Identification of novel drug targets using model organisms

Anthony Brzoska [1], Ryan Withers [1], Kylie Turner [2], Andrew Robinson [3], Nick Dixon [3], Liz Harry [2] & Peter Lewis [1]

1. School of Environmental and Life Sciences, University of Newcastle, Callaghan NSW 2308
2. Institute for the Biotechnology of Infectious Diseases, University of Technology, Sydney NSW 2007
3. School of Chemistry, University of Wollongong NSW 2522

Antibiotic resistance is an endemic problem within hospitals worldwide, and is becoming an increasing problem within the general community. Traditionally, physicians and the public have relied on the belief that as bacteria acquired resistance to one antibiotic, new drugs would be made available that could be used to combat those infections. The appearance of vancomycin-resistant Enterococcus (VRE) infections in the 1990s, combined with the withdrawal of funding for antimicrobial drug discovery and development by big Pharma, has led to the realisation that we can no longer assume that all infections can be treated with a 'magic bullet'. Recent years have seen the emergence of infections that are resistant to all clinically available antibiotics, including newly released drugs such as tigecycline [1, 2]. The cupboard is bare. Or at least it is heading that way.

Fear not, all is not lost! There is a considerable amount of activity in molecular microbiology labs worldwide directed towards the establishment of new targets for antibiotic development, with some very promising drugs in the pipeline. Traditionally, enzymic reactions such as those involved in cell wall synthesis (e.g. penicillin), ribosome activity (e.g. tetracycline) or DNA topology (e.g. quinolones) have been targets of successful clinical antibiotics. Many of the new generation anti-microbials are derivatives of these drugs (tigecycline is a modified tetracycline), which may account for the perception that antimicrobial development is fulfilling the law of diminishing returns. However, an area receiving increasing attention in all areas of drug design, including antimicrobials, is the targeting of protein-protein interactions, and this is being greatly aided by extensive molecular microbiology experience with model organisms.

Cell division, DNA replication and transcription are essential processes dependent on extensive protein-protein interactions that involve many proteins unique to bacteria. Hence they offer outstanding opportunities for targeting protein-protein interactions for new approaches to antimicrobial development. Cell division complexes contain several highly conserved elements across the eubacterial kingdom (e.g. the tubulin homologue FtsZ), as well as some restricted to a much smaller range of distribution [3]. Many cell division proteins are also extracytoplasmic, making them excellent targets for validation as any potential drug does not need to cross the cytoplasmic membrane to gain access to its target. DNA replication requires a complex and dynamic assembly of proteins to ensure the catalytic subunits are able to progress rapidly and processively throughout the replication cycle, and protein-protein interactions are involved in the coordination of many essential processes such as lagging strand priming, replication fork progress, single strand capture during lagging strand synthesis, and coupling of leading and lagging strand synthesis [4].

Likewise, there are many essential protein-protein interactions in transcription, the best characterised being the interaction of the initiation σ factor with RNA polymerase that ensures transcription starts at the correct location upstream of a gene [5]. Transcription factors are also required for the efficient production of rRNA, coupling transcription and translation, coupling transcription with DNA repair, and efficient termination [6].

There is much we do not yet understand about the dynamic assembly and disassembly of these various complexes, as well as whether we have identified all the protein actors. By undertaking comprehensive protein-protein interaction studies on these essential processes, we will gain a greater understanding of microbial biology, and also identify potential new 'Achilles heels' for antimicrobial development.

In addition to identifying novel proteins, it is also essential to understand the molecular events in the assembly of multi-protein complexes. Cell division in nearly all bacteria requires the activity of the tubulin homologue FtsZ, a GTPase that polymerises into a ring at mid cell; this ring represents the earliest detectable sign that cell division has been initiated. The FtsZ ring then constricts concomitantly with cytoplasmic membrane invagination and septum synthesis (Figure 1). FtsZ-FtsZ and FtsZ-division protein interactions are required to ensure the success of this process. Coccoid cells such as those produced by *Staphylococcus aureus*

only synthesise peptidoglycan when forming division septa, and so cell division is also essential for growth in such organisms. If FtsZ ring formation or constriction is prevented in rod-shaped cells, they only survive three to four generations until they die by cell lysis (Figure 1).

For other bacteria such as *S. aureus,* cell division is the sole mode of cell growth so antibiotics targeting cell division will be bactericidal. Several compounds from both natural libraries and made through rational design that inhibit FtsZ ring formation have now been identified [7, 8]. These compounds hold promise for development of a completely new class of effective antimicrobials.

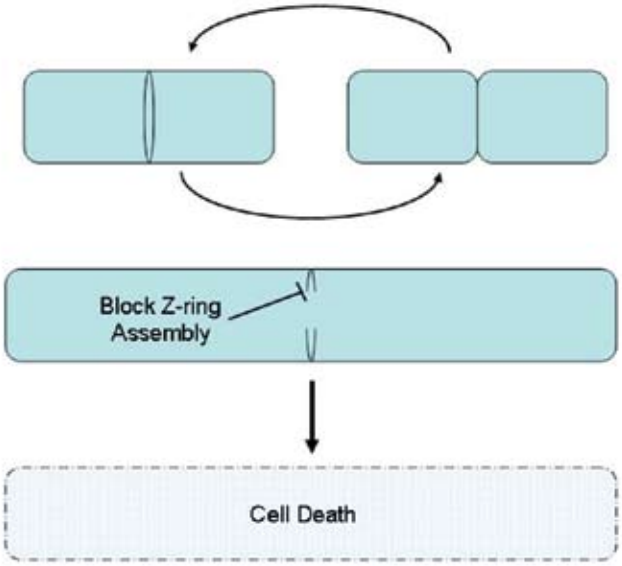

Figure 1. Inhibition of cell division leads to death. FtsZ forms a ring at mid cell early in the division cycle. Extensive interactions with other cell division proteins are required for the formation of a functional division apparatus. If FtsZ ring, or assembly of the division complex is blocked, rod-shaped cells form filaments which ultimately lyse.

Another essential protein-protein interaction showing promise as a target is that between RNA polymerase and its initiation factor σ (Figure 2). If RNA polymerase is unable to interact with σ, it cannot initiate transcription and cells die. Crystal structures of RNA polymerase in complex with σ [9] indicate that there are extensive contacts across a large interface, which might not traditionally be considered a good target. Nevertheless, Andre *et al.* [10] identified a series of small molecules that inhibit the RNA polymerase-σ interaction, indicating that even an extensive interaction surface can be effectively targeted. Ribosomal assembly is also being targeted for drug development [11], as is the assembly of the DNA replication apparatus (Figure 3 [12]).

Further understanding of essential processes in model organisms such as the Gram-negative *E. coli* and Gram-positive *B. subtilis* is crucial for the success of this type of work because of the vast amount of information already available on them, our general expertise in manipulating them genetically, and the large collections of strains available in private and public collections. To gain a comprehensive understanding of similarities and differences among these processes across the eubacteria, additional models need to be explored, such as the highly tractable *Acinetobacter baylyi* [13]. There is ample evidence that molecular microbiology research projects utilising carefully chosen model organisms can provide important information, not just on the fundamental aspects of biological processes, but also on the validation of targets for the development of desperately needed antibiotics.

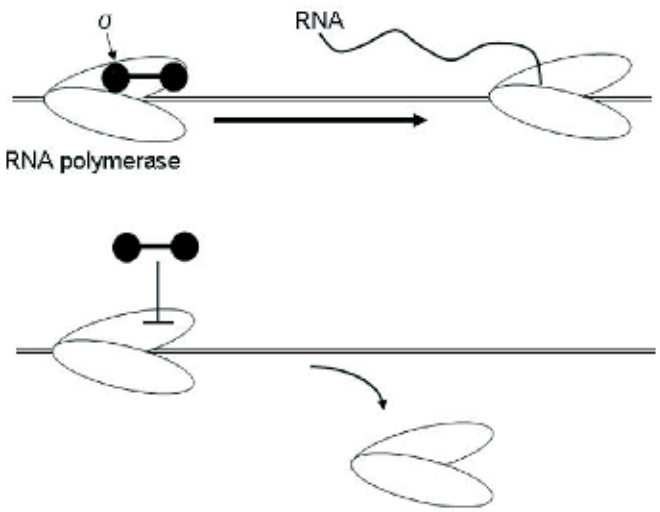

Figure 2. Targeting assembly of transcription complexes. RNA polymerase interacts with a σ factor in order to correctly initiate transcription. Prevention of formation of an initiation complex through inhibition of the interaction of σ with RNA polymerase will lead to a cessation of transcription and cell death.

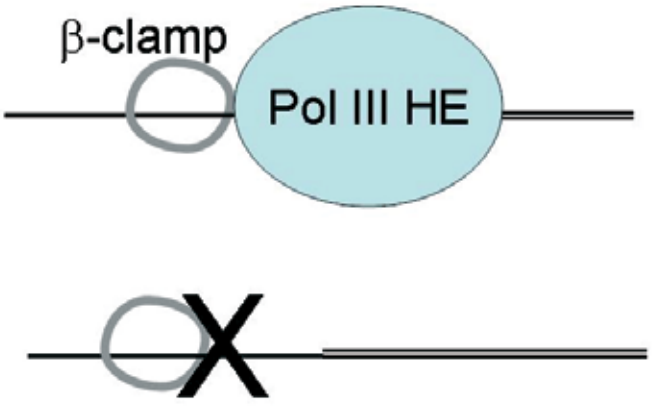

Figure 3. Inhibition of the interaction of the ß-clamp with DNA polymerase III holoenzyme. DNA polymerase III holoenzyme requires interaction with the ß-clamp in order to assemble on DNA to initiate DNA replication. Inhibition of this process leads to cell death due to lack of chromosome replication.

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A/Prof Peter Lewis is team leader of the Molecular Microbiology lab at the University of Newcastle, NSW.

Prof Nick Dixon is an ARC Professorial Fellow and team leader of the Biological Chemistry lab at the University of Wollongong, NSW

A/Prof Liz Harry is team leader of the Medical and Molecular Biosciences lab at the University of Technology, Sydney NSW

Drs Anthony Brzoska, Kylie Turner and Andrew Robinson are NHMRC-funded postdoctoral scientists in the labs of Peter Lewis, Liz Harry and Nick Dixon, respectively.

Ryan Withers is a University of Newcastle funded PhD student.

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