SESSION VIII—KEYNOTE ADDRESS

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CLOSTRIDIUM DIFFICILE: SPORULATION, GERMINATION AND TOXIN GENE EXPRESSION

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Clostridium difficile, the primary causative agent of antibiotic-associated diarrhea and pseudomembranous colitis, is a strictly anaerobic, Gram-positive, spore-forming bacterium. The primary factors in *C. difficile* pathogenesis are two large toxin proteins that glycosylate members of the Rho family of host cell GTPases. Synthesis of the toxin proteins is tightly regulated in response to various environmental conditions, particularly the quality of the nutritional environment. During growth in a complex medium in the laboratory, the toxin genes are induced at the end of exponential growth phase, as the cells sense limitation of certain key nutrients. At least four regulatory factors control the expression of the toxin genes. (i) Transcription of the genes is directly dependent on recognition of their promoters by TcdR, an alternative sigma factor for RNA polymerase. (ii) TcdR is expressed at low level during exponential growth phase, but this low level of TcdR does not permit toxin gene expression because TcdR is inhibited by TcdC, an anti-sigma factor that is made preferentially during exponential growth phase. Interestingly, the recent outbreak of highly virulent *C. difficile* strains is associated with mutations that inactivate TcdC. (iii) The induction of *tcdR* and toxin gene expression during early stationary phase is due to inactivation of both TcdC and a global regulatory protein, CodY, that represses *tcdR* when cells are growing rapidly. CodY responds to two co-repressors, GTP and the branched-chain amino acids isoleucine, valine, and leucine. It is presumed that the intracellular pools of these effectors decrease when cells make the transition from exponential growth to stationary phase. (iv) Finally, toxin synthesis is blocked even in stationary phase when cells are grown in a medium that contains an excess of a rapidly metabolized carbon source, such as glucose. The mechanism of this regulation is unknown.

Although toxin proteins made by early stationary phase cells are the major virulence factors for *C. difficile*, the infectious form of the bacterium is the dormant spore. Thus, for exposure to *C. difficile* to lead to disease, the spores must germinate in the intestinal tract and grow out as vegetative cells. Germination requires the bacteria to sense the presence of certain nutrients. For *C. difficile* spores, one such germinant has proved to be the combination of glycine and a primary bile salt. Some secondary bile salts, such as deoxycholate, are also germinants (with glycine), but the secondary bile salts also inhibit growth of *C. difficile*. Thus, the secondary bile salts, which are produced by metabolism carried out by members of the normal intestinal flora, may be important for protection of healthy individuals against *C. difficile*-associated disease.