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Cellular Structures and Functions That Aid in Antibiotic Resistance among Bacteria

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Antibiotic resistance is a vital issue because numerous bacterial strains are becoming resistant to many of the antibiotics that we have available today. Bacteria are building a tolerance against these medications because of how often humans overuse them. Microbiologists are to target the specific cellular structures and functions that are allowing microorganisms the ability to withstand the toxins that are usually fatal to them. Studies have shown that the resistance that certain bacteria exhibit arises from genetic mutations. Phenotypic heterogeneity occurs when different mutations in the same gene give rise to variations in the phenotype of the microorganisms. Scientists are currently experimenting with different bacterial species to see if there are any activities that may cause these mutations.

Introduction

Antibiotic treatments are crucial to all advanced life forms. Bacterial infections have become harder to control and cure because of the increased antibiotic resistance to medications. To keep these treatments around for our use we must try to understand what makes the resistant bacterial cells unaffected from the toxins produced by the antibiotics. It is hypothesized that some bacterial strains have the ability to adapt under extreme conditions. "It is thought that this heterogeneity has evolved to ensure the longevity of a population threatened with a potentially catastrophic event such as lethal antibiotic exposure" (Girgis 12740). When bacteria are introduced to antibiotics, the majority of the colony will be affected. A few microorganisms will survive and these cells have mutated genes that make them resistant. The next step is to understand what happens chemically within the cell that initiates the mutation. Hindre states that fitness enhancing mutations affect gene sequences that control gene expression. The genes that are expressed directly affect how the organisms act in a certain environment. The final step to understanding bacterial resistance is to locate which metabolic and structural enzymes specifically are different from those of bacteria cells that are not resistant. Studies are getting closer to these steps through the work being conducted on *Mycobacterium Tuberculosis.* Zheng expresses that *WhiB* proteins can be found in the M. tuberculosis genome, these proteins are not found in other species of organisms, therefore, *WhiB2* and *WhiB7* could serve as a template for drug resistance within *Mycobacterium.* Singling out these *WhiB* proteins as potentially being where the mutations occur is essential to stopping antibiotic resistance. Scientist can now monitor these proteins in cells that are resistant and those that are not to see what chemically changes between gene transfer, cell to cell contact, and biofilms.

Recent Progress

Microbiologists have recently moved onto gene specification testing as they have singled out many sites on the genome where these mutations could be occurring. "We isolated several mutants with insertions in the 3′ region of *metG*. The persistence frequency of one of these mutants was evaluated by generating a kill curve, and remarkably, this *metG* mutant showed a level of survival that is very similar to the *hipA7* mutant" (Girgis 12742).

MetG and *hipA7* are both genes that were found to be mutant within the wild type or original field sample of bacteria. Wild type bacteria with these mutations showed persistence in the presence of antibiotics while the non-mutant cells died. When extracted mutant *metG* and *hipA7* genes are inserted into normal cells, we could expect to see a higher rate of resistance among the new mutant colonies. Just as predicted, the isolated mutant strains were re-plated and had a significantly larger amount of colonies that were able to grow in the presence of antibiotics than each plate before. In another study, Zheng found that "immunocompetent mice challenged with *M. tuberculosis* H37Rv *whiB3* deletion mutant showed a prolonged survival than those infected with wild-type strain" (104). The mutant cells within this experiment were able to survive longer than the wild type strain. *WhiB* proteins must be associated with the expressed alleles within *M. tuberculosis* because when it was deleted from a normal cell, the cell became resistant. The expressed phenotypes among organisms are important to monitor because they are the determining factors of resistant or susceptible attributes of the cells. In addition to this type of experiment, scientists have found a way to produce artificial or synthesized organisms to compare to live microorganisms. Evolutionary theories state that mutations occur over time as organisms adapt to survive constantly fluctuating environments. Therefore, synthetically producing bacterial cells can help target at a time frame during cell division in which mutations actually occur. This also provides greater insight on what happens within a cell when adaptations are prompted to occur. Hindre highlights in his studies that gene sequencing affects global regulatory genes in certain bacteria. He also states that these sequence mutations occur at the beginning of a sequence at the promoter and sigma factor. Global regulatory networks are used to determine which alleles need to be expressed in order for bacterial adaptation to ensue, which is necessary for survival in a new environment.

Discussion

The experiments listed above have given us answers and insight on many topic related issues, but there are still unanswered questions about antibiotic resistance that we need to understand before we can finish solving the issue at hand. The first experiment dealt with the *metG* gene that was deleted from a mutant *E. Coli* cell and inserted into normal cell to see if the normal cell would become resistant to the antibiotic once receiving the new DNA. In result, the isolated mutant bacteria grew much better than the wild type. This experiment provided vital information of this issue, but to take it a step further we must try to understand what exactly about *metG* is causing this change in phenotype. We also need to discover which stress factors prompt the adaptation within the cell initially. From there, we would see if we could inhibit the receptors that receive the stress factors because if the signal for adaptation is not received then the mutations may not take place.

The second experiment conducted was one that was designed to discover the significance of the *WhiB* gene in the bacterial cells that have been linked to the resistant mutant cells. When the *WhiB* gene was deleted from the *M. tuberculosis* cells, the cells became more resistant to the antibiotics. This experiment was tested even further than the others because they discovered molecules that could be the causes of stress to the cells. "NO and CO are presumably inevitable stresses for M. tuberculosis. Redox disorder within M. tuberculosis-infected macrophages might be due to the biased reduction equivalent of NADH, NADPH resulting from host fatty acids metabolism" (Zheng 105). By identifying the stresses that cause the cells to adapt and mutate, we now may be able to successfully block the nitrogen monoxide and carbon monoxide receptors so that the cells do not realize that they are in danger. If the cells cannot sense that there is any danger to their survival, no mutation will occur and antibiotics will have an effect.

The final experiment conducted above used synthesized bacteria cells and compared them to live bacterial cells to see if the results of the live bacteria could be duplicated. This experiment is important because researchers can keep record of the genes in the synthesized bacterial cells. This experiment also proposes that the environmental changes might not be the only thing that triggers a mutation. Hindre expresses that antibiotics target specific cell functions as their tactic to kill bacteria. Therefore, when the synthesized bacteria are not affected in the presence of similar antibiotics as their parent cells were, they have already undergone some form of mutation. From that point, he observed which proteins changed within the new cells. Most bacteria seen today have mutated far from their ancestors because they are constantly prompted to protect themselves against antibiotics.

These three experiments have all tied together to provide helpful insight on what needs to be done in order to reduce antibiotic resistance. People overuse these drugs and as they do, bacterial cells are becoming better and better at equipping themselves against them. The medications that we are taking today are essentially building super bacteria that have resistance to many of our drugs that we use to kill them. Now we are at the stage that we need to either produce stronger antibiotics or to find a way to make the bacterial cells susceptible to the medications again. However, those are short-term and long-term solutions. Making stronger antibiotics is a short-term resolution because that may eventually cause even stronger super bacteria to be produced. The best way to challenge this issue is to find a way to incorporate the

stress receptor inhibitors into our antibiotics. This way we can take the same medication, but it will be more efficient by far as the bacteria won't be able to adapt to the medications. Scientists are already well on their way to a solution. Bacterial antibiotic resistance is a very important issue and will be until we find a cure because no matter what we do bacterial infections are never going to vanish.

References

Girgis, Hany S., Kendra Harris, and Saeed Tavazoie. "Large mutational target size for rapid emergence of bacterial persistence." PNAS 109 (2012): 12740-2745.

Hindre, Thomas, Carole Knibbe, Guillaume Beslon, and Dominique Schneider. "New insights into bacterial adaptation through in vivo and in silico experimental evolution." Nature Reviews 10 (2012): 352-65.

Zheng, Fei, Quanxin Long, and Jianping Xie. "The Function and Regulatory Network of WhiB and WhiB-Like Protein from Comparative Genomics and Systems Biology Perspectives." Cell Biochym Biophys (2012): 103-07.

