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Review

Tracking bacterial pathogens with genetically-encoded reporters

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ABSTRACT

During the infectious process, bacterial pathogens are subject to changes in environmental conditions such as nutrient availability, immune response challenges, bacterial density and physical contacts with targeted host cells. These conditions occur in the colonized organs, in diverse regions within infected tissues or even at the subcellular level for intracellular pathogens. Integration of environmental cues leads to measurable biological responses in the bacterium required for adaptation. Recent progress in technology enabled the study of bacterial adaptation in situ using genetically encoded reporters that allow single cell analysis or whole body imaging based on fluorescent proteins, alternative fluorescent assays or luciferases. This review presents a historical perspective and technical details on the methods used to develop transcriptional reporters, protein–protein interaction assays and secretion detection assays to study pathogenic bacteria adaptation in situ. Finally, studies published in the last 5 years on gram positive and gram negative bacterial adaptation to the host during infection are discussed. However, the methods described here could easily be extended to study complex microbial communities within host tissue and in the environment.

1. Introduction

In their host, microbes are exposed to varying growth conditions that are chiefly influenced by intrinsic bacterial factors such as the density and aggregation state, the subcellular distribution within host cells, the localization within a given tissue and the organ(s) colonized (Fig. 1). Throughout evolution, bacteria have acquired the capacity to sense their environmental input in order to generate the appropriate biological output for adaptation. This review will focus on genetically encoded reporters used for studying adaptation of bacterial pathogens to their environment within host cells tissues. Examples of the most recent studies on the use of such reporters in gram negative and gram positive pathogens will be presented after a short historical and technical perspective on the design of the various types of reporters that have been developed.

1.1. Transcriptional reporters

The quest for assays to monitor the adaptation of microbes to their environment is an endeavor that originated from the very cradle of molecular biology, as illustrated by the studies on *Escherichia coli* adaptation to growth on galactose that led to the description of the operon model by Jacob and Monod [1]. The potential of β-galactosidase, encoded by the lacZ gene, for monitoring the activity of ectopic promoters was rapidly understood (reviewed in [2]). However, β-galactosidase needs to first be recovered by lysis of the bacterium before it can be assayed, thus β-galactosidase reporters do not preserve contextual information and allow only for interrogating bacterial adaptation or interactions with their host indirectly. With the discovery of Green Fluorescent Protein (GFP), and particularly its optimization for expression and maturation in bacteria [3,4], studies of host pathogen interaction in situ using bacterial transcriptional reporters became a reality and led, for example, to the identification of genes expressed by *Salmonella typhimurium* and *Mycobacterium tuberculosis* specifically inside macrophages [5,6]. In addition, due to its intrinsic fluorescence and therefore the simplicity to measure promoter activity, GFP-based assays are amenable to exhaustive studies of promoters activity within a genetically tractable bacterial model [7].

Currently GFP-based assays are preferable to structurally homologous Red Fluorescent Protein (RFP) from cnidarians because of the slower maturation of the latter [8,9]. Further discovery and optimization of far-red fluorescent proteins (FP) with fast

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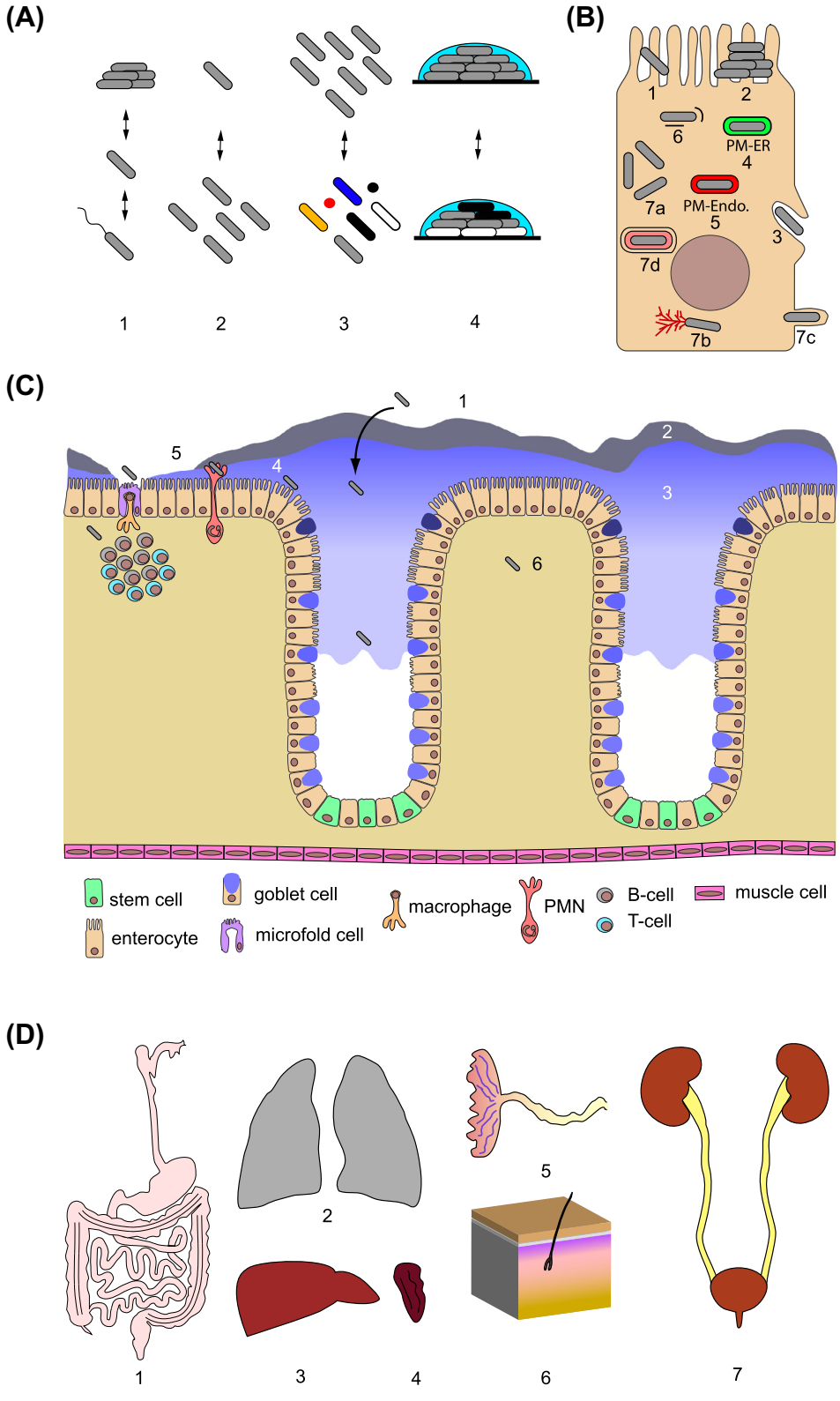

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