Transcript: Phage-inspired antibacterial target discovery

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Welcome to the online lecture on phage-inspired antibacterial target discovery. The first part of the lecture will explain why there is a high need for the discovery of new antibacterial targets and define what an antibacterial target actually is. In the second part, the search for antibacterial targets, using phages as a source of inspiration, will be carefully explained step by step. Finally, a sneak peek is given on how antibacterial targets are used to make new antibiotics to fight bacterial infections.

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Lately, the media has been focusing on the problem of growing resistance of bacterial pathogens against the currently available antibiotics. This means that the bacteria become unresponsive to currently available antibiotics and bacterial infections become much more difficult to treat. Therefore, the world health organization recently called for help by publishing a list of the bacteria for which new antibiotics are urgently needed. Among others, *Pseudomonas aeruginosa* and *Acinetobacter baumanii* are considered the most critical group of resistant bacteria, for which new antibiotics are essential. The goal of this list is to encourage researchers to focus on the development of new antibiotics, which specifically inhibit antibacterial targets. These targets are defined as proteins or processes which are essential to bacterial survival, like for example cell-wall synthesis or the ribosomes. Moreover, these targets need to be susceptible to inhibition, meaning that antibiotics can be found that inhibit the protein or process and thereby inhibit bacterial growth. One way to find new antibacterial targets is to use phages as a source of inspiration. As the natural enemies of bacteria, they express several toxic proteins during infection that inhibit essential bacterial processes and convert the host into a phage-producing machine. These toxic phage proteins have been selected through evolution and are therefore highly efficient. Identifying the bacterial targets of the toxic phage proteins and mimicking their toxic effect proteins can lead to the development of new antibiotics.

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The discovery of the antibacterial targets that are inhibited by the toxic phage proteins can be subdivided into three stages: First bacteria-specific phages are sequenced and their genes are predicted. Next, the genes that exert an antimicrobial effect are identified and finally the bacterial targets of the toxic phage genes are traced. These three stages will now be discussed in detail.

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First, a phage is chosen that infects the bacteria for which you want to develop a new antibiotic, for example a phage that specifically infects *Pseudomonas aeruginosa.* After collecting a number of phages of this type, the phage genomes are extracted and the nucleotide sequence of the phage genome is determined. In the final steps, computer programs are used to predict the genes of the genome that code for the phage proteins.

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The second stage involves finding out which of the predicted phage genes encode proteins that are toxic to the bacterium of interest. In the first step, an expression plasmid is constructed for each individual predicted phage gene. This is a small circular DNA molecule that contains a single phage gene. Next, the plasmids are brought into the cytoplasm of the target bacteria through transformation. In the third step, the expression of the phage genes is activated, resulting in the production of the phage proteins in the bacterial cytoplasm. To identify the proteins that are toxic to the bacteria, the growth of the bacteria containing a certain phage protein in its cytoplasm, is interpreted.

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Interpretation of bacterial growth to identify toxic phage proteins can be done as follows. For each phage gene, growth is interpreted for bacteria that do not express the protein and bacteria that do express the protein. It is expected that bacteria that only contain the plasmid, but not produce the phage proteins, grow normally. On the other hand, two options are possible for bacteria that do produce the phage proteins. If the bacteria can still grow normally, the produced proteins are not toxic. Alternatively, when the phage proteins are toxic, the bacteria won't be able to grow. By exerting this growth experiment for each identified phage genes, the genes can be selected which encode for toxic phage proteins.

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How can we now find the bacterial target proteins that are inhibited by the identified toxic phage proteins? One way to do this, is with a pull-down experiment. In this experiment, a certain toxic phage proteins is used as a bait to capture it's bacterial target protein. First, The phage protein is immobilized on the inside of a column. Then a mixture of all the proteins that are produced by the bacteria of interest, is collected and allowed to enter the column at the top. As the bacterial proteins move throughout the column, they are allowed to interact with the immobilized phage proteins. The bacterial proteins that are targeted by the phage proteins will be retained in the column, while the non-target proteins will not be retained by the phage proteins and instead run through the column. Finally, the retained bacterial proteins can be eluted and identified. Alternatively, other techniques can also be used to identify antibacterial targets, after a toxic phage protein has been identified. One example is the protein-protein interaction technique "yeast two-hybrid", which is used for this purpose in the Wagemans *et al.* paper.

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How to use these antibacterial targets of toxic phage proteins to develop a new antibiotic? This involves the identification of a small molecule that mimics the action of the toxic phage protein, which means that the small molecule exerts a similar inhibiting effect on the bacterial target. Phage proteins themselves cannot be used as antibiotics, because proteins are quickly broken down during digestion in the intestinal tract and they cannot penetrate the bacterial cells. Small molecules on the other hand, cannot be digested and are small enough to penetrate and exert their toxic effect.

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If you would like to read more about antibiotic resistance and the use of toxic phage proteins to identify new antibacterial targets, the following literature is recommended. Thanks you for following this online lecture.