Cell-to-Cell Transfer of Bacterial Outer Membrane Lipoproteins

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interaction between IκBα and RelA were probed in HeLa cells pretreated with TNF-α, which inhibited the endocytic uptake of MNPs, as described (*20*). MNPs coated with antibody to phosphorylated Ser^{32} of IκBα or with FK506 were microinjected into TNF-α–pretreated HeLa cells expressing IκBα-mRFP or IκBα-ECFP-FKBP12/EYFP-RelA/mRFP-βTrCP, respectively (*8*). Signal-induced phosphorylation and interaction of the NF-κB/IκB pathway were readily detected by MAGIC in this experimental setting (fig. S6).

MAGIC offers several advantages over target ID methods currently in use. First, it directly translates a physical molecular interaction into a clear readout signal, unlike indirect readout methods that are dependent on intermediary interactions (*22*), overall expression profiles (*23*), or complex biological phenotypes (*24*). Thus, intrinsic false positives/negatives or error-prone deductions about molecular target(s) of a small molecule are obviated. Second, by probing such interactions in a physiologically relevant context, misleading outcomes produced by an artificial experimental setting (*22*, *24*–*28*) can be greatly diminished. Third, it is amenable to dynamic, single-cell analysis of interactions. Finally, MAGIC can be used to detect a variety of biological interactions and protein modifications within live cells in a broad range of tissues and disease states. With the great advantage of being able to detect dynamic interactions between the biomolecules within mammalian cells, this technology could be exploited in genome-wide interaction screens.

The benefits of MAGIC may be best achieved through efficient and nondisruptive introduction of MNPs into cells. Prolonged incubation of cells with TAT-HA2–conjugated MNPs may affect cellular physiology, and microinjection cannot be used for large populations of cells. Other technologies for delivering biologically active cargos into cells (*10*) will be helpful to complement MAGIC.

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Supporting Online Material

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Cell-to-Cell Transfer of Bacterial Outer Membrane Lipoproteins

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Myxococcus xanthus **cells can glide forward by retracting type IV pili. Tgl, an outer membrane lipoprotein, is necessary to assemble pili. Tgl mutants can be transiently "stimulated" if brought into end-to-end contact with *tgl*$^+$ donor cells. By separating the stimulated recipient cells from donor cells, we found that Tgl protein was transferred from the donors to the rescued recipient cells. Mutants lacking CglB lipoprotein, which is part of a second gliding engine, could also be stimulated, and CglB protein was transferred from donor to recipient cells. The high transfer efficiency of Tgl and CglB proteins suggests that donor and recipient cells briefly fuse their outer membranes.**

Myxobacteria move by gliding on surfaces—an effective mode of translocation in their soil habitat (*1*). *Myxococcus xanthus* moves smoothly in the direction of its long axis, then reverses and moves in the opposite direction. It has no flagella and cannot swim; its gliding is propelled by two polar engines that are encoded by the A and the S genes (*2*). The A engines appear to extrude polymer chains from the trailing ends of cells, which gelate, pushing the cells forward (*3*). Slime is extruded from the trailing end only (*4*). The S genes encode hairlike appendages projecting from the leading end of the cell—the type IV pili (*5*, *6*)—and they pull a cell forward by retracting (*7*). Many proteobacterial pathogens have type IV pili (*8*). *M. xanthus* type IV pili are polar (*6*), and S motility is limited to cells within pilus-contact distance of each other (*9*).

A common set of 10 proteins is necessary for the assembly and retraction of type IV pili in *M. xanthus*, *Pseudomonas aeruginosa*, and *Neisseria gonorrhoeae* (*8*). One of those proteins, known as PilA in *M. xanthus*, constitutes the pilus filament, a helical array of PilA monomers (*10*). The filament is thought to pass through the outer membrane within a gated channel formed by the PilQ secretin protein (*11*). Secretins are multimeric channels large enough to pass folded proteins (*12*). Secretins often have cognate lipoproteins that facilitate their assembly in the outer membrane (*13*, *14*).

The Tgl lipoprotein is necessary to assemble a detergent-resistant PilQ secretin in *M. xanthus* (*15*). Nevertheless, *tgl* mutants can be stimulated to assemble pili and to glide after transient contact with *tgl*$^+$ donor cells (*16*, *17*). Cells with a *tgl* mutation lack S motility but have normal A motility. Tgl stimulation specifically restores their S motility (*2*). To render stimulation visible by its effect on motility, we transferred a mutant *tgl* locus into an A$^-$ background, creating a nonmotile strain. Strain DK8602 (table S1) lacked both A and S motility and formed a smooth-edged colony (Fig. 1A). When DK8602 was mixed with a nonmotile *tgl*$^+$ donor strain (Δ*pilA*, *aglB1*), the mixed population initially had a smooth colony edge reflecting both strains' lack of A and S motility (Fig. 1A). Recipient cells swarm beyond the original colony edge as flares when they are stimulated (Fig. 1B), which shows that the cells had assembled retractile pili.

To distinguish donor from recipient cells in a Tgl-stimulation mixture, we expressed the green fluorescent protein (GFP) in one population or the other. When the recipient strain

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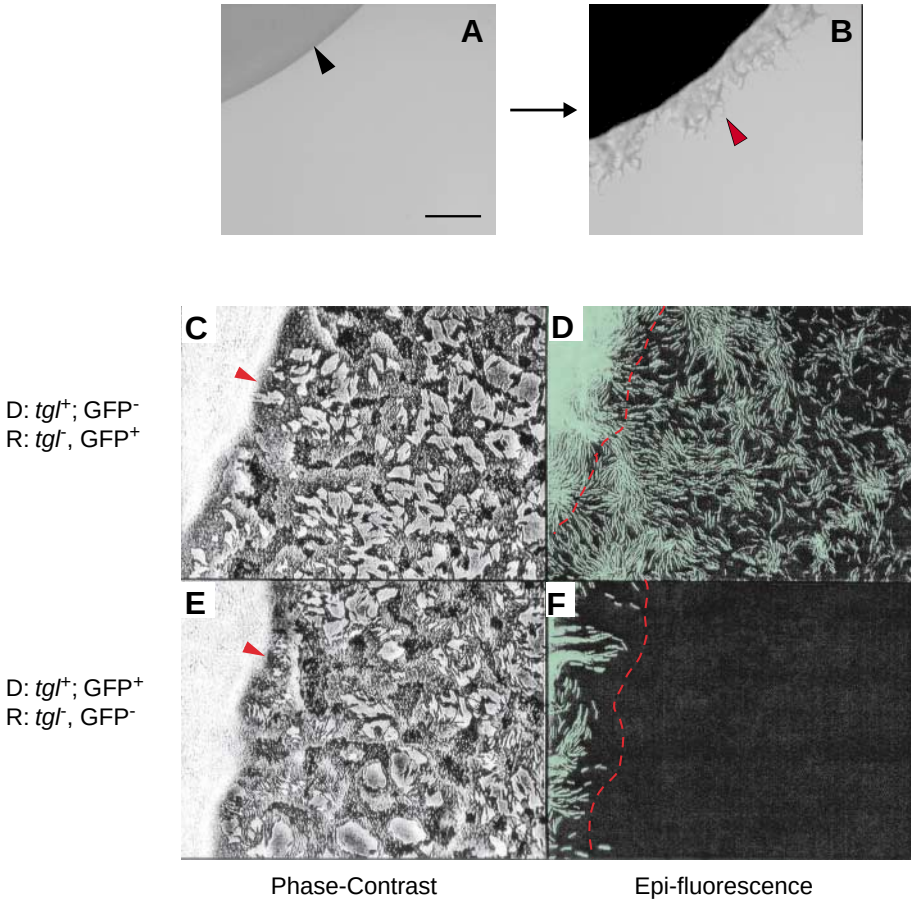

Fig. 1. Tgl stimulation. Stimulated recipient cells became motile and swarmed outward, the donor strain remained nonmotile; it was found inside the original edge of the colony. A *tgl* mutant carrying an A⁻ mutation (A⁻S⁻ strain DK8602) was mixed with another nonmotile *tgl*⁺ mutant strain (DK8601) and spotted on agar. **(A)** At 0 hours, the mixed colony had a smooth edge (black arrowhead) because there were no motile cells (Scale bar, 500 μm). **(B)** After several hours, the *tgl*⁺ donor cells activate S motility in the *tgl* mutant recipient cells by stimulation. The outward swarming of the stimulated recipients after 4 days is indicated by the red arrowhead. This motility was transient; it lasted only a week (fig. S2). **(C)** Phase-contrast image of DK8601 (GFP⁻ donor cells) mixed 1:1 with a mixture of DK8607 [GFP⁺ recipient cells (table S1)] and DK8602 (GFP⁻ recipient cells), the two last-named strains at a 1:50 ratio. The original colony edge is indicated by the red arrowhead. **(D)** Epifluorescent image of the field shown in (C). The original colony edge is indicated by the dashed red line. **(E)** Phase-contrast image of DK8602 (GFP⁻ recipient cells) mixed 1:1 with a mixture of DK8606 (GFP⁺ donor cells) and DK8601 (GFP⁻ donor cells) at a 1:50 ratio. **(F)** Epifluorescent image of (E).

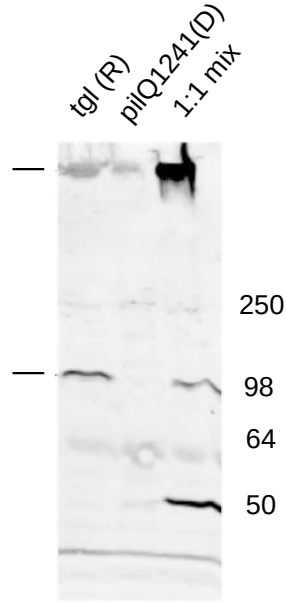

Fig. 2. Stimulated assembly of PilQ multimers. A mutant strain with an in-frame deletion in *tgl* failed to assemble PilQ multimers (top line to left of gel), although it produced the 98-kD PilQ monomer (other line on left). When the Δ*tgl* strain was stimulated with the donor strain *tgl*⁺ *pilQ*^{1241}(a pilQ allele that produces unstable PilQ monomers in lane 2), boiling SDS–resistant PilQ multimers apparently formed at the expense of monomers (lane 3).

DK8607 (Δ*tgl*, *aglB1*) contained GFP, it was seen to move out in characteristically S-motile flares (Fig. 1, C and D). By contrast, when GFP was expressed in the donor strain DK8606 (Δ*pilA*, *aglB1*), it was not found beyond the original colony edge (Fig. 1F). Because only stimulated recipients swarmed out, it was possible to harvest pure cultures of stimulated cells. The genotype of such harvested cells was tested by colony polymerase chain reaction (PCR) that specifically amplified the *tgl* gene. As expected, pure cultures of donor cells had a *tgl* gene, and pure cultures of Δ*tgl* recipient cells lacked it (fig. S1). Furthermore, harvested stimulated recipient cells lacked the *tgl* gene (fig. S1).

Because Tgl is required for the assembly of (detergent-resistant) PilQ multimers (*15*), the S motility of stimulated cells implies that those cells have assembled PilQ multimers, because they are able to extend and retract pili. To investigate the assembly of PilQ in stimulated recipients, a donor strain was needed that lacked such assemblies. The Δ*pilQ tgl*⁺ strain is not an efficient donor for Tgl stimulation (*15*). However, although a *pilQ*^{1241} nonsense mutant did not assemble stable PilQ multimers (*15*), it could nevertheless serve as a Tgl donor. When a *tgl*⁺ *pilQ*^{1241} donor strain was mixed with *tgl* recipients, assembled PilQ multimers were readily detected by their resistance to dissociation after being boiled in SDS-containing buffer (Fig. 2). Unassembled PilQ monomers were also found in the stimulated recipients, as well as a 50-kD PilQ fragment that always accompanied PilQ assembly (*15*). The recipients before stimulation had only unassembled PilQ monomers and no 50-kD fragment (Fig. 2). The donor did not have assembled PilQ multimers, 50-kD fragment, or PilQ^{1241} monomers (Fig. 2). It appears that the mutant monomers whose carboxy ends had been truncated were degraded.

We tested the possibility that Tgl protein was transferred from donor to recipient cells. As expected, Tgl protein was found in the donor cells, but not in the recipient cells (Fig. 3A). However, after stimulation, large amounts of Tgl protein were also found in the harvested stimulated recipient cells (Fig. 3A). Thus, transient contact between Tgl⁺ donor cells and Tgl⁻ recipients results in the transfer of Tgl protein from donor to recipient. To challenge the specificity of transfer, we examined a cytoplasmic protein, GFP, in donor cells, recipient cells, and stimulated recipient cells. When only recipients contained GFP, it was detected only in the recipient cells and the harvested stimulated recipient cells (Fig. 3A). Conversely, when only donors contained GFP, it was detected only in the donor cells (Fig. 3A), which demonstrated the absence of contaminating donor cells in the harvested stimulated recipient cells. This experiment also shows that GFP is not transferred.

Transfer of Tgl protein was also demonstrated in cells separated by flow cytometry. The (GFP⁻) donor strain DK8601 was mixed 1:1 with the fluorescent GFP⁺ recipient strain DK8607, and the mixture was spotted on agar.

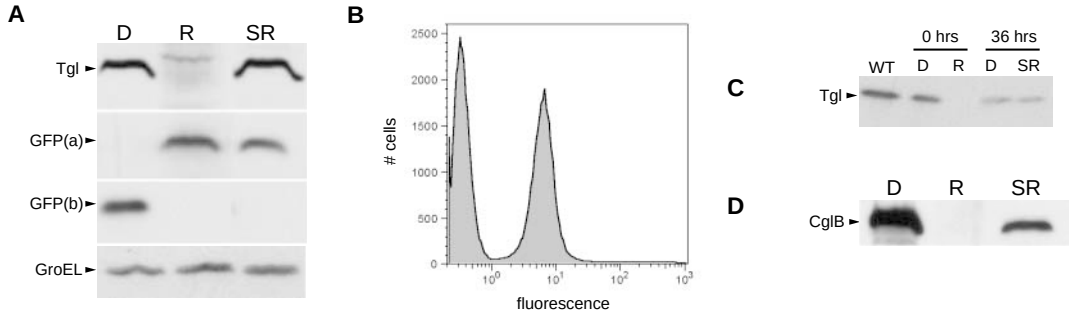

Fig. 3. Detection of Tgl protein transfer from donor cells to recipient cells. (**A**) Immunoblots lane 1, donor cells, DK8606; lane 2, recipient cells, DK8602; and lane 3, the harvested stimulated recipient cells (SRs). The GFP(a) row is a fluorogram of GFP$^-$ donor strain, DK8601, GFP$^+$ recipient, DK8607, and their harvested SRs. The GFP(b) row shows GFP$^+$ donor, DK8606, the GFP$^-$ recipient, DK8602, and their harvested SRs. GroEL is the loading control. (**B**) Fluorescence profile of a mixture of GFP$^-$ donor cells (strain DK8601) with GFP$^+$ recipient cells (strain DK8607), separated by fluorescence-activated cell sorting (FACS) 36 hours after mixing. (**C**) Tgl Immunoblot of fractions from the FACS separation in (B), 0 hours after mixing and 36 hours after mixing. (**D**) Transfer of the CglB protein from donor (strain DK6204) to recipient cells (strain ASX1). The two peaks evident in B were harvested separately.

After 36 hours, the cells remained in a 1:1 ratio in two distinct populations: GFP$^-$, which has 0.35 units of autofluorescence, and GFP$^+$, which has 8 units of GFP fluorescence (Fig. 3B). The presence of the Tgl protein was evaluated before and after stimulation. As expected, before stimulation, Tgl was present in the donor cells and absent in the recipients (Fig. 3C). After 36 hours of mixed swarming, Tgl was detected in both the donor cells and the population of stimulated recipient cells (Fig. 3C).

The only gene that can be stimulated in the S motility system is Tgl. However, five A motility genes can be stimulated: *cglB*, *cglC*, *cglD*, *cglE*, and *cglF* (*17*). The *cglB* gene has been cloned and sequenced; it encodes a lipoprotein that has no similarity to *tgl*, apart from a type II signal sequence (*18*). To test protein transfer in A motility stimulation, stimulated *cglB* recipient cells were separated from the donor cells after they had spread beyond the edge of the original spot. Indeed, the harvested, stimulated *cglB* mutant recipients contained large amounts of the CglB protein (Fig. 3D).

The concentration of Tgl and CglB proteins in stimulated cells was similar to the concentration in donor cells (Fig. 3), as if the donor and recipient cells shared their outer membrane lipoproteins equally. Myxobacteria may have evolved an efficient sharing of outer membrane lipoproteins, because they need to reverse their gliding direction frequently, 20 or more times per division cycle (*19*). Frequent reversal means frequently reconstructing the A and the S engines (*4*). Tgl and CglB (and perhaps CglC, D, E, and F) are needed to specify which cell poles have pili and which are active in slime secretion. This sharing of outer membrane lipoproteins among the thousands of cells in a swarm creates a primitive tissue.

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Ubiquitination on Nonlysine Residues by a Viral E3 Ubiquitin Ligase

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Ubiquitination controls a broad range of cellular functions. The last step of the ubiquitination pathway is regulated by enzyme type 3 (E3) ubiquitin ligases. E3 enzymes are responsible for substrate specificity and catalyze the formation of an isopeptide bond between a lysine residue of the substrate (or the N terminus of the substrate) and ubiquitin. MIR1 and MIR2 are two E3 ubiquitin ligases encoded by Kaposi's sarcoma–associated herpesvirus that mediate the ubiquitination of major histocompatibility complex class I (MHC I) molecules and subsequent internalization. Here, we found that MIR1, but not MIR2, promoted down-regulation of MHC I molecules lacking lysine residues in their intracytoplasmic domain. In the presence of MIR1, these MHC I molecules were ubiquitinated, and their association with ubiquitin was sensitive to β_2-mercaptoethanol, unlike lysine-ubiquitin bonds. This form of ubiquitination required a cysteine residue in the intracytoplasmic tail of MHC I molecules. An MHC I molecule containing a single cysteine residue in an artificial glycine and alanine intracytoplasmic domain was endocytosed and degraded in the presence of MIR1. Thus, ubiquitination can occur on proteins lacking accessible lysines or an accessible N terminus.

Ubiquitination is a highly regulated process conserved in all eukaryotes (*1*, *2*) that regulates many fundamental cellular processes. Many pathogens mimic, block, or redirect the activity of the ubiquitin system. The modulators of immune recognition (MIR) 1 and 2, two proteins encoded by Kaposi's sarcoma–associated herpesvirus (KSHV), specifically down-regulate the expression of MHC I from the surface of infected cells, presumably to prevent lysis of infected cells by cytotoxic T lymphocytes (*3*–*6*). MIR1 and MIR2 are highly homologous structurally and functionally, and they belong to a large family of E3 ubiquitin