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Proteolytic Regulation of CtrA, the Master Regulator of Cell Cycle in Caulobacter crescentus

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Abstract
Cell cycle progression in *Caulobacter crescentus* depends on the master regulator, CtrA. During the transition from swarmer to stalk cell (G1 to S phase), CtrA is degraded by the AAA+ protease ClpXP and levels rise again in the predivisional stage. The focus of this work is to explore how cyclic, regulated degradation is controlled. CtrA is known to bind to the origin of replication, thereby suppressing replication, so we first asked if DNA binding had an effect on CtrA stability. CtrA is readily degraded by ClpXP on its own, but when bound to DNA containing the proper binding

sites, degradation is inhibited. Stabilization is dependent on DNA binding, as CtrA mutants deficient in DNA binding show the same degradation regardless of addition of DNA, as does CtrA in the presence of a mutant origin sequence lacking CtrA binding sites. Looking closely at CtrA degradation in the presence of auxiliary factors suggests that higher order complex formation may be a mechanism of protecting critical cell cycle regulators from premature proteolysis. *In vivo* study of over-expression of CtrA mutants revealed that accumulation of non-degradable CtrA, CtrA-DD, perturbs the cell cycle, leading to filamentation and a G2 arrest. Over-expression of the DNA-binding domain alone showed filamentation but no G2 arrest, suggesting that CtrA-DD is detrimental for reasons including, but likely not limited to, its ability to bind DNA. Exogenously expressing other domains of CtrA may further elucidate the mechanism of its regulated degradation *in vivo*.

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