mechanism may help to address the increase or antimicrobial resistance as bacteria become resistant to conventional antimicrobial drugs over time.

Earlier attempts to characterize the mechanism of Pol II inhibition by the ABU by comparison of whole genome sequences of *E. coli* 83972 and the uropathogenic strain, *E.coli* CFT073 have failed to identify specific factors associated with this (I. Amibite et al. Pathogens, 2016, 5, 49; doi:10.3390), indicating that this is not a simple matter. However, as a result of the serendipitous occurrence of attenuated Pol II inhibitory activity in a re-isolate, obtained from a patient inoculated with a prototype ABU, the applicants have been able to make a significant breakthrough in identification of the factors which may be useful in immunosuppression.

Summary

There is provided an inhibitor of host RNA polymerase II (pol II inhibitor) for use in therapy, wherein said inhibitor is a moiety which targets a protein selected from PAF1C or CDK12. According to a first aspect, there is provided an inhibitor of RNA polymerase II for use in therapy, wherein said inhibitor is a bacterial NlpD protein, or a variant thereof, or an active fragment of either of these, or a bacterial Sigma S protein or a variant thereof or an active fragment of either of these.

Moieties which target these proteins may be known in the art or may be identified or designed using conventional screening methods. They may for example comprise antibodies or binding fragments thereof, aptamers or small molecules, which bind to CDK12 or PAF1C so as to inhibit RNA polymerase II in a host cell.

Specific examples of such moieties may be obtained from bacteria, such as the commensal bacteria or asymptomatic carriers such as asymptomatic bacteriuria.

As a result of the isolation of a spontaneous, loss of function mutant of the ABU strain *E. coli* 83972, the applicants have identified bacterial proteins able to act as direct inhibitors Pol II phosphorylation in infected hosts. Mutations were localized by comparative genome sequencing of the *E. coli* 83972 WT and mutant strain and identified sequence variants were systematically introduced into the *E. coli* 83972 WT strain for analyses of effects on Pol II phosphorylation.

In a particular example, the inhibitor is a polypeptide expressed by a gene selected from IldD, IldR, nlpD or rfaH of a bacterial species, or a variant of said polypeptide.

subjected to analysis to confirm RNA polymerase II inhibitor activity. Filtration of the supernatant for example using centrifugal or flow-through filters suitable for separating proteins can be used to remove non-active fractions which tend to be high molecular weight components, thus concentrating the inhibitor activity.

The inhibitor may be purified from the concentrate using conventional methods and the amino acid sequence determined, also using conventional methods. When this has been done, the inhibitor may be produced synthetically, for example using recombinant DNA technology. In another aspect, there is provided a method for preparing an inhibitor of RNA polymerase II as defined herein which comprises culturing suitable bacteria in a culture medium, and isolating a bacterial NlpD protein, or a variant thereof, or an active fragment of either of these, or a bacterial Sigma S protein or a variant thereof or an active fragment of either of these from the supernatant.

For use in modulating the immune system, the pol II inhibitor of the disclosure is suitably formulated into a pharmaceutical composition in which it is combined with a pharmaceutically acceptable carrier. Such compositions form a part of the disclosure. In another aspect, there is provided a pharmaceutical composition comprising an inhibitor of RNA polymerase II as defined herein in combination with a pharmaceutically acceptable carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravesical, intravenous, subcutaneous, intramuscular, intravascular or infusion) as a sterile solution, suspension or emulsion, for topical administration as an ointment or cream or for rectal administration as a suppository.

In general the above compositions may be prepared in a conventional manner using conventional excipients.

Methods of using the RNA polymerase II inhibitor will depend upon a variety of factors such as the disease being treated and the nature of the particular inhibitor being used. These will be determined in accordance with clinical practice.

The disclosure provides a method of treating a patient in need therefore with an effective dosage of an inhibitor of RNA polymerase II as described above. Suitable dosages will be determined in accordance with normal clinical practice, but will generally be in the range approximately from 0.01 g/kg to 25 g/kg but with considerable variation between individuals and disease conditions.

subsequently mutated in *E. coli* 83972, by homologous recombination and the mutants were screened for effects on Pol II Ser 2 phosphorylation. The genes lldD and lldR, whose products are involved in aerobic L-lactate metabolism, were shown to significantly affect Pol II phosphorylation. To confirm these effects, a biochemical approach was used. Supernatant of bacteria incubated in tissue culture medium were shown to contain inhibitory activity and was further fractionated to define the molecules responsible for these effects. Weak acids of <3 kD molecular weight, formic and acetic, were identified to have inhibitory effect of Pol II phosphorylation. In addition, Pol II phosphorylation was affected by deletion of nlpD, which encodes a lipoprotein with a potential function in cell wall formation and rfaH and cysE with products of both genes involved in biofilm formation. In another aspect, there is provided an inhibitor of RNA polymerase II which comprises an isolated polypeptide expressed by a gene selected from nlpD of a bacterial species, or a variant of said protein, and wherein the inhibitor of RNA polymerase II is synthetic.

In particular, the applicants have identified a new mechanism by which bacterial proteins disturb the formation of the Pol II complex and attenuate the Pol II activation in human cells. The bacterial TATA box binding protein, encoded by rpoS and its transcriptional regulator Nlpd as bacterial genes responsible for this effect through competitive inhibition of the human TATA box binding protein TBP and a reduction in PAF1C, CDK 9 and CDK12. The findings define a new, potent mechanism for cross-regulation of the transcriptional machinery between eukaryotic and prokaryotic cells. Without being bound by theory, the findings may illustrate a new general mechanism of bacterial adaptation and survival in infected hosts. In another aspect, there is provided a bacterial protein selected from Sigma S protein or NlpD protein or an active fragment thereof for use in therapy.

The encounters between bacteria and host cells are guided by specific molecular interactions. Pathogen attack is often executed by virulence factors via receptor-mediated interactions, involving conserved receptors like the TLRs and pathogen specific recognition mechanisms with specificity for unique sets of virulence factors. Adhesive interactions determine the tissue specificity and site of infection. Exotoxins bind cell surface receptors, such as GM1 (cholera toxin) or Gb3 (Shiga toxin) and after internalization, the toxins interfere with key cellular functions. Endotoxins activate TLR4 signaling cascades and by

Claims

1. An inhibitor of RNA polymerase II for use in therapy, wherein said inhibitor is a bacterial NlpD protein, or a variant thereof, or an active fragment of either of these, or a bacterial Sigma S protein or a variant thereof or an active fragment of either of these.

2. An inhibitor of RNA polymerase II for use according to claim 1 wherein the bacteria is a commensal bacteria or an asymptomatic carrier.

3. An inhibitor of RNA polymerase II for use according to claim 2 wherein the bacteria is asymptomatic bacteriuria (ABU).

4. An inhibitor of RNA polymerase II for use according to claim 2 or 3 wherein the bacteria is an *E.coli* strain.

5. An inhibitor of RNA polymerase II for use according to claim 4 wherein the bacteria are *E. coli* 83972.

6. An inhibitor of RNA polymerase II for use according to claim 1 which is a Sigma S protein or a variant thereof or an active fragment of either of these.

7. An inhibitor of RNA polymerase II for use according to claim 6 wherein the Sigma S protein is of SEQ ID NO 1 or SEQ ID NO 2 or a variant thereof, or an active fragment of either of these, such as a peptide of SEQ ID NO 3.

8. An inhibitor of RNA polymerase II for use according to claim 1 which is a bacterial NplD protein, or a variant thereof, or an active fragment of either of these.

9. An inhibitor of RNA polymerase II for use according to claim 8 which is a protein of SEQ ID NO 4.

10. An inhibitor of RNA polymerase II for use according to any one of claims 2 to 5 wherein the inhibitor is secreted by the bacteria.

11. An inhibitor of RNA polymerase II for use according to any one of the preceding claims wherein the NlpD protein is of less than 3kDa in molecular weight.

12. An inhibitor of RNA polymerase II for use according to any one of claims 2 to 11 which is synthetic.

13. An inhibitor of RNA polymerase II for use according to any one of the preceding claims wherein the therapy is immunosuppression, anti-inflammatory or anti-infection therapy.

14. A method for preparing an inhibitor of RNA polymerase II as defined in claim 10 or claim 11 which comprises culturing suitable bacteria in a culture medium, and isolating a bacterial NlpD protein, or a variant thereof, or an active fragment of either of these, or a bacterial Sigma S protein or a variant thereof or an active fragment of either of these from the supernatant.

15. A pharmaceutical composition comprising an inhibitor of RNA polymerase II as defined in any one of claims 1 to 13 in combination with a pharmaceutically acceptable carrier.

16. An inhibitor of RNA polymerase II which comprises an isolated polypeptide expressed by a gene selected from nlpD of a bacterial species, or a variant of said protein, and wherein the inhibitor of RNA polymerase II is synthetic.

17. An inhibitor according to claim 16 wherein the bacteria are commensal bacteria or an asymptomatic carrier.

18. An inhibitor according to claim 17 wherein the bacteria are asymptomatic bacteriuria (ABU).

19. An inhibitor according to any one of claims 16 to 18 wherein the bacteria is an *E.coli* strain.

20. An inhibitor according to claim 19 wherein the bacterial strain is *E. coli* 83972.

21. An inhibitor according to any one of claims 16 to 20 wherein the inhibitor is a protein secreted by the bacteria.

22. An inhibitor according to claim 19 which is of less than 3kDa in molecular weight.

23. A bacterial protein selected from Sigma S protein or NlpD protein or an active fragment thereof for use in therapy.

24. A bacterial protein for use according to claim 23 wherein the Sigma S protein is of SEQ ID NO 1 or SEQ ID NO 2, or a fragment of SEQ ID NO 3, and the NlpD protein is of SEQ ID NO 4.