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Substrate-Activated Conformational Switch on Chaperones Encodes a Targeting Signal in Type III Secretion

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SUMMARY

The targeting of type III secretion (TTS) proteins at the injectisome is an important process in bacterial virulence. Nevertheless, how the injectisome specifically recognizes TTS substrates among all bacterial proteins is unknown. A TTS peripheral membrane ATPase protein located at the base of the injectisome has been implicated in the targeting process. We have investigated the targeting of the EspA filament protein and its cognate chaperone, CesAB, to the EscN ATPase of the enteropathogenic *E. coli* (EPEC). We show that EscN selectively engages the EspA-loaded CesAB but not the unliganded CesAB. Structure analysis revealed that the targeting signal is encoded in a disorder-order structural transition in CesAB that is elicited only upon the binding of its physiological substrate, EspA. Abrogation of the interaction between the CesAB-EspA complex and EscN resulted in severe secretion and infection defects. Additionally, we show that the targeting and secretion signals are distinct and that the two processes are likely regulated by different mechanisms.

INTRODUCTION

The type III secretion (TTS) system is a multiprotein machinery that has evolved to deliver bacterial virulence proteins directly into eukaryotic cells through an organelle termed the injectisome (Cornelis, 2006; Galán and Wolf-Watz, 2006). The TTS substrates (needle-forming proteins, effectors, and translocators) are targeted to the cytoplasmic base of the injectisome and hierarchically secreted through the channel (Izoré et al., 2011). In the cytosol, TTS substrates are typically found as complexes with their cognate chaperones (Birtalan et al., 2002; Page and Parsot, 2002; Feldman and Cornelis, 2003; Francis, 2010), which have established roles as antiaggregation and stabilizing factors for TTS substrates. It has been hypothesized that chaperones may also act as signals for targeting and hierarchy-determining factors (Birtalan et al., 2002; Lilic et al., 2006; Rodgers et al., 2010; Lara-Tejero et al., 2011).

A key protein in TTS systems is the ATPase (Woestyn et al., 1994; Pallen et al., 2005), a peripheral membrane protein located at the entrance of the injectisome. Biochemical experiments have provided evidence that the TTS ATPase protein, which is ubiquitous to all TTS systems, may serve to recognize and engage the TTS proteins at the injectisome (Gauthier and Finlay, 2003; Akeda and Galán, 2005; Thomas et al., 2005; Boonyom et al., 2010; Cooper et al., 2010). The ATPase is located at the cytoplasmic base of the injectisome and forms a ring structure (Müller et al., 2006) that resembles the F_1F_0-ATPase (Pallen et al., 2006; Imada et al., 2007; Zarivach et al., 2007). The molecular basis for the targeting of TTS substrates to the ATPase remains completely unknown.

We studied this targeting process in enteropathogenic *E. coli* (EPEC), the archetype of a group of pathogens that adhere to host enterocytes via the formation of attaching and effacing lesions and cause extensive host cell cytoskeletal rearrangements (Dean and Kenny, 2009). When secreted, EspA undergoes self-polymerization, thereby forming a long extracellular filamentous extension that coats the needle and connects it to the translocation pore in the eukaryotic plasma membrane, and most likely acts as a molecular conduit for TTS protein translocation (Knutton et al., 1998). EspA has a high tendency to self-oligomerize and, thus, is retained in a monomeric, soluble state in the cytoplasm by forming a complex with the CesAB chaperone (Creasey et al., 2003; Yip et al., 2005).

Here, we show that the homodimeric CesAB chaperone exists in a partially unfolded state and does not interact with the EscN ATPase. In contrast, the formation of the CesAB-EspA chaperone-substrate complex results in a strong affinity for EscN. Structural analysis demonstrated that the binding of EspA to CesAB results in the extensive folding of many regions in the chaperone. The induced structure in one of these regions is specifically recognized by EscN, and it mediates the formation of the ternary EscN-CesAB-EspA complex. Interestingly, a homodimeric CesAB variant designed to adopt a folded structure

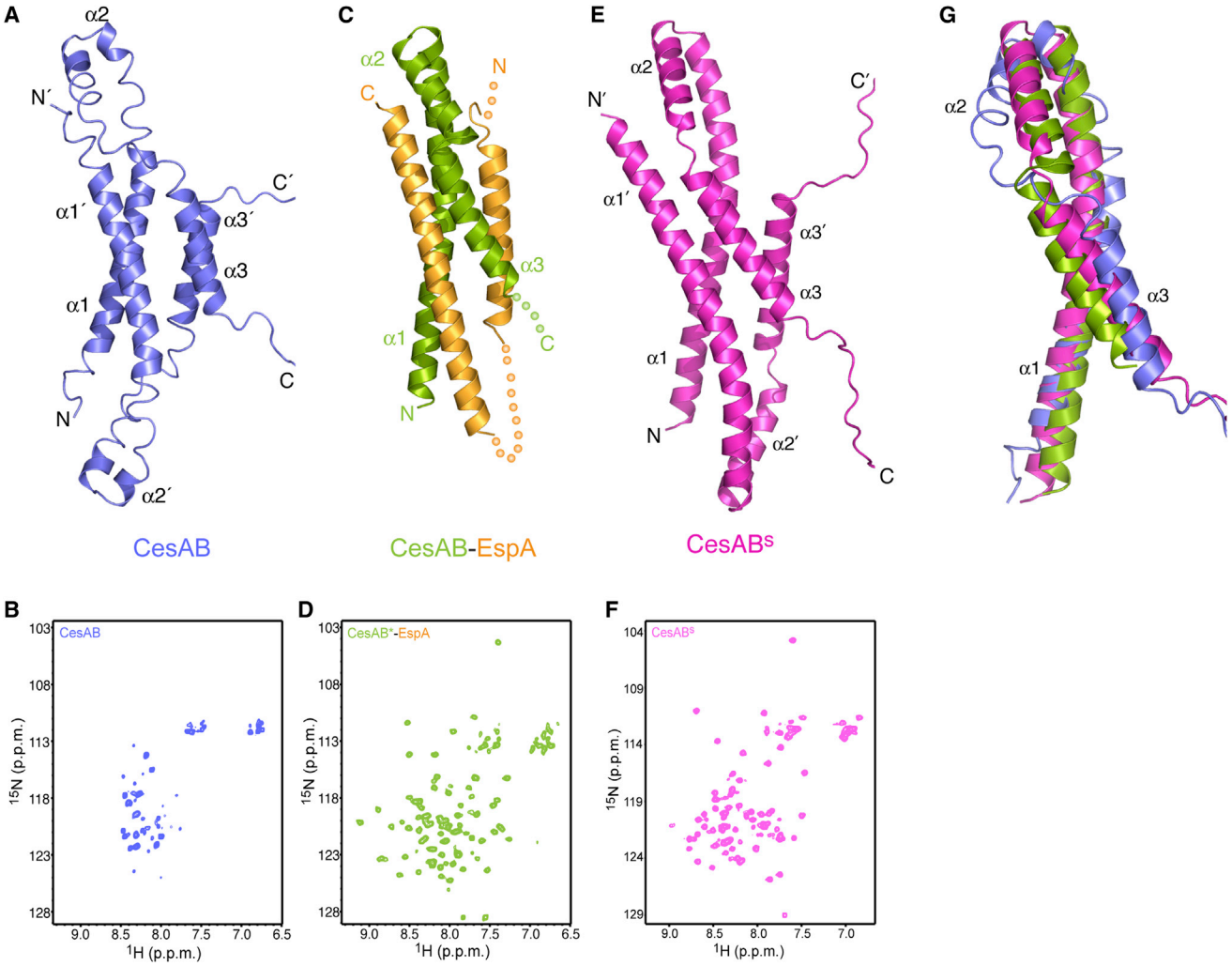

Figure 1. Structures of CesAB, CesAB-EspA, and CesAB^S

(A) The solution structure of the homodimeric CesAB, which adopts a molten-globule-like structure in solution (Chen et al., 2011).

(B) The ^{1}H-^{15}N HSQC spectrum of CesAB.

(C) The crystal structure of the heterodimeric CesAB-EspA (Yip et al., 2005). Regions of the proteins that were not crystallographically resolved are represented as dotted lines.

(D) The ^{1}H-^{15}N HSQC spectrum of CesAB-EspA. The CesAB subunit is ^{15}N labeled, whereas the EspA subunit is unlabeled.

(E) The solution structure of the CesAB^S variant (D14L-R18D-E20L), as determined in this work.

(F) The ^{1}H-^{15}N HSQC spectrum of CesAB^S.

(G) Superposition of the CesAB subunit of CesAB, CesAB-EspA, and CesAB^S.

similar to the one induced by EspA binding is capable of interacting with EscN. Amino acid substitutions in the EscN-interacting CesAB region abrogate the targeting of CesAB-EspA to EscN, resulting in severe secretion and infection defects.

RESULTS

The Substrate-Free CesAB Chaperone Does Not Interact with the EscN ATPase

In the absence of its substrate EspA, CesAB exists as a loosely packed, conformationally dynamic homodimer in solution (Chen et al., 2011) (Figures 1A and 1B). CesAB adopts a four-helix bundle structure with each of the subunits in an all-helical conformation consisting of three helices of variable stability (Chen et al., 2011) (Figure 1A). We used nuclear magnetic resonance (NMR) spectroscopy, which is a very sensitive reporter of even transient binding interactions (Takeuchi and Wagner, 2006), to test whether CesAB interacts with the ATPase EscN (Gauthier and Finlay, 2003; Zarivach et al., 2007). For this reason, we prepared full-length EscN, which, as we show here, forms a stable hexamer in solution with stimulated ATPase activity (Figures S2A and S2C). The NMR data show that none of the CesAB resonances are affected by the addition of EscN (Figure S2D), thereby demonstrating that there