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## **Role of the c-di-GMP in the biofilm induction by UVA in *Pseudomonas aeruginosa***

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*Pseudomonas aeruginosa* is a versatile opportunistic pathogen known for causing severe infections in immunocompromised individuals. Its adaptability to various environments is attributed to a complex regulatory network that modulates gene expression in response to stress. Also, *P. aeruginosa* exhibits a remarkable ability to form biofilms, which are crucial for its pathogenicity. In natural environmental, *P. aeruginosa* is exposed to solar UVA radiation (400-320 nm), which is the primary fraction of UV light reaching Earth's surface. High doses of UVA are lethal due to reactive oxygen species, while low doses cause oxidative damage and trigger adaptive responses, including biofilm formation. The transition from planktonic to biofilm mode is regulated by cyclic diguanylate (c-di-GMP), a central messenger molecule. The synthesis and degradation of c-di-GMP are controlled by diguanylate cyclase (DGCs) and phosphodiesterase (PDEs) enzymes. This study explores the role of c-di-GMP in biofilm formation induced by UVA. *P. aeruginosa* PAO1 was grown under UVA or dark conditions, and biofilm formation was assessed. Using the *PcdrA-gfp* reporter, which is positively regulated by c-di-GMP, we measured intracellular c-di-GMP levels. UVA exposure significantly increased fluorescence (15, 30 minutes  $p < 0.005$ ; 60, 90 min  $p < 0.05$ ). We then examined whether UVA-induced c-di-GMP levels correlate with changes in DGC and PDE gene expression. UVA exposure resulted in significant upregulation of PA3177, PA1120 ( $p < 0.0005$ ), *sadC*, *wspR* ( $p < 0.05$ ), but no changes in *siaD*. Conversely, *bifA* and *rdbA* expressions were significantly reduced ( $p < 0.005$ ,  $p < 0.05$ ) under UVA. Considering that c-di-GMP interacts with other signaling systems, including the stringent response mediated by ppGpp, we investigated if ppGpp regulates c-di-GMP induction by UVA. In a *relA* mutant strain, deficient in ppGpp production, biofilm induction was not observed under sublethal UVA. The *PcdrA-gfp* reporter in the *relA* mutant showed no fluorescence increase in response to UVA. Additionally, in the *relA* mutant, UVA did not upregulate PA3177, PA1120, *sadC*, or *wspR*, and there were no changes in *siaD* expression, similar to wild-type observations. However, *bifA* and *rdbA* were significantly downregulated by UVA as in the wild type. In summary, these results highlight the crucial role of c-di-GMP in biofilm formation following UVA exposure. UVA radiation enhances c-di-GMP levels through the modulation of DGCs and PDEs, and this process is at least partially regulated by ppGpp.

Palabras clave: *Pseudomonas aeruginosa*- ultraviolet radiation (UVA)- biofilm- c-di-GMP