inter-branch loops. Free movement of GFP was observed throughout the loops and, after photobleaching of the internal region of stabilized loops, symmetrical fluorescence recovery occurred from both sides of the loop. Although fasciculation could play a role in the formation of such loops, these observations suggest that inter-branch fusion can also occur in an EFF-1-dependent fashion. Whereas these loops were induced and analyzed under conditions in which EFF-1 activity was experimentally manipulated throughout development, the authors found through studies of archival transmission electron micrographs that loops occur in menorahs of adult wild-type neurons, suggesting that fusion is important for normal dendritic development in these cells. Taken together, these data suggest that EFF-1 activity can promote branch retraction, fission, as well as inter-branch fusions, all of which help to ensure proper patterning of PVD dendrites.

The recent studies of PVD neuron arborization open interesting directions in the study of dendrite formation, retraction and maintenance. Several questions remain to be answered about the role of EFF-1 in these processes. One important issue will clearly be to understand how EFF-1 is capable of mediating both branch retraction and fusion. Oren-Suissa and colleagues [7] propose a model in which trans-interaction between EFF-1 could result in autofusion whereas larger EFF-1 complexes on dendrites could promote retraction. Can these roles be dissociated molecularly, and if so, what is the significance of fusion for the normal physiology of the

dendrites of PVD neurons? Given the findings of Smith *et al.* [13], it would be interesting to know what role, if any, fasciculation between PVD and other nerve fascicles plays in PVD arborization and, given the disorganization of dendrites in *eff-1* mutants, whether EFF-1 participates in such fasciculation. It could also be informative to perform rescue experiments with EFF-1 mutants lacking the PLA_2 consensus or the EHP to determine whether the domain requirements are the same as for rescue in other cellular contexts [9]. Interestingly, EFF-1-dependent neurite fusion can also occur during regeneration of laser-damaged axons in *C. elegans* [14]. Understanding these newly emerging roles for EFF-1 in the nervous system should therefore further our understanding of neurite formation, regression, and regeneration. The PVD neurons will undoubtedly provide a powerful emerging model to study not only these problems, but also general molecular mechanisms of dendritic patterning and the relation to sensory function [7,13]. It seems that many surprising insights lie ahead.

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Synthetic Biology: Now that We're Creators, What Should We Create?

A 'synthetic' microbe has been created by introducing the artificially produced genome of one species into the cytoplasm of another. The technology allows the introduction of easily transferable adaptive units, as well as sets of genes that have likely never been transferred successfully.

Frederick M. Cohan

Craig Venter and colleagues [1] have realized their long-touted ambition to build a synthetic organism, an

accomplishment with significant implications for biotechnology and scientific investigations of microbial physiology, ecology, and evolution. This technology may eventually allow

us to construct a bacterium with any desired set of changes from an existing organism, including huge but precisely engineered deletions or insertions of genes from other organisms, as well as targeted changes in existing genes. The technology will allow investigation of issues that are not accessible through more standard introductions of limited sets of linked genes [2] or even massive but uncontrolled introductions of thousands of genes [3].

The Venter team first synthesized the entire genome of a donor organism,

a strain of *Mycoplasma mycoides*, from a computer record of the genome sequence. They facilitated spontaneous entry of the synthesized genome into a closely related recipient, a strain of *Mycoplasma capricolum*; this whole-genome uptake was enabled by the team's choice of *Mycoplasma* for its lack of a cell wall. After entering the recipient cell, the donor genome spontaneously replaced the native genome (by a mechanism not yet understood) and produced a self-replicating cell lineage. By 30 generations, this synthetic lineage had become phenotypically indistinguishable from the native donor cell. The synthetic cell was also genomically indistinguishable from the native donor strain, except for some DNA intentionally inserted (such as a DNA-encoded email address) and some known errors of synthesis, included to distinguish the synthetic genome from that of the native donor strain.

One scientific issue motivating the work was to test whether the chromosomes contain all the instructions required to re-direct the physiology of a cell [4]. By showing that the donor cell's physiology and biochemistry could be produced exactly by substituting only the chromosome in a recipient cell, the authors demonstrated a completely genome-based determination of cell physiology, at least in this case.

It is not clear, however, whether all donor–recipient combinations, particularly highly divergent combinations, will succeed in replicating even once, or if they do, whether the full repertoire of the donor genome will be activated. This is partly because the promoter sequences next to each gene on a donor genome may be incompatible with the native regulatory molecules of the recipient cell (which bind to promoters and activate the genes). Indeed, homologous genes of divergent bacteria can differ in the strength with which their promoter sequences bind to a given regulatory molecule [5]; moreover, bacteria can differ in the sets of transcription factors responsible for regulating a given gene [6]. Thus, the recipient's native transcription regulation machinery may fail to effectively and appropriately transcribe the full extent of the donor genome, so that some of the donor cell's proteins may

not get expressed. If these donor proteins are required for induction of some donor regulatory molecules, these regulatory molecules may *never* be expressed.

Also, the native cell structures of the recipient may not support the function of all the proteins produced by the donor genome. For example, there appears to be an incompatibility between the photosynthetic pigments and membrane structures of different divisions of photosynthetic bacteria [7]. Similarly, a donor genome may code for membrane-binding proteins that will not function when transplanted into a distantly related recipient that bears all the recipient's native membrane structures. If functionality of such a donor protein is required for induction of donor membrane features, then the membrane will remain recipient-like and the proteins requiring a donor-like membrane will forever fail to function.

To the extent that synthetic cell creation is significantly limited by the divergence between donors and recipients, synthetic cell technology will require development of techniques that allow different bacterial taxa to serve as genome recipients. The technology would then have to move beyond genomic transformation of the cell-wall-lacking *Mycoplasma*.

What fundamental issues of microbial physiology, ecology, and evolution may be addressed with a synthetic-cell approach? One scientific motivation for the authors was their long-term interest in discovering the minimum genome and physiology required for cellular life, which up to now has focused on the small genomes of *Mycoplasma* [8]. The synthetic approach should foster exploration of minimalism by readily allowing elimination of genes from different starting points (other small-genome organisms, unrelated to *Mycoplasma*).

Beyond the search for the minimal organism, one could also use synthetic organisms to investigate the physiological, ecological, and evolutionary consequences of *inserting* genes. Bacteria frequently evolve into new ecological niches by acquiring sets of functionally related genes from other organisms by horizontal genetic transfer (HGT) [9]. Indeed, functionally related genes that work well in a diversity of foreign genetic backgrounds have evolved to form

consecutive, co-regulated gene sets (operons) that are easily transferable [10]. Synthetic organisms could allow exploration of the adaptive horizons potentially opened by HGT. For example, one could ask which of the thousands of operons previously known to have been transferred by HGT would be compatible and functional within a particular recipient organism of focus. This could provide an encyclopedia of predictions for future ecological diversity within a clade of bacteria.

Nevertheless, metabolic functions that can be placed on transferrable operons may constitute only a small part of physiological and ecological differences among bacteria. To my mind, the most potentially exciting discoveries will come from exploring the transfer of extremely complex physiological and structural components, which are not found on transferable operons, and might never be transferable in the context of ordinary, natural genetic exchange. The various divisions of bacteria, much like the phyla of animals, have profound differences in their organismal architecture (bauplans), and these differences (frequently in membrane structure) have been stable for billions of years [11,12]. For example, the Gram positive cell wall has not been transferred to other organisms, even though it would foster drought and re-wetting resistance [11,13].

With the potential to engineer large introductions of never-before-transferred and unlinked genetic material in synthetic cells, we may be able to explore completely novel ecological diversity in bacteria. Moreover, we may be able to retrace the evolutionary steps toward the bauplans of the major bacterial divisions. The challenge will be to identify the genes contributing to the bauplan; lacking a history of HGT, the component genes are not likely to be identifiable as pre-packaged transferable units. I expect that efforts to synthesize combinations of bauplans will complement ongoing bioinformatic efforts [14] to identify the functions of all a genome's components.

The debate on the safety and ethics of constructing synthetic microbes [15] will have to take into account that the wholesale recombination of genes in synthetic organisms may be far outside

of what has been possible in either nature or the laboratory. The safety of any proposed recombination will have to be weighed against its novelty and the possibility that it may never have occurred in the 3.5 billion years of evolutionary history.

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Bacterial Invasion: Entry through the Exocyst Door

Salmonella entry into host cells involves rearrangements of actin and mobilization of membranes. Here we discuss new findings showing that *Salmonella* recruits the exocyst complex, which plays a role in vesicle secretion, to the site of invasion to promote its entry.

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Exocytosis is a fundamental cellular process by which a cell secretes proteins or lipids. It involves the tethering, docking and fusion of intracellular vesicles with the plasma membrane in order to release their contents. Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are involved in the fusion of secretory vesicles with the plasma membrane (reviewed in [1]). The exocyst complex is thought to mediate the tethering of vesicles to the membrane prior to fusion and is a multimeric protein complex highly conserved from yeast to mammals [2]. It was first identified in the budding yeast *Saccharomyces cerevisiae*, where it plays an essential role in exocytosis. The exocyst is composed of eight proteins: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 [2].

In yeast it has been shown that Sec3 [3] and Exo70 are localized at the plasma membrane [4], whereas the other subunits are localized at the membrane of the secretory vesicles [4] (Figure 1A). Recent studies have shown that Sec3 and Exo70 are recruited to the plasma membrane by interacting with phosphatidylinositol 4,5-biphosphate (PI(4,5)P$_2$) [5,6]. In particular, the carboxy-terminal domain of Exo70 (domain D) contains a succession of basic residues that are essential for the recruitment of the exocyst to the plasma membrane (Figure 1A). Mutations of those residues lead to mislocalization of the exocyst and a severe defect in secretion [5]. Similarly, the amino-terminal domain of Sec3 contains a cluster of basic residues that are responsible for the interaction with PI(4,5)P$_2$ [6].

The localization and function of the exocyst is tightly regulated by interactions with several small GTPases from the Rab, Rho, Arf and Ras families (for extensive review, see [7]). Of particular interest is a member of the Ras family, the small GTPase RalA. Yeast two-hybrid screen and pulldown assays showed that Sec5 interacts with the GTP-bound form of RalA [8]. The first functional studies on RalA showed that inhibition of RalA expression disturbs assembly of the exocyst complex, leads to a mislocalization of basolateral membrane proteins and impairs delivery of secretory vesicles to the plasma membrane [8]. Since then, numerous studies have reported a critical involvement of RalA not only in secretion but also in membrane trafficking [9]. In a recent issue of *Current Biology*, Nichols and Casanova [10] describe a new role for the exocyst and RalA as targets of the bacteria *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium).

S. Typhimurium is a Gram-negative bacterial pathogen and a significant cause of food poisoning and gastrointestinal inflammation. Upon interaction with the plasma membrane of the host cell, *S.* Typhimurium activates the expression of a type three secretion system (T3SS) encoded by the *Salmonella* pathogenicity island 1 (SPI-1). The T3SS is a needle-like structure complex, which allows the bacteria to translocate bacterial proteins (called effectors) directly into the host cytoplasm. SPI-1 effectors are involved in actin rearrangements and formation of membrane ruffles that facilitate the internalization of the bacteria [11]. Actin rearrangements