Pathogens on aspirin: promising research and therapeutic applications

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An aspirin-modulated gene expression control circuit provides a powerful method to regulate expression of bacterial genes inside an infected host. This should provide a safe and easy way to study host-pathogen interactions, and may have direct therapeutic applications.

The majority of microbiology research during the last century has been the study of microbes under well-defined laboratory conditions. Despite the value of these studies, pathogens exist in dynamic and complex environments in their hosts that are difficult to mimic in the laboratory. A typical infection cycle is a finely tuned process that may include all or some of the following events: attachment, invasion, colonization, multiplication, persistence and transmission[1]. Commonly, a particular virulence gene is required for one of the above events, which takes place in a specific location and at a defined time before, during or after the infection.

Understanding the precise role of a virulence gene in disease is a difficult task because of the many complexities of disease. In this issue of *Nature Methods*, Eduardo Santero and coworkers describe a creative approach that allows examination of the function of virulence genes inside the complex environment of the host organism and may even have relevance for cancer therapy[2].

To date, the most common approach to study gene function is a typical gene-to-phenotype strategy by means of gene inactivation, looking for a gross phenotypic defect. This 'all-or-none' approach is not always appropriate for the analysis of a complex or subtle phenotype as it does not allow accurate temporal and spatial analysis of the contribution of the gene product. Experimental approaches for functional investigation of genes using finely controlled expression systems ('on-off') *in vivo* are highly desirable but are not well developed for microbial studies *in vivo*.

Salmonella enterica is an example of a Gram-negative bacterial pathogen that infects animal and human hosts. The subspecies *S. enterica* serovar Typhimurium causes gastroenteritis in humans and calves, whereas in mice it leads to a typhoid-like systemic infection or intestinal inflammation in streptomycin-pretreated mice. Studies suggested that at least 4% of the *S. enterica* serovar Typhimurium genome is required for full virulence in mice[3]. Besides *S. enterica* being a common pathogen, it is also an excellent model to study host-pathogen interactions.

Several inducible expression systems that respond to external stimuli such as the tetracycline responsive bacterial tetracycline repressor (TetR) have been developed previously[4]. However, their use for studying host-pathogen interactions is limited by toxicity, side effects of their inducers and leakiness of their expression. In contrast, Santero and colleagues came up with an innovative approach using a common external stimulus to tightly control the expression of a heterologous gene carried by *S. enterica* during infection in mice[2]. They worked with an improved inducible system that uses acetyl salicylic acid (also known by the trade name aspirin) or salicylate as an external stimulus. These compounds are well studied, are inexpensive and are much safer inducers. This approach can be used to study the function of potential virulence genes *in vivo*, and to understand their time and place of action, and contribution to the infection process.

Several bacterial species including *Bifodobacterium*, *Clostridium* and *Salmonella* have been shown to infiltrate and selectively replicate within solid tumors upon systemic administration[5]. To demonstrate the feasibility of the new method, the authors exploited the ability of *S. enterica* to colonize tumor tissues and the anti-tumor activity of a prodrug-converting enzyme, cytosine deaminase (encoded by the *Escherichia coli codA* gene). Cytosine deaminase, which is not found in mammalian cells, converts the nontoxic compound 5-fluorocytosine into the active anti-tumor agent 5-fluorouracil[6]. Thus, only tissues in which cytosine deaminase is expressed (that is, *S. enterica*–colonized tissues) will be exposed to the cytotoxic derivative.

To achieve tight control of the cytosine deaminase expression, the researchers integrated into the chromosome of *S. enterica* a regulatory module, which is based on a circuit cascade of metabolic regulatory pathways from the bacterium *Pseudomonas putida* that has been previously used for the expression of heterologous genes[7]. Using this control circuit, the authors report high induction rates of reporter-gene expression after stimulation with salicylate in bacterial culture, macrophages, epithelial cells and tumor cells.

When they tested the salicylate-induced system *in vivo* they found that induction of cytosine deaminase expression by salicylate showed superior anti-tumor activity compared to that of a tetracycline-induced system, as judged by growth reduction of solid tumors colonized by *codA*-carrying *S. enterica* (**Fig. 1a**). These results demonstrate the ability of such a system to tightly control the expression of any gene of interest carried by a pathogen inside the host.

Such a conditional expression system could be applied for cancer therapy or diagnostic imaging, using *S. enterica* expressing reporter molecules to mark tumors. Despite the encouraging potential of this study, there are many questions that need to be addressed before clinical use. The efficiency of this technique in humans is not known, although the attenuated *S. enterica aroA* strain has been tested for safety and efficacy in clinical trials. Other questions include: 'What is the fate of the bacteria within the tumor? Are

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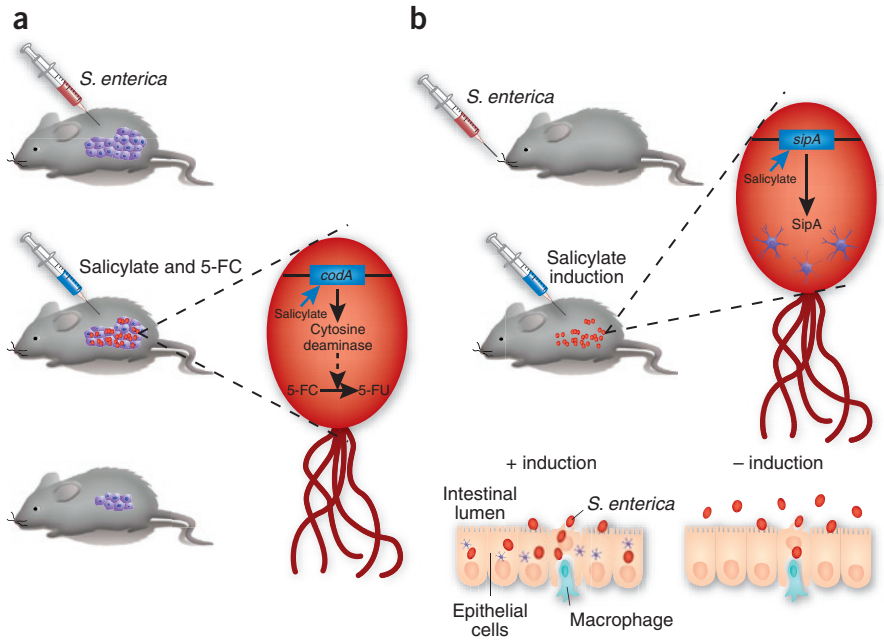

Figure 1 | Possible applications of the salicylate-modulated gene expression control circuit in *S. enterica* infecting a host. (**a**) In a therapeutic application, induction of *S. enterica codA* results in conversion of 5-fluorocytosine (5-FC) into the antitumor agent 5-fluorouracil (5-FU), and tumor growth is reduced. (**b**) In a possible research application, the effect of inducing expression of an invasion gene, such as *sipA*, on bacterial uptake into intestinal epithelial cells could be studied.

the bacteria subject to immune responses inside the tumor? And what is the effect of colonizing bacteria and cytosine deaminase expression in systemic sites, such as the spleen and liver?' Certainly answers to these and many other questions require additional studies, but the proof of principle, as well as the prospect of an effective cancer therapy approach and *in vivo* gene functional studies, are well documented in this report.

From a basic research standpoint this technology could be used to determine at what stage of a host-pathogen interaction a virulence factor is needed. For example, SipA is an *S. enterica* virulence effector protein required for invasion, which is injected into host cells by *S. enterica*[8]. In the absence of SipA, *S. enterica* invasion through intestinal epithelial cells is attenuated in a manner that can affect subsequent stages of the infection such as *S. enterica*–induced intestinal inflammation (enterocolitis). By replacing *sipA* with a salicylate-inducible copy of this gene an investigator could switch on its expression at sequential time points to reveal where and when during the infection process SipA is required to cause disease in mice (**Fig. 1b**).

Establishing the mechanisms by which pathogens interact with their hosts is essential for the rational design of new antimicrobial drugs and vaccines. The approach presented here, as a tool to study virulence genes during the infection, could lead to the identification of new antimicrobial drug targets and to new antigens that might be useful as vaccine components. Another clinical use could be the development of an *in vivo*–regulated system that allows controlled expression of heterologous antigens for use in live vaccines. At the very least, application of this technology will be extremely useful to better define where and when a particular virulence factor is required for the disease process.

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