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Transgenic commensals as mucosal protectants

Kevin J. Whaley and Larry Zeitlin

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As our first line of defense against the majority of infections, mucosal surfaces in the genitourinary, oral, gastrointestinal, and respiratory systems are obvious sites for delivering protective agents for preventing infectious disease. Unfortunately, the technology for mucosal protection is severely limited; for example, the world community has had few options for preventing sexual transmission of HIV. With the report by Beninati et al. in this issue of *Nature Biotechnology*[1], an additional option may be realized. The authors used a single-chain antibody fragment (scFv) for treating vaginal *Candida albicans* infections in a rat model. What makes this report distinctive is the combination of a novel use of an antibody (antiidiotype scFv) and a unique delivery system (transgenic commensal bacteria expressing the scFv).

Candida albicans, an opportunistic fungus, causes recurrent vaginal infections in a large percentage of women as well as oral and esophageal infections in the immunocompromised. By exploiting idiotypic networks, the investigators generated an scFv antiidiotype antibody (H6) that mimics the activity of a killer toxin from *Pichia anomala*[2]. The toxin has activity against a variety of fungal pathogens but is unsuitable for topical microbicides because of its instability at physiologic pH and temperatures. The investigators generated recombinant *Streptococcus gordonii*, an oral commensal bacteria, that either secreted or displayed H6 on the surface. These engineered bacteria were then used therapeutically to colonize the vaginas of rats with existing candidal infection. Although their study focused on *Candida* as an example, the authors point out that this technology may be broadly applicable to infections on all mucosal surfaces.

Delivery of agents such as antibodies and peptides to mucosal surfaces for hours to days of protection has been achieved using solutions, gels, aerosols, tablets, or films (Fig. 1A). In the vagina, antibodies have been incorporated into controlled-release devices for weeks to months of protection[3]. Alternatively, as described in this report, transgenic commensal bacteria can be used for sustained delivery (Fig. 1B). By this means, protection may last as long as the transgenic commensal remains. In this study, the transgenic commensals continued to colonize the rat vaginas for at least 15 days. It remains to be seen whether long-term colonization of the human vagina with *S. gordonii* can be maintained in the presence of intercourse, menses, and related daily fluctuations of vaginal flora[4]. It may prove preferable to use a vaginal commensal, e.g. *Lactobacillus*, that is adapted to the acidic pH of a healthy vagina. A further concern with the use of transgenic commensals that will need to be addressed is the stability of the antibody gene and its ability to transfect wild-type bacteria. This can at least in part be studied using green fluorescent protein as a reporter gene as described recently[5]. Finally, as with any new approach, the means for large-scale production must be developed and the regulatory pathway for transgenic commensals will need to be defined.

The importance of developing microbicides for protecting mucosal surfaces, in particular the vaginal and cervical mucosa, was recently highlighted at the Microbicides

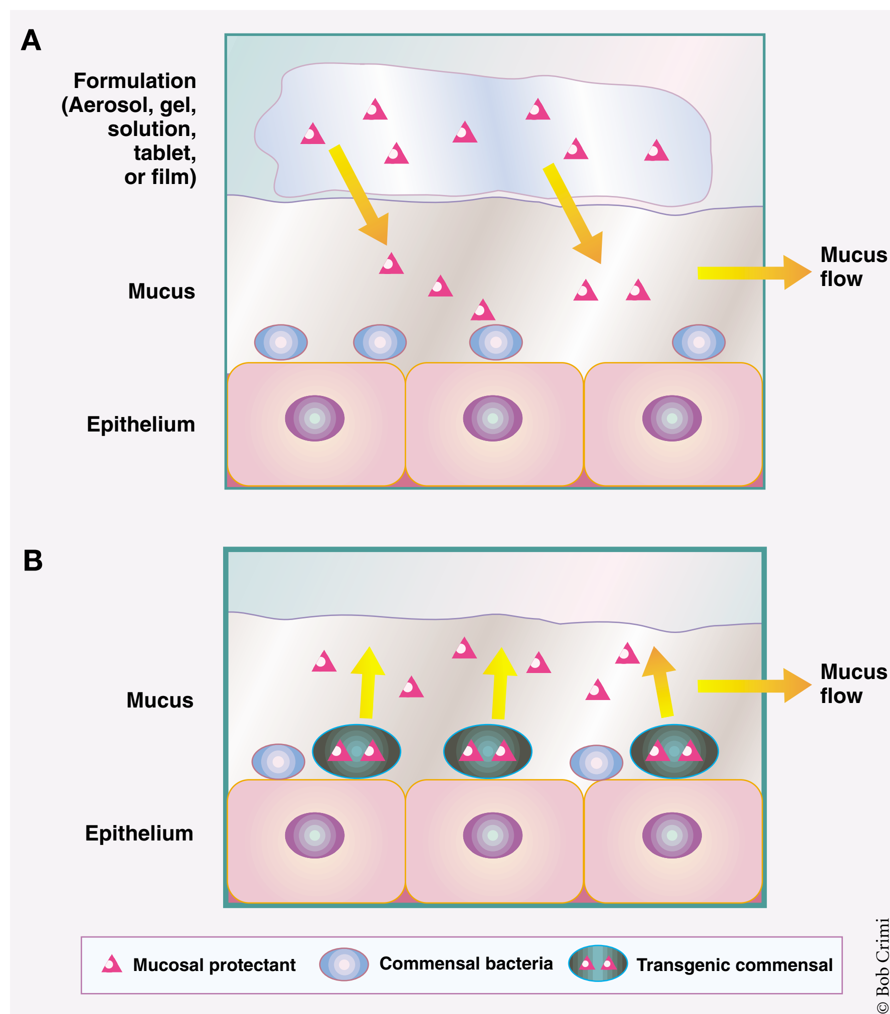

Figure 1. Protection afforded by different methods of delivery. (A) Conventional delivery of a aersol or tablet formulation affords only hours to days of protections. (B) Transgenic microbe delivery to the mucus affords days to months of protection.

Kevin J Whaley is director and research scientist at Epicyte Pharmaceutical (San Diego, CA), ReProtect LLC (Baltimore, MD), Johns Hopkins University (Baltimore, MD) (kwhaley@epicyte.com). Larry Zeitlin is research scientist at Epicyte Pharmaceutical (San Diego, CA) (lzeitlin@epicyte.com).

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2000 Conference sponsored by national and international agencies[6]. The development of mucosal protectants for vaginal microbicides is starting to increase markedly. In fact, the authors of the current study reported on an HIV-inactivating protein expressed in *S. gordonii* for use as a vaginal microbicide[7]. According to the Alliance for Microbicide Development (Silver Spring, MD), at least 60 vaginal microbicidal products (antibodies, peptides, acidic buffers, sulfated polymers) are in various stages of development.

There are currently no vaccines available for preventing sexually transmitted diseases (with the exception of hepatitis B) or reproductive health ailments such as urinary tract and *Candida* infections. Interestingly, the investigators in the current study have also used this transgenic commensal for delivery of vaccine antigens to mucosal surfaces[8]. This use of transgenic commensals for active as well as passive immunization highlights the intersection between the science of antibody-based mucosal microbicides and mucosal vaccines. Some of the best characterized antibodies, discovered through vaccine development efforts, are the same ones being developed as mucosal protectants. Conversely, evaluating the efficacy of antibodies delivered to mucosal surfaces may help determine the mucosal antibody concentrations necessary for vaccines to be protective[9].

One historic obstacle to evaluating the clinical efficacy of mucosal antibodies has been their high cost and limited production capacity. Now we can add the use of transgenic commensals to deliver therapeutic antibodies mucosally, to methods to produce antibodies in plants or animals[10] that have been developed to overcome this limitation. Another recent report describing delivery of therapeutic peptides[11] suggests that these reports are just the beginning of an exciting and potentially generally applicable strategy for delivery of a variety of protectants to mucosal surfaces.

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The single cell as a microplate well

K. Dane Wittrup

Given our incomplete understanding of protein structure/function relationships, directed evolution is currently the most rapid route to engineer desired protein properties, such as stability, affinity, activity, specificity. In particular, new applications of antibodies and enzymes have blossomed with the broad availability of inventive screening and mutagenesis strategies. In this issue, Olsen et al.[1] describe a bacterial display/cell surface capture approach to enzyme evolution that they applied to engineering of an unusual protease specificity.

Protein evolution requires linkage of a protein and its gene by some display framework, whereas enzyme evolution further requires trapping of a diffusible reaction product (Fig. 1). Although there is an abundance of display frameworks available[2], linkage options have been more limited for enzymatic reaction products. In the present work, Olsen et al. take clever advantage of the intrinsic negative charge of the bacterial cell wall to electrostatically trap reaction products from the cleavage of fluorogenic protease substrates. Given the nonspecific nature of this charge interaction, there is surprisingly little fluorophore crosstalk among cells, enabling single-pass enrichments of 5,000-fold. Electrostatic capture modules such as these may also find general use with a wide range of nonpeptide fluorogenic substrates. A key contribution of this work is that it makes enzyme screening amenable to the use of flow cytometry, a powerful but as yet underutilized screening tool for directed evolution.

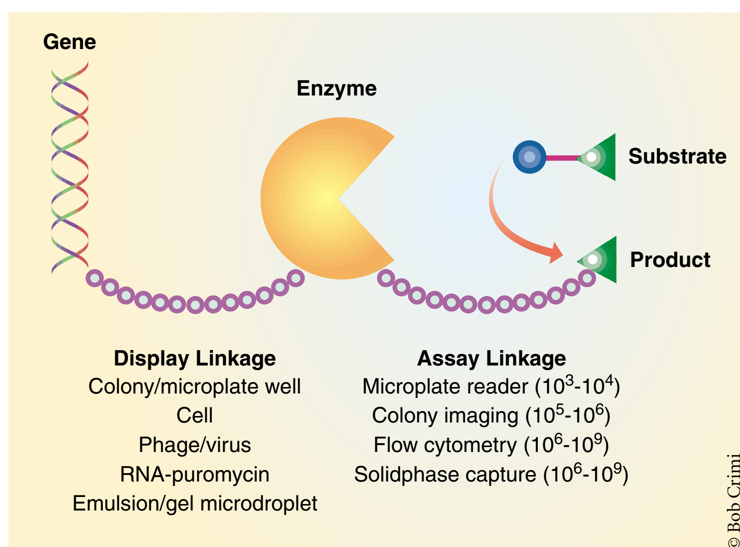

Figure 1. Necessary display and assay linkages for directed evolution of enzymes. The numbers next to the different assay types reflect typical library sizes, not theoretical limits.

The primary advantages of flow cytometry[3] lie in numbers—in screening of large libraries, and in quantitative assays. Superlative prefixes struggle to keep pace with the throughput of screening technology, as yesterday's HTS is eclipsed by today's UHTS. Following this trend, how many *U*'s must be appended to describe commercially available flow cytometers that quantify cellular multicolor fluorescence and sort 50,000 cells/s (the equivalent of about one million 1,536-well microplates per workshift)? In order to take advantage of this blazing speed, the challenge is to develop assays that confine the gene, enzyme, and reaction product to a cell-sized particle.

Is such exorbitant throughput necessary, when enzyme properties have been significantly improved using screens of only 10^3 or

K. Dane Wittrup is the J.R. Mares Professor of Chemical Engineering and Bioengineering at the Massachusetts Institute of Technology, Cambridge, MA 02139. (wittrup@mit.edu).

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