projections and cytoplasmic melanosomes (Fig. 2A), whereas cells transfected with BAP1 siRNA lost these features, developed a rounded epithelioid morphology, and grew as multicellular nonadherent spheroids, strikingly similar to the features of class 2 clinical biopsy samples (Fig. 2A). Microarray gene expression profiling of 92.1 UM cells transfected with control versus BAP1 siRNA showed that most of the top genes that discriminate between class 1 and class 2 tumors shifted in the class 2 direction in BAP1-depleted cells compared with control cells (fig. S4). Similarly, depletion of BAP1 shifted the gene expression profile of the multi-gene clinical prognostic assay toward the class 2 signature (Fig. 2B). BAP1 depletion caused a reduction in mRNA levels of neural crest migration genes (*ROBO1*), melanocyte differentiation genes (*CTNNB1*, *EDNRB*, and *SOX10*), and other genes that are down-regulated in class 2 tumors (*LMCD1* and *LTA4H*) (*19*). In contrast, BAP1 depletion caused an increase in mRNA levels of *CDH1* and the proto-oncogene *KIT*, which are highly expressed in class 2 tumors (*20*). Similar results were seen in other UM cell lines and with an independent BAP1 siRNA (*10*).

GNAQ mutations occur early in UM and are not sufficient for malignant transformation (*4*), but they may create a dependency of the tumor cells on constitutive GNAQ activity. In contrast, *BAP1* mutations occur later in UM progression and coincide with the onset of metastatic behavior. Thus, simultaneous targeting of both genetic alterations might have synergistic therapeutic effects. One potential strategy to counteract the effects of BAP1 mutation would be to inhibit the RING1 ubiquitinating activity that normally opposes the deubiquitinating activity of BAP1 (*16*). Our findings strongly implicate mutational inactivation of *BAP1* as a key event in the acquisition of metastatic competence in UM, and they expand the role of BAP1 and other deubiquitinating enzymes as potential therapeutic targets in cancer.

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Direct Exchange of Electrons Within Aggregates of an Evolved Syntrophic Coculture of Anaerobic Bacteria

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Microbial consortia that cooperatively exchange electrons play a key role in the anaerobic processing of organic matter. Interspecies hydrogen transfer is a well-documented strategy for electron exchange in dispersed laboratory cultures, but cooperative partners in natural environments often form multispecies aggregates. We found that laboratory evolution of a coculture of *Geobacter metallireducens* and *Geobacter sulfurreducens* metabolizing ethanol favored the formation of aggregates that were electrically conductive. Sequencing aggregate DNA revealed selection for a mutation that enhances the production of a c-type cytochrome involved in extracellular electron transfer and accelerates the formation of aggregates. Aggregate formation was also much faster in mutants that were deficient in interspecies hydrogen transfer, further suggesting direct interspecies electron transfer.

Interspecies exchange of metabolites is crucial to the balanced functioning of many microbial communities (*1*–*5*). The canonical example of an essential microbial exchange is interspecies hydrogen transfer, in which two cell types, neither of which is independently capable of anaerobically oxidizing an organic compound, cooperatively exchange electrons through the production and consumption of hydrogen in order to degrade the substrate (*4*, *5*). The concept of interspecies hydrogen transfer was developed from studies of *Methanobacillus omelianskii*, a coculture of the "S organism" that converted ethanol to acetate and hydrogen gas (Eq. 1), and the methanogen *Methanobacterium ruminantium*, which consumed hydrogen with the reduction of carbon dioxide to methane (Eq. 2) (*6*):

$$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + 2H_2 + H^+ \quad (1)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (2)$$

The S organism is no longer available in culture, but *Pelobacter carbinolicus* functions similarly (*7*). *P. carbinolicus* evolved from Fe(III)-reducing members of the Geobacteraceae family to grow as an ethanol-oxidizing syntroph (*8*). When Fe(III)-reducing microorganisms deplete Fe(III) in anaerobic soils and sediments, it is advantageous for them to form syntrophic associations with microorganisms that can accept the electrons that were formerly transferred to Fe(III).

To investigate how Fe(III) reducers in the Geobacteraceae family switch to syntrophic growth, we initiated nine replicate cocultures (*9*) with *Geobacter metallireducens*, an ethanol-oxidizing Fe(III) reducer closely related to *P. carbinolicus* (*10*). *Geobacter sulfurreducens* (*11*), which cannot metabolize ethanol, was added as a hydrogen-consuming partner with fumarate as the electron acceptor, which *G. metallireducens* cannot use. In such a coculture, *G. sulfurreducens* could presumably make ethanol metabolism by *G.*

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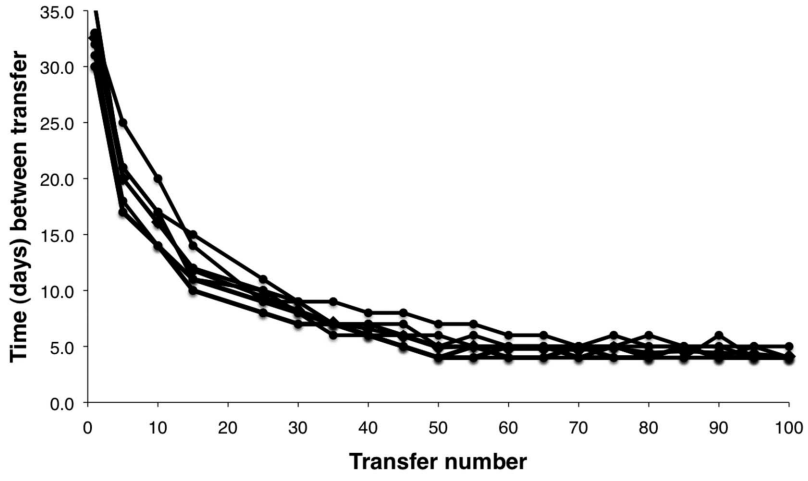

Fig. 1. Time required for sequential transfers of each of the individual cocultures of *G. metallireducens* and *G. sulfurreducens* to metabolize at least 70% of the ethanol provided.

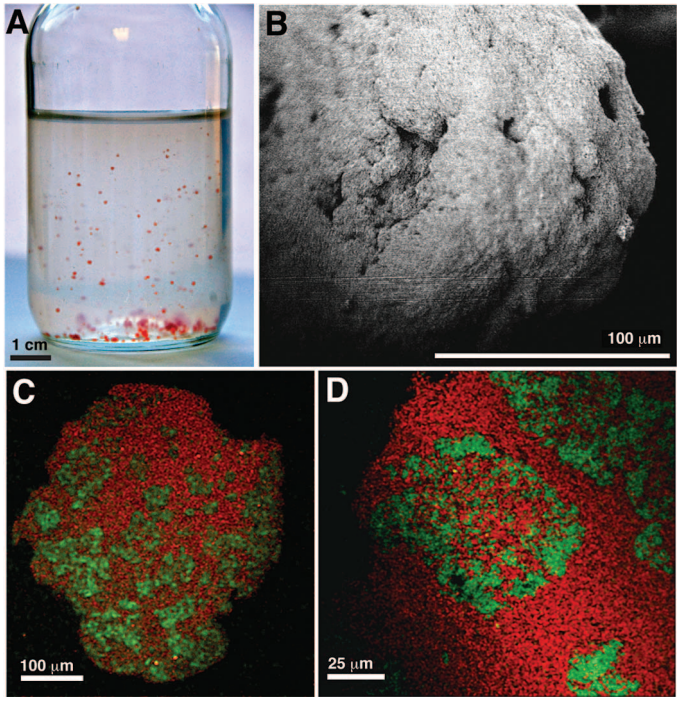

Fig. 2. Aggregates in evolved coculture of *G. metallireducens* and *G. sulfurreducens*. (**A**) Aggregates in culture bottle. (**B**) Scanning electron micrograph of a typical aggregate. (**C**) FISH of a semi-thin section of an aggregate treated with green-fluorescing *G. metallireducens* probes and red-fluorescing *G. sulfurreducens* probe. (**D**) FISH analysis at higher magnification.

metallireducens possible by consuming the hydrogen produced by *G. metallireducens* with the reduction of fumarate to succinate (Eq. 3); *G. sulfurreducens* would also consume the acetate that *G. metallireducens* produced from ethanol (Eq. 4):

$$H_2 + C_4H_2O_4^{2-} \rightarrow C_4H_4O_4^{2-} \quad (3)$$

$$CH_3COO^- + 4C_4H_2O_4^{2-} + 2H_2O + H^+ \rightarrow 2CO_2 + 4C_4H_4O_4^{2-} \quad (4)$$

Initially the coculture grew very slowly and required about 30 days to metabolize ~70% of the ethanol provided. During this phase, measured hydrogen concentrations in the culture were ~10 parts per million (ppm). With continued transfer (1% inoculum) into fresh medium after the cocultures had metabolized ~70% of the ethanol, the coculture adapted to consume at least 70% of the ethanol within 4 days (Fig. 1). This increase in metabolic rate was accompanied by the formation of large spherical aggregates (diameter ~1 to 2 mm) that began to appear as small flocks by transfer 12 and had formed large, tight spherical associations by transfer 30 (Fig. 2A). Hydrogen concentrations were ~6 ppm at this stage. The aggregates were morphologically similar to those that typically form during anaerobic treatment of wastewater (*12*) with channels to promote exchange of materials with the bulk environment (Fig. 2B).

Quantitative polymerase chain reaction targeting 16*S* rRNA gene sequences indicated that *G. metallireducens* accounted for ~15% of the cells in the aggregates (fig. S1). Examination of thin sections of the aggregates by fluorescence in situ hybridization (FISH) with species-specific oligonucleotide probes revealed that the two species formed distinct clusters within the aggregates (Fig. 2, C and D).

To evaluate the role of interspecies hydrogen transfer within the aggregates, we initiated cocultures with a strain of *G. sulfurreducens* in which the gene *hyb* was deleted. This gene encodes a hydrogenase subunit, and previous studies have shown that the strain with *hyb* deleted is unable

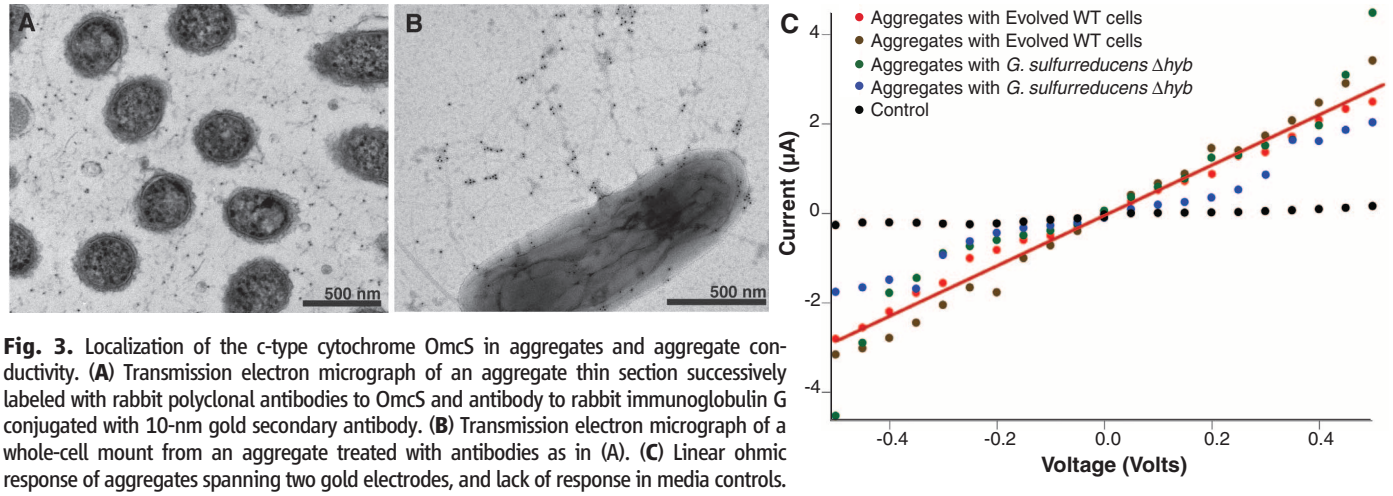

Fig. 3. Localization of the c-type cytochrome OmcS in aggregates and aggregate conductivity. (**A**) Transmission electron micrograph of an aggregate thin section successively labeled with rabbit polyclonal antibodies to OmcS and antibody to rabbit immunoglobulin G conjugated with 10-nm gold secondary antibody. (**B**) Transmission electron micrograph of a whole-cell mount from an aggregate treated with antibodies as in (A). (**C**) Linear ohmic response of aggregates spanning two gold electrodes, and lack of response in media controls.

to consume hydrogen (*13*). The coculture metabolized ethanol (fig. S2). Furthermore, it formed large aggregates within 21 days, rather than the 7 months required for the cocultures containing wild-type *G. sulfurreducens*. Interspecies formate transfer is a potential alternative to interspecies hydrogen transfer for electron exchange between some organisms (*5*), but this was not a possibility in the *Geobacter* coculture because *G. sulfurreducens* is unable to use formate as an electron donor (*11*), and aggregates were incapable of formate-dependent fumarate reduction (fig. S3). These results suggest that an alternative mechanism of electron transfer between the two *Geobacter* species, enhanced by close cell association, confers a growth advantage when interspecies hydrogen transfer is no longer possible.

Sequencing of genomic DNA extracted from one of the aggregate cultures evolved from wild-type cells after the 15th transfer (~100 cell generations) revealed a single mutation in *G. sulfurreducens* and none in *G. metallireducens*. The mutation in *G. sulfurreducens*, which was also present in the other eight replicate cocultures but was not detected in the common inoculum, was a deletion of a single base pair in the gene encoding PilR, which functions as an RpoN-dependent enhancer-binding protein and regulates the expression of a variety of genes in *G. sulfurreducens* (*14*). This deletion resulted in a frame shift changing the amino acids starting at position 337 and introduced a stop codon at position 342, resulting in a truncated PilR protein that lacked the helix-turn-helix domain required for DNA binding (fig. S4). Although this mutation was not detected in the common inoculum used to initiate the cocultures, it is conceivable that it was a rare variant in that culture. Furthermore, genome resequencing may fail to detect transposition or other genome rearrangements. Therefore, to determine whether a mutation in *pilR* was sufficient to promote rapid syntrophic metabolism, we initiated the cocultures with a strain of *G. sulfurreducens* in which *pilR* was deleted. Within 21 days, all three replicate cocultures formed aggregates in the initial culture tubes, which suggests that inactivation of PilR was sufficient to promote aggregate formation.

One of the primary impacts of deleting *pilR* in *G. sulfurreducens* is enhanced expression of the gene encoding OmcS (*14*), a multiheme c-type cytochrome that promotes electron transfer to insoluble Fe(III) oxides (*15*) and electrodes (*16*). OmcS is primarily associated with pili (*17*). The multiple hemes in OmcS are thought to facilitate the transfer of electrons exported from the cell along the pili, which are electrically conductive (*18*, *19*), onto electron acceptors, such as Fe(III) oxide (*17*). Immunogold labeling of OmcS revealed that OmcS was dispersed throughout the intercellular matrix (Fig. 3A) and was associated with pili (Fig. 3B). Heme staining and Western blot analysis indicated that OmcS was the most abundant c-type cytochrome in the aggregates and that OmcS was much more abundant in the evolved aggregates than in monocultures of *G. sulfurreducens* (fig. S5). Deletion of the genes encoding OmcS or PilA, the structural pilin protein, prevented any visible growth even after long-term incubation (>9 months).

These results show that OmcS is essential for effective syntrophic electron exchange and that selective pressure for syntrophic growth selected for a mutation that enhanced OmcS production. The lack of detailed study of *G. metallireducens* and the lack of homology between outer-surface cytochromes in the *Geobacter* species (*20*) has precluded definitive verification of the mechanisms for extracellular electron transfer in *G. metallireducens*, but it is expected that *G. metallireducens* also uses a network of conductive pili and cytochromes, similar to the closely related *G. sulfurreducens*. Thus, a likely model for electron exchange between *G. sulfurreducens* and *G. metallireducens* is that OmcS of *G. sulfurreducens* can accept electrons from outer-surface c-type cytochromes of *G. metallireducens* that are either localized on the cell or along pili. This direct exchange of electrons would alleviate the need for interspecies hydrogen transfer and would explain the effective syntrophy between *G. metallireducens* and *G. sulfurreducens*, even when *G. sulfurreducens* was unable to consume hydrogen. Indeed, the aggregates exhibited ohmic conductance when placed between two gold electrodes (Fig. 3C and fig. S6).

Our results suggest that selective pressure for effective electron exchange in this coculture favored direct electron transfer between consortium members, rather than interspecies hydrogen transfer. This is consistent with previous speculation on the possibility of direct electron transfer between cells (*18*, *21*), but in the absence of experimental evidence, this possibility has been met with skepticism (*4*, *5*), especially considering the preponderance of evidence from studies with defined cocultures in which interspecies transfer of hydrogen or formate predominates. Previous coculture studies, however, typically examined dispersed (i.e., not aggregated) syntrophic partners (*22*). The lack of cell aggregation dictates that interspecies hydrogen or formate transfer predominates over direct cell-to-cell electron transfer under those conditions. In contrast, aggregation of cells involved in syntrophic anaerobic metabolism appears to be the rule in most environments (*1*, *3*, *23*) and is key to the successful anaerobic treatment of wastewater (*12*). Modeling of electron transfer (*22*, *24*–*26*) in such aggregates, as well as experimental evidence (*26*, *27*), suggests that interspecies hydrogen transfer is not the primary mechanism for electron exchange. Rapid direct electron exchange is consistent with the findings in those studies, and in fact, extracellular c-type cytochromes may play an important role in aggregates catalyzing syntrophic methane oxidation (*28*).

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