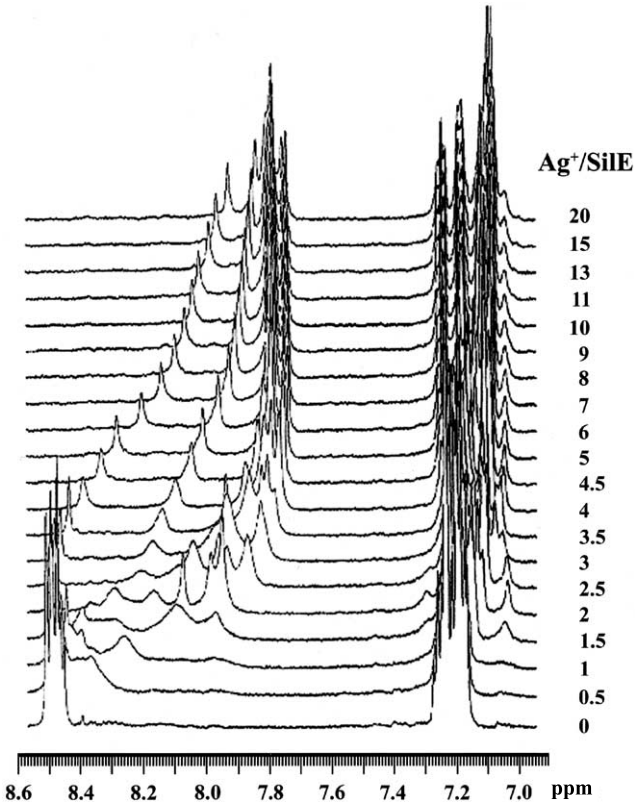


Fig. 6. Changes in C2 and C4 proton NMR spectra of histidine residues of the SilE protein (modified from [72] with permission).

The last two imidazole proton pairs to bind increasing Ag(I) additions [72] are thought to be those toward the N-terminus of the protein (Fig. 5). In a parallel fashion, four Ag(I) bind at lower pH with a fifth at neutral pH, as measured by AAS [72]. Acidifying the folded, mostly α-helical, SilE protein with bound five Ag(I) resulted in a loss of secondary α-helix structure as measured by CD [72] and on reneutralizing the SilE solution the α-helical structure reformed. This combination of Ag(I) binding, CD and NMR studies [72] together with initial studies with purified SilE protein altered in one or another histidine position has provided support for the tentative detailed model of SilE binding of Ag(I) shown in Fig. 5. Although the *silE* gene confers low level Ag(I) resistance by itself [18,72], it has never been found in nature without the other *sil* genes.

## 3. Uses of silver compounds in medicine and health

### 3.1. Silver as a topical antimicrobial agent for burns

The widest and best known use of silver preparations in medicine is as preferred antimicrobial agents for treatment of serious burns [40–44,87–90,125]. A topical cream that contains 1% silver sulfadiazine plus 0.2% chlorhexidine digluconate in a water immiscible cream base is the most widely used product for human use and veterinary medicine, marketed as Silvazine in the USA (by Marion-Hoechst-Russell Laboratories, Kansas City, MO, USA) and as Flamazine in other countries, largely in the UK (Smith and Nelson Company; Roche), Canada and continental Europe. From the initial use of silver sulfadiazine creams, there has been more recent incorporation of the silver sufadiazine directly into bandages used on burned skin surfaces and similar large open wounds [91–101]. Use of direct current electricity to accelerate the release of Ag(I) from the covering into the damaged tissue and then penetration into the tissue has been shown beneficial [92–95], although this appears without wide use. There are many hundreds of PubMed hits for silver sulfadiazine in recent searches, indicating its wide range of uses as an effective biocide. It is the silver, Ag(I), that is biocidal with sulfadiazine functioning to keep the Ag(I) in a stable form less subject to blackening by reduction, than with applications of $AgNO_3$, which also has been used effectively as a biocide on burns – with, however, the unwelcome side effect of turning the burned tissue black from reduced Ag(0).

### 3.2. Bandages for trauma and diabetic wounds

Ag-coated bandages are increasingly being used to cover

burn wounds and traumatic injuries of humans [49] and large animals [50,51]. Silver sulfadiazine-coated methacrylate sheet material that provides a stable base for sustained release of Ag(I) over days is also being investigated. Two new commercial products being used in North America are Acticoat and Silverlon. Acticoat is a silver-coated polyethylene polymer sheet that releases Ag(I) and 'Ag nanoparticles' [101]. Silverlon is a product consisting of Ag-coated polyamide fibers. These silver-containing fabrics are easier to apply and remove from large burns and wounds than is the residue of a cream. It is clear that these products are effective clinically and that the released Ag is broadly bactericidal. It is less clear that the released Ag(I) provides a direct therapeutic benefit. Some studies seem to indicate more rapid wound healing with Ag present than with control coverings [126], while others conclude that it is only the maintenance of a moist sterile covering that is needed, and that the Ag(I) itself does not add to the benefit.

Additional clinical uses of Ag(I) include aseptic coverings for plastic surgery, traumatic wounds, leg ulcers, skin grafts, incisions, abrasions, and minor cuts.

### 3.3. Silver-coated catheters and medical devices

Silver-impregnated polymers of medical devices such as catheters and heart valves have widely been used to prevent the growth of bacterial biofilms [45–48,102–110]. One recent use of 'silverized' fabrics as a biocidal bandaging material is with implanted heart values [96].

Plastic indwelling catheters coated with silver compounds are intended to retard the formation of microbial biofilms on the catheters and infection by nosocomial bacteria. The use of Ag-coated nylon threads in electroretinograms has allowed the detection of tissue damage without fear of infection (http://www.silverinstitute.org). Silver salts have traditionally been administered to the eyes of newborn infants to prevent neonatal eye infections, so that this author's birth certificate states 'eyes treated with 1.5% silver nitrate as required by law'.

"Another new product of considerable interest is silver-zeolite, which is a hydrated aluminosilicate powder which can bind up to 40% of its weight as $Ag^+$, which can be incorporated into medical and dental objects. The $Ag^+$ is subsequently released slowly to result in antibacterial activity [127,128]. The activity of silver-zeolite generally requires air and is considered to involve reactive oxygen species such as superoxide [127]. However, silver-zeolite is also affective against anaerobic oral bacteria [128], indicating a broad potential."

### 3.4. Dental 'silver amalgams'

Dental amalgams, so-called 'silver fillings', contain about 35% Ag(0) and 50% Hg(0), but there is no evidence that sufficient Ag(0) is released and oxidized to Ag(I) to have an antimicrobial effect. It is known, however, that the release of Hg(II) from dental amalgams selects for metal-resistant bacteria.

It is worth starting this section with several declarative statements: (a) there has been much more concern with release of mercury from amalgams [111] than with silver. Thus silver has basically gone along for the ride with mercury. (b) Mercury from amalgam wastes from dental offices is a major source in urban water systems, accounting for perhaps as much as 60% of mercury in municipal waste waters [112]. Presumedly (although not measured) a near-equivalent discharge of silver also occurs. (c) The mercury released from amalgams in the mouth and in dental office waste water is sufficient in amounts and bioavailability to be of health concern. Hg(0) is oxidized to H(II) by catalase, an enzyme abundant in bacteria [113] and in human tissues. It is reasonable that Ag released as Ag(0) is also oxidized abiotically or biotically to bio-active Ag(I). The mercury released can be methylated in the oral cavity and in the gut [61–63] and the amounts of mercury released are sufficient to select for mercury-resistant bacteria in gut flora of animals with silver/mercury amalgams [62]. The amounts are significant and may be the largest source of mercury (and silver) exposure for populations with high incidents of mercury amalgams.

In spite of the statements above and the very active 'mercury zero campaign' by environmental activist groups, e.g. [111,112], there is no credible evidence for any adverse medical affect from released mercury on patients or even dentists and dental office staff. If there are minimally observable or unobservable effects of released Hg(II), then certainly there is no medical concern with Ag released from amalgams. However, the amounts are likely to be comparable for Ag as for Hg, but Ag is less 'mobile' both chemically and biologically. Ag is not subject to methylation, and monomethylmercury is the major toxic form of Hg in fish. Therefore, I conclude that there is no basis to consider the biocidal effects of slow release Ag as an effective oral cleanser nor to be concerned with adverse clinical effects of Ag released from amalgams, either in our mouths or in dental waste water. The impact of release of dental silver on aquatic environments [114] should not be of concern.

Dental amalgams are the major source of mercury (and equivalent silver) release into domestic water systems, with a current release of about 100 tonnes per year in North America [112]. This can be roughly calculated from a North American population of about $3.5\times10^8$ individuals with an average of about 10 amalgams per person with about 0.5 g each Ag(0) and Hg(0). The total is then about 1750 tonnes each and with a 'replacement' or loss time average of about 15 years; then the 100 tonnes per year becomes immediately intuitive. A release of 4 tonnes per year into New York Harbor alone has been estimated [112]. The Ag(0) that is released may be to some extent 'bioaccumulated' [114].

### 3.5. Argyria

Argyria is the permanent irreversible gray coloring of the skin due deposits of silver granules, perhaps silver sulfide precipitates in the dermis, especially in regions around hair follicles and sweat ducts [115–117]. Rosemary Jacobs, whose photo showing the gray face of argyria is shown in [115], is a prominent current critic of human use of silver compounds. Her story of taking silver nose droplets for allergies or viral colds from age 11 is detailed on her Internet homepage (http://homepages.together.net/~rjstan/rose2.html), which is an excellent introduction to the heated exchanges between proponents of widespread use of silver preparations (see next) and their equally opinionated opponents.

### 3.6. Other uses and misuses of silver compounds in human health and homeopathic medicine: 'snake oil'

There has been a continuing battle between advocates of uses of Ag(I) preparations for health and medical benefits and government agencies regulating claims and products [118–121] for more than 100 years [122]. The American government view is that potential legitimate benefits have decreased dramatically over time and that what remains is a lack of established effectiveness for marketed silver products plus a potential (if not remarkable) toxicity of the products. The U.S. FDA (Food and Drug Administration) issued a 'final rule' on silver drugs that appears in the Federal Register at http://www.access.gpo.gov/su_docs/aces/aces140.html#frbrowse (to locate this section, check off '1999' and 'final rules'. Then search for 'colloidal silver', with quotation marks around 'colloidal silver'). Nevertheless, the ardent touting of such products continues [120,121] and wherever such human uses occur, there is a real potential for selection of silver-resistant microbes.

On famous (but questionable) use of silver compounds in North America was in the preparation called 'Argyrol' that was marketed from 1902 to 1996 by a Philadelphia-based company founded and owned by a medical doctor Alfred Barnes [121]. This over-the-counter preparation became so popular that the name 'argyrol' still appears in on-line dictionaries, defined as 'a trademark for a silver-protein compound used as a local antiseptic' or alternatively as 'mild silver protein'. Barnes became wealthy from sales of this product and he retired in 1929, and later he placed his private art collection in a private museum and garden complex still available in suburban Philadelphia ([121], www.barnesfoundation.org/). Whether argyrol was useful as a local antiseptic as intended, or not effective but rather harmless is still unclear 100 years later. Rosemary Jacobs, who actively debunks current silver proteinate products, claims the product was long known to be without beneficial effect or even harmful; see http://www.silverfacts.org/pages/argyrol.html.

A source of less caution is Protects, Inc., www.silversolutions.com, which was still active in February 2003 and is used by Mark Metcalf [59] to list 680 diseases, including lupus and AIDS, which are cured by silver proteinate preparations. There are not many conditions missing from the list. This site includes a letter of apparent endorsement from Magic Johnson. As the efficacy of silver compounds for health or as snake oil is currently debated more on Internet than in the peer-reviewed literature, people interested in uses of Ag compounds and the microbiology of silver resistance cannot ignore these sources. A series of privately published books [58,59,119,120] also reflect the current picture.

It seems purposeless to continue a microbiology review with more detailed discussion of basically anti-scientific 'snake oil' remedies based on silver immobilized in protein coacervates. Nevertheless, the details above are useful to show non-users of silver products just how invasive silver exposure and silver products have become in this time of health fads and Internet. A web search engine search will quickly show that the need for magic cures of medical problems continues and thrives in our time and that Ag-containing preparations are prominent among 'snake oil' remedies offered in health food shops, pharmacies and supermarkets.

## 4. Silver as a biocide; non-medical uses of silver

Our primary concern remains Ag(I) usage in medicine and the possibility that selection for Ag(I) resistance will lessen the usefulness of Ag(I)-containing products. However, the wide use of silver products as biocides adds to the potential problem of selection for resistance. Silver-containing products are used in hospital and hotel water distribution systems to control infectious agents (for example, *Legionella*). Silver was used to sterilize recycled drinking water aboard the Russian MIR space station and on the NASA space shuttle. Supermarket home-water purification units in the USA contain silverized activated carbon filters and ion-exchange resins (Brita Company). While widely used in North America and Europe (http://www.sanosil-disinfectants.com/-des-application-clinics.htm), the available published literature is equivocal about the usefulness of 'silverizing' water carbon filtration units as a biocide [53,54].

Although 'folk remedies' and 'snake oil' preparations are not the same, they are coupled here as representative of an amazingly broad range of uses of suspected benefit (http://www.silverinstitute.org). Silver is a health additive in traditional Chinese and Indian Ayurvedic medicine (http://www.reach4life.com/colloidalsilver.html). Over-the-counter Ag(I) health food supplements are probably not effective and are frequently mislabeled (US Federal Register, 15 Oct. 1996, 16 (200), 53685–53688; FDA Health Fraud Bulletin #19, Colloidal Silver, Oct. 7, 1994). In Mexico, Microdyn (colloidal silver in gelatin) is

sold in supermarkets to disinfect salad vegetables and drinking water. Johnson Matthey Chemicals (UK) has an inorganic composite with immobilized slow release silver as a preservative in cosmetics and toiletries. Some of these uses of silver products as biocides are not listed on labels (and are learned of only as difficult to verify stories), but some are clearly labeled. The Japanese sell mint-flavored 'Jin Tan' Silver Pills (Jin, or Gin as in Ginza is the author's family name) for 'heartburn, nausea and vomiting, motion sickness, hangover, dizziness, bad breadth, choking, indisposition, and sunstroke' (from the label). Also in Japan, a silver compound (Amenitop, silica gel microspheres containing a silver-thiosulfate complex; http://www.yourlifewell.com/index4.shtml) is mixed into plastics for lasting antimicrobial protection of telephone receivers, calculators, toilet seats, and children's plastic toys. It is said that Ag(I) compounds for slow silver release are incorporated into infants' dummies ('pacifiers' in the USA), public toilet seats and public telephones in Japan. One might wish one's child's rubber dummy to be self-cleaning and one would not wish to use unsanitized public facilities when silver compounds are available.

Since silver-impregnated bandages are available for medical uses, it was a short step to embedding Ag in sports fabrics, including sleeping bags and sports socks. This use has been suggested as a means of retarding microbial growth for hygiene and lessening smell. Supermarket surfaces used for meat storage and display are possibly 'silverized', again as a possibly useful biocide. Metallic silver–copper-containing ceramic disks ('Clean Power Plus') are marketed as an alternative for users who might be allergic to laundry detergents. After the 2001 anthrax scare in the USA, the Mayor of Tampa Florida publicly called for adding Ag(I) to municipal drinking water as protection against anthrax and HIV (unlikely to be effective, but equally unlikely to do harm). The potential uses of Ag materials seem endless, and this is but a partial list of the increasing uses of silver as an antimicrobial agent. In essentially all situations, open public testing to demonstrate efficacy or harm is not available.

What is needed for research? With the availability of the genes for silver resistance, we have identified closely related genes in bacteria from environmental and clinical environments and from diverse geographical locations (A. Gupta et al., in preparation). These findings should eliminate recent skepticism about the existence of silver-resistant bacteria. Now that the means for identifying silver resistance determinants in Enterobacteriaceae is available, similar efforts are needed with other common pathogens on large burns (specifically Pseudomonads and Staphylococci). The wide and uncontrolled use of silver products may result in more bacteria developing resistance, analogous to the world-wide emergence of antibiotic- and other biocide-resistant bacteria [36,37]. Such resistant microbes would be detrimental to clinical and hygienic uses that depend on the microcidal properties of silver.

Table 1
Silver solubility products (M) [124]

| | |
|---|---|
| AgBr | $7.7\times10^{-13}$ (at 25°C) |
| AgCl | $3.7\times10^{-11}$ (at 9.7°C) |
| AgCl | $1.6\times10^{-10}$ (at 25°C) |
| AgI | $1.5\times10^{-16}$ (at 25°C) |
| AgS | $1.6\times10^{-49}$ (at 18°C) |
| $AgNO_3$ | 0.7 (at 0°C) |
| $Ag_3PO_4$ | $1.6\times10^{-5}$ (at 20°C) |

## 5. A little silver chemistry

Symbol Ag [123]. Atomic weight: 107.868, 51.35% Ag107 and 48.65% Ag109; atomic number: 47; electron shells: 2, 8, 18, 18, 1, filling orbital: 4d10; covalent radius: 1.34 Å; atomic radius: 1.75 Å. Oxidation state: 1. Density (at 293 K): 10.5 g $cm^{-3}$; sometimes available radionuclide $Ag^{110m}$ *t*/2 253 days, internal transition plus strong 2.99 MeV β-emission.

With Ag(I) cations, the question of solubility in clinical and environmental settings is crucial, especially with halides and other anions present. Table 1 gives solubility products and the relative insolubility of Ag(I) halides and AgS.

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