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► To cite this version:

Guillaume Duménil. Type IV Pili as a Therapeutic Target. Trends in Microbiology, 2019, 27, pp.658 - 661. 10.1016/j.tim.2019.05.005 . hal-03487955

HAL Id: hal-03487955

<https://hal.science/hal-03487955>

Submitted on 20 Dec 2021

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Type IV pili as a therapeutic target

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In the age of antibiotic resistance, strategies targeting virulence traits of bacteria are the focus of intense study. Two such studies came out independently a week apart showing that bacterial type IV pili are a promising therapeutic target.

Keywords: antibiotic resistance, anti-virulence, type IV pili, sepsis, meningitis

Anti-virulence as a solution to antibiotic resistance?

Antibiotic resistance has been recognized as a major problem for many years and is now frequently encountered in clinical settings. Available efficient and broad-spectrum antibiotics are increasingly limited in number. Industrial attempts to develop new molecules are thwarted by the time and effort needed and also precisely because of their limited use due to the rapid emergence of resistance. Endeavors coming from academic laboratories to fully describe molecular mechanisms of infection provide valuable leads towards the development of innovative therapies. In addition, therapies designed to block virulence rather than killing bacteria may slow down the emergence of resistance [1]. Virulence-related strategies likely impose less selective pressure on bacterial communities by affecting only a limited number of species and by restricting survival of bacteria only in specific environments. Given the increasing realization of the importance of commensal bacteria, treatment that preserves these bacterial communities should be favored.

Bacterial nanomachines as a target for innovative anti-virulence therapeutics

Disease-causing bacteria have a number of properties that allow them to colonize specific niches in the host, cross barriers in tissues and avoid the immune system. Such properties are made possible by the expression of a diverse set of virulence factors. Of particular interest from the therapeutic point of view, bacterial pathogens have evolved a number of surface-localized complex nanomachines to interact with their host, which broadly fall into two classes: secretion and piliation systems [2]. Release of proteins as well as other types of molecules through secretion systems into the extracellular milieu and/or directly into host cells allows pathogens to modulate their environment to their benefit. Several inhibitors of type III and type IV secretion systems have been identified [2]. Piliation systems that allow bacteria to interact with each other, to adhere to and move on cellular surfaces have also been targeted. Pilicides are a typical example of such inhibitors that affect the biogenesis of the chaperone-usher class of pili by blocking the function of chaperones necessary for the insertion of subunit components into the pilus fiber [3].

Type IV pili are essential virulence factors in numerous human pathogens

Despite their broad distribution, inhibitors of type IV pili had not been identified until two recent studies [4, 5]. These adhesive filamentous structures (Figure 1) have the unique property to be highly dynamic by undergoing repeated and rapid cycles of extension and retraction [6]. Type IV pili *a priori* constitute a target of choice as they are necessary for the infectious process of a long list of human pathogens. For

instance, the key role of type IV pili is well documented in *Vibrio cholerae* during gut colonization in an infant mice model as well as in humans [7, 8]. In a subcutaneous route of infection with *Francisella tularensis*, the agent of tularemia, infection doses required to cause a lethal infection with non-piliated mutants is 10^5 fold greater than the wild type strain [9]. In a xenograft-based animal model of infection with *Neisseria meningitis*, type IV pili are essential for vascular colonization and disease progression [10]. In the closely related bacterium *Neisseria gonorrhoeae* piliation is required for infection of human male volunteers [11]. Other human pathogens including enterotoxigenic *E. coli*, enteropathogenic *E. coli* and *Pseudomonas aeruginosa* also express type IV pili. Potential applications of type IV pili inhibitors are thus very broad.

New additions to the anti-virulence therapeutic toolbox

Two studies seized this opportunity by providing several inhibitors of type IV pili [4, 5]. In one case the authors fortuitously observed that certain members of the phenothiazine family of molecules including trifluoroperazine and thioridazine induce the disassembly of type IV pili (Figure 1). In the second study by Aubey *et al.* [3], compounds such as P4MP4 (1-[(piperidin-4-yl)methyl]piperidin-4-ol, called compound B in the publication) were identified by a phenotypic screen on the ability of *N. meningitidis* to adhere to endothelial cells. In both cases treatment of bacteria with the drugs leads to the decrease in the number of pili at the bacterial surface. The effect of both compounds is strikingly rapid, taking place within a few minutes and reflects the unusually rapid dynamics of type IV pili which undergo cycles of extension and retraction within minutes. Accordingly, a *pilT* mutant that fails to retract its pili is resistant to all these new compounds. The exquisite dynamics of type IV pili that confers to the bacteria motility on surfaces, liquid-like aggregate formation and competence turns out to also be its Achilles' heel [6].

Mechanism of action of the type IV pili disrupting drugs

P4MP4 targets the PilF ATPase. Pilin subunits of type IV pili are stored in the inner membrane and can then be captured by the assembly complex composed of about 15 proteins leading to its incorporation into the helical pilus fiber. The PilF ATPase provides the energy required for the assembly of pili. P4MP4 does not, however, inhibit the PilT retraction ATPase thus locking the system in a retraction-only mode eventually leading to a bacterium without pili. The drug thus affects the delicate balance between extension and retraction of the pili without affecting bacterial viability. Future structural studies will reveal where the compound is binding on PilF and how this prevents the function of this complex hexameric enzyme.

The phenothiazine derivatives function in a different way, at least initially. They affect the function of the Na^+ -pumping NADH:quinone oxidoreductase (Na^+ -NQR) [12]. Because trifluoroperazine is partially toxic to bacteria at concentrations triggering type IV pili disassembly, Denis *et al.* [4] elegantly identified this transporter by selecting mutants that survive this treatment. Bacterial toxicity and pilus disassembly are thus tied together in this case although the authors provide ample evidence that antibiotics mediated bacterial killing is not sufficient to trigger pilus retraction and block pilus-mediated phenotypes. The reaction catalyzed by Na^+ -NQR is similar to the one carried out by the H^+ -pumping NADH:quinone oxidoreductase from the respiration complex I found in mitochondria for instance. In both cases, these enzymes participate in the generation of the electrochemical gradient that is essential to subsequently drive ATP synthesis. These two respiratory enzymes are not

homologous, however, and likely have a different mechanism of action. Importantly, the Na⁺-NQR enzyme can be found in the genome of numerous pathogenic bacteria but has no eukaryotic homolog, underlining its potential as a therapeutic target. Trifluoroperazine would thus first induce pilus retraction, perhaps by lowering intracellular stores of ATP, eventually altering the bacterium viability. Exactly how the Na⁺-NQR transporter modulates type IV pilus dynamics and whether the balance between PilF and PilT activities is affected remains to be determined.

The added value of drugs targeting type IV pili over antibiotics

Perhaps the most important finding of the study by Denis *et al.* [4] is the demonstration that in a humanized animal model, treatment with the phenothiazine family in addition to antibiotics therapy performs better than antibiotics alone. During *N. meningitidis* infection of this animal model antibiotics alone kill the majority of the bacteria but a significant amount of inflammation and coagulation remains. The phenothiazines favor bacterial clearance from the blood and increase mouse survival following infection. Both treatments combined not only lead to complete bacterial clearing but also to reduced coagulation and inflammation. This exciting finding opens the possibility of a synergistic double treatment. Since the phenothiazine family of drugs has been used for years as an antipsychotic drug typically used to treat schizophrenia, a potentially rapid translation towards the clinic can be envisioned.

Concluding Remarks

Two independent studies together make a strong argument that type IV pili are a druggable target and provide several of such inhibitors. Although initially identified for *Neisseria meningitidis* both studies show that these compounds are efficient on other type IV pili expressing bacterial pathogens (*Neisseria gonorrhoeae* and *Pseudomonas aeruginosa*) thus offering a broad application for these drugs. Much work is needed to optimize these drugs and understand their precise modes of action but the possibility to make a difference in the treatments of patients is now within reach.

Figure 1. New drugs lead to type IV pili disassembly in several pathogenic bacteria

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