Abstract

Toxin-antitoxin systems are small genetic elements with a wide variety of proposed biological functions, uses in biochemistry and potential applications in medicine. Of the eight types currently known, type II is the most studied and abundant toxin-antitoxin system type, consisting of a protein toxin which is inactivated by direct binding of its protein antitoxin. This thesis was centred on the regulation mechanism of these type II toxin-antitoxin systems. Their regulation is notable due to its often-complex nature. Most commonly, antitoxin binds and represses its own promoter region. If toxin is present, its binding to antitoxin affects antitoxin-DNA binding capabilities. We focused on two effects that toxin binding can achieve: 1. toxin association weakens DNA-binding and causes de-repression (anti-cooperative regulation) and 2. toxin association can enhance DNA-binding up to a certain molar ratio followed by de-repression (conditionally cooperative regulation).

While many type II regulation mechanisms have been studied in great detail *in vitro*, there are few extensive investigations of their *in vivo* behaviour. We aimed to establish an expression system which would allow for toxin/antitoxin ratio-dependent control of reporter protein production. For this purpose, we tested IPTG and L-arabinose as potential antitoxin and toxin inducers, respectively. IPTG and L-arabinose are prone to producing an “all-or-nothing” response when added to cells. In order to achieve tuneable gene expression, we used *LacY*-deficient strain Tuner[DE3] and constant expression of *araE* transporter gene. These modifications proved to be effective and allowed us to independently express *sfGFP* in a tuneable manner. Based on these results, we constructed a pRAT-sfGFP vector base for the study of type II toxin-antitoxin systems in *E. coli*.

The work of our research group and collaborators then allowed us to elucidate the anti-cooperative regulation mechanism of *Vc*HigBA2. *In vitro* data detailing the disorder-based anti-cooperativity was corroborated by data obtained from constitutive expression of system in *E. coli* along with pRAT-sfGFP-based experiments. The main driving force of anti-cooperativity is antitoxin N-terminus, being both a stabilizing agent in DNA-binding and the main toxin-antitoxin interface. When bound to toxin, this N-terminus no longer stabilizes DNA-binding and thus leads to de-repression.

We then investigated systems *Pv*HigBA, *Pp*GraTA, *Ec*MqsRA, *Ec*RelBE, *Ec*MazEF, *Ec*CcdBA and *Ep*PhdDoc using pRAT-sfGFP. We were able to compare promoter strength and expression profiles of these systems. We found that plasmid-borne system promoter strength is stronger. We also noticed that regulation mapping of previous work qualitatively aligned with our results in the case of *Pv*HigBA and *Pp*GraTA, whereas *Ec*MqsRA, *Ec*RelBE, *Ec*MazEF, *Ec*CcdBA and *Ep*PhdDoc require further investigation.

While promising, there are still issues that need to be addressed for more accurate regulation mapping, namely the potential residual toxicity of enzymatically inactive toxins, the correct inducer levels for conditional cooperativity, antitoxin leaking and the detection of toxin and antitoxin protein molecules from expression experiments. Still, our results provide an interesting new framework for the study and comparison of type II toxin-antitoxin systems.

Keywords: toxin-antitoxin, tuneable gene expression, *Vc*HigBA2, regulation mapping, IPTG, L-arabinose