PROKARYOTE GENETICS

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Summary

Prokaryotes are the most common organisms in the world and are most likely, assuming

that like is not unique on the Earth to be the most common style of living organism in the Universe, even if the chemistry is different elsewhere. Therefore, iyt is most likely also that prokaryotes on Earth were the first to use sex for evolution and therefore also give rise to the science of genetics. Because they are simple organisms, made up usually of only a single cell, because they growth relatively quickly in most cases and because they can grow on simple growth media in many cases, they were from the 1940's onwards the target for the use of molecular biology to understand genetics. The results have turned out much more complex than might have been expected by the pioneers. Just about everything that a eukaryote can do has already been done by a prokaryote, which perhaps says something about the origin of eukaryotes. Initially, bacteria were believed to have only circular chromosomes and plasmids, while eukaryotes used linear molecules. Linear genetic elements merely proved to be rarer than circular. The same is true for the presence of more than one chromosome in prokaryotes, although the definition of what is a chromosome can be somewhat difficult. The DNA transfer mechanisms that power the genetics of prokaryotes are remarkably similar to those of eukaryotes, although the context for uptake of naked DNA, transfer by viruses and conjugation are quite different. Therefore, this outline of prokaryotic genetics is really a pathfinder for the study of the genetic of eukaryotes and without prokaryotic genetics as a tool, eukaryotic genetics would not be so far advanced

1. Mechanism of DNA Mutation, Transfer and Recombination in Bacteria

The diversity of prokaryote microorganisms, with perhaps less than 1% or less than 0.1% of the diversity of such organisms on the Earth having been even just looked at, provides a basis for evaluating the importance of genetics to prokaryotes. As has been clearly stated elsewhere in this Theme, there is strong evidence that there was a single progenitor for all life on Earth. The importance and complexity of higher organisms should not blind the reader to the effects of 4 billion years evolution on the simplest organism. With their fast generation time, they have evolved to fill just about every possible environment niche that it is possible for life to exist in on Earth. The mechanisms available for this evolution are the basis of prokaryote genetics and include mutation, transfer of genetic material between organisms and the introduction of such material into that organism by recombination. It is now normal to separate evolution which occurs by mutation and evolution that occurs by genetic exchange within the prokaryote world into vertical evolution and horizontal evolution respectively. This distinction should not be taken as absolute. Mutation can have an effect on the ability of a prokaryote to undergo genetic exchange and be caused indirectly by genetic exchange. Similarly, genetic exchange can result directly in mutations.

The mechanisms of mutation remain relatively constant for all prokaryotes although their rate of mutation may vary quite dramatically depending on their ability to identify and repair the mistakes that cause the mutations. However, the mechanisms of genetic exchange vary tremendously from prokaryote to prokaryote. It is possible to split the prokaryotes into a number of major groups which allow a general classification of their methods of genetic exchange. These are: the Gram -ve bacteria; the low G+C% Gram +ve bacteria; the high G+C% Gram +ve bacteria; the Archaea and the viruses. But it should be borne in mind that, if we have only identified less than 0.1% of all prokaryotes, we have studied the genetic exchange in only a very small proportion of these. Novel means of

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genetic exchange and not impossible, but, in general, the mechanisms described below probably cover the systems used by most prokaryotes.

2. Mutation

Mutation is the major way that genetic diversity occurs in all organisms including prokaryote and eukaryote microorganism. Mutation consists of changes to the genetic code of the organism. This can be one of the following changes to a genome: the changing of one base for another; the addition or deletion of one or a few bases; the addition or deletion of large pieces of nucleic acid and the movement of a piece of DNA from one position to another in the genome. The processes by which these changes occur can be classified as either direct DNA damage or via the repair systems that attempt to correct DNA damage. The phenotypic results of mutation can vary dramatically. If the changes occur in a non-functional area of DNA or, because of the redundant nature of the genetic code, do not change a gene at the level of the protein sequence, the mutation will be silent. Such a mutation will not have any phenotype although such mutation can accumulate and change the genetic structure of subsequent generations. Even if a mutation causes a change in the amino acid sequence of a protein, the consequences to the organism may be negligible. For example, conservative amino acid changes may not affect the activity of a mutated enzyme or the enzyme may not be required in the environmental niche in which the organism is growing. In the former case, such a change is an evolutionary step affecting the protein and may have consequences on protein activity at a latter stage of evolution change. In the latter case, if the environment changes, the lack of the enzyme may become critical. In general, changes at the amino acid level in a protein will have a consequential effect on the protein's activity, usually detrimental. Thus, an important metabolic pathway may be blocked by the lack of a functional enzyme resulting in identifiable phenotypes. These phenotypes can be classified into four main groups: auxotrophic mutations, substrate utilization mutations, resistance mutations and essential mutations.

2.1. Types of mutations

Auxotrophic mutations are mutations that knock out genes that are involved in anabolic pathways inside the bacterial cells. The metabolic pathways within cells synthesize a wide range of amino acids, nucleic acids, lipids, cofactors, etc that are central to the normal growth of the cell. Obviously, all bacterial species do not synthesize all such cellular requirements and for a particular species, some additions to a basic medium which provides a carbon, nitrogen and energy source are needed. Such a medium is called a defined minimal medium and it is the simplest medium on which bacteria can grow. An auxotrophic mutation, because it interrupts a central anabolic function, would now require an addition chemical added to its minimal medium. Thus, a mutation that inactivates an enzyme in the pathway that synthesizes histidine will result in a requirement for histidine to be added to the minimal medium for bacterial growth. Similarly, an interruption of the purine biosynthetic pathway would need the addition of purines. Such mutations are easily identified by the failure of such a strain to group on the minimal medium for that species but the strain will grow in the presence of the added factor.

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Substrate utilization mutations are mutations that knock out genes that are involved in catabolic pathways inside and outside the bacterial cell. Different species of bacteria can utilize different complex substrates as sources of energy, carbon, nitrogen, etc. Such utilization needs the production of enzymes capable of breaking down the complex substrates to simple compounds that can be taken up by the cell. A mutation in either an enzyme involved in the breakdown or involved in the uptake will result in the loss of the ability to utilize such a resource. For example, a mutation in beta galactosidase in *E. coli* stops utilization of lactose as a carbon and energy resource. Similarly, a mutation that affects a cellulase gene will reduce to some extent degradation of cellulase by that organism. Interestingly, in this case, because most cellulase utilizing bacteria contain a number of cellulase genes, the utilization will probably still continue, but at a lower rate.

Resistance mutations are a class of mutations which can be considered artificial for the most part although they can occur in nature. The presence of antibiotics, heavy metals and other toxic substances in the environment means that they do occur in specific environments. In general, they can be divided into three types: firstly, mutations that inhibit uptake of the toxic substance, usually by inactivating a gene involved in uptake; secondly, mutations that change the biological target of the toxic substance so that it is less affected by the substance; and finally, by changing an enzyme so that it can now degrade the toxic substance, either by switching the gene on, if it is already present or by natural selection of a new gene activity.

2.2 DNA damage

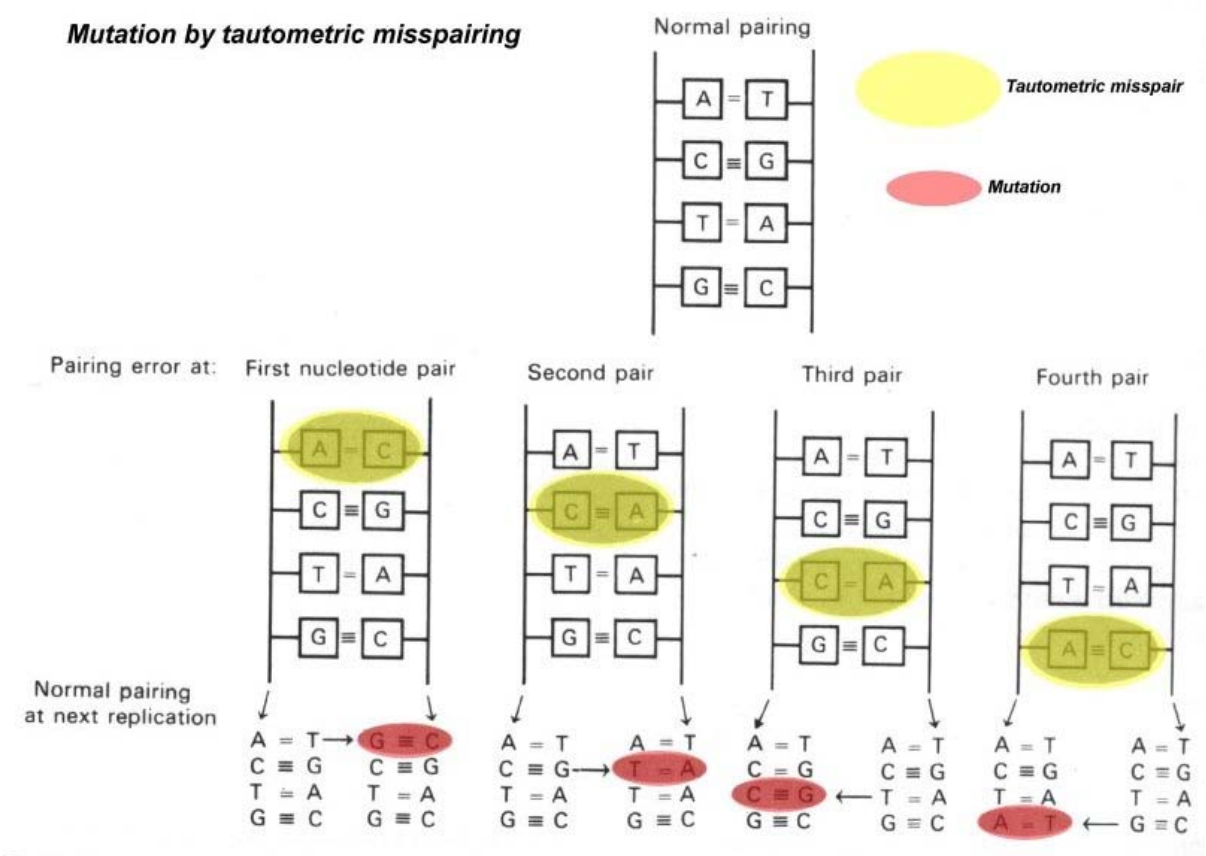

Figure 1. Mutation by tautometric mutation

Although DNA is naturally quite resistant to change because of its structure, aspects of its structure can lead limited change. Figure 1 shows the tautometric changes that can occur with certain bases which allow miss-pairing during DNA replication. Such miss-pairing results in a change in the base on one strand of the daughter DNA molecule. A second round of replication will result in the fixing of such a mutation. Usually, however, the miss-pairing is picked up by the DNA repair systems of the organisms. At that point, there is a 50% chance that the repair mechanisms will fix the mutation.

Another form of DNA damage at the base level is the formation of thymidine dimers by ultra-violet light. As shown in Figure 2, two adjacent thymidines can be cross linked and such a complex stops DNA replication. Again, DNA repair comes in to remove the problem (See 2.3).

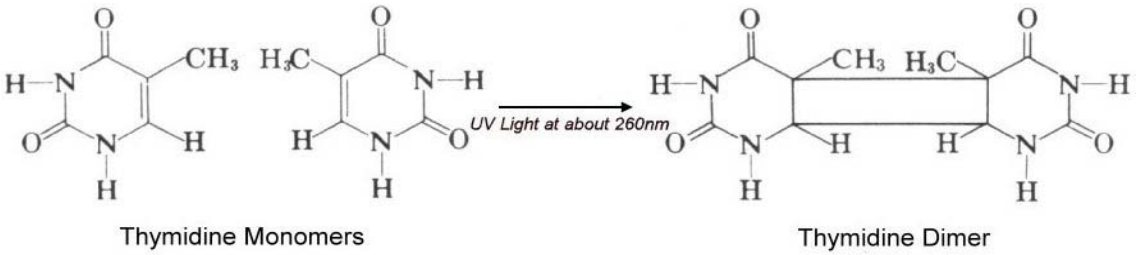

Figure 2. Formation of a thymidine dimer

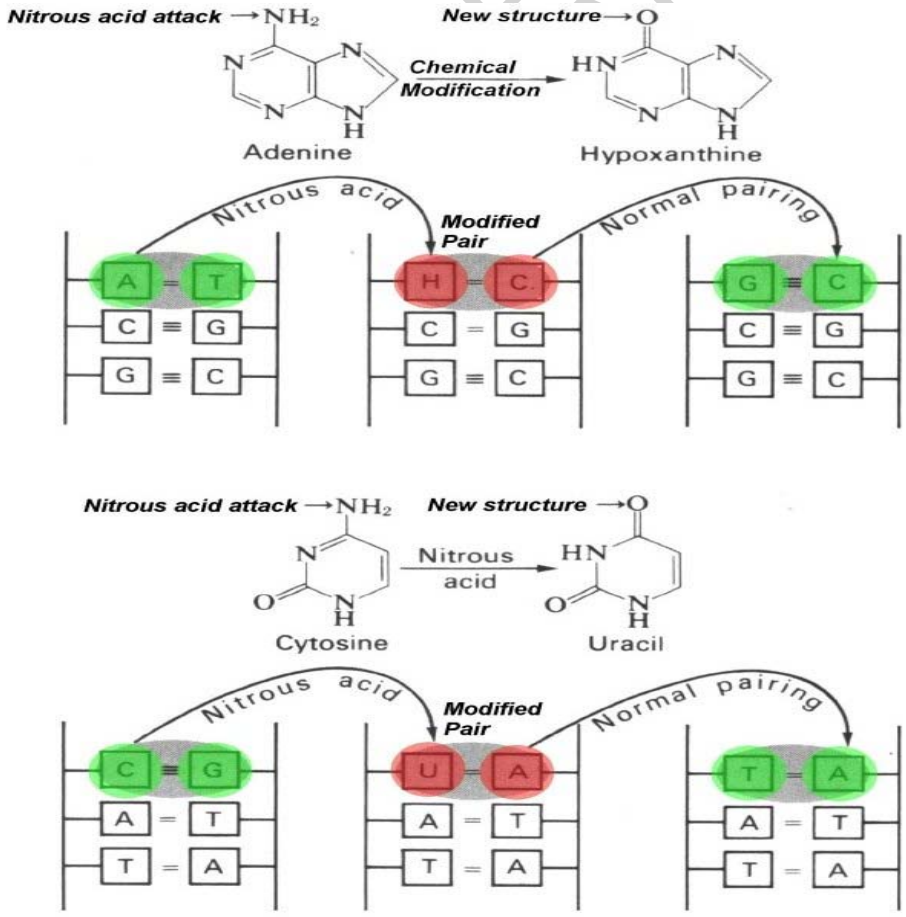

Figure 3. Base pair modification caused by nitrous acid

Finally, at the base level, a variety of chemicals can damage DNA by modification of the base. This is shown in Figure 3. The resulting change may alter DNA pairing and result in a base sequence change.

Large scale changes can be induced by environmental factors such as ultra-violet light, nuclear radiation and chemicals. These can result in DNA strand breakage and rejoining; this causes change in the order of genes and the control of gene expression.

The end point can be so major that normal sexual reproduction becomes impossible. The latter is exploited when insects are sterilized by irradiation *en mass* and then released as a control measure for insect borne disease.

2.3 DNA Repair

Although the mutation mechanisms described in 2.2 occur, the most significant agent for mutation in a prokaryote is DNA repair. This paradox is due to the fact that DNA repair is an induced emergency mechanism which has evolved to remove mutation that may cause the death of the cell. Because of this, some DNA repair mechanisms are error prone and as such cause further but less lethal DNA changes. An example of a DNA repair mechanism that is error free is the removal of thymidine dimers by photoreaction. Here, the enzyme photolyase uses visible light to split the linked bases and restore the ability of the DNA to replicate (see Figure 4). However, error-prone repair in prokaryotes involves the enzyme DNA polymerase 1 which has a higher error rate than the DNA polymerase the cell uses for normal DNA replication. Thus, when DNA polymerase fills in the area where the mutation has been removed, as shown in Figure 5, the rate at which the wrong base is inserted is 100 to 1000 fold higher than in normal DNA replication. Because prokaryotes are haploid, such a mutation immediately become part of the genetic makeup of the cell unlike eukaryotic diploids where a normal second copy of the gene usually protects the cell from the potentially lethal effect of such a mutation.

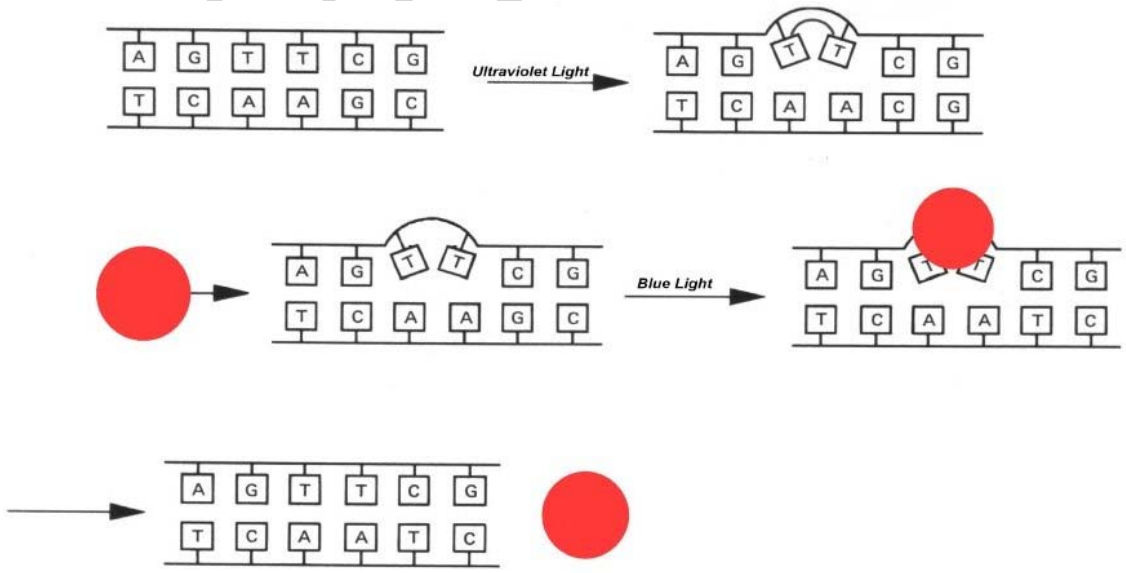

Figure 4. Removal of thymidine dimers by photoreaction.

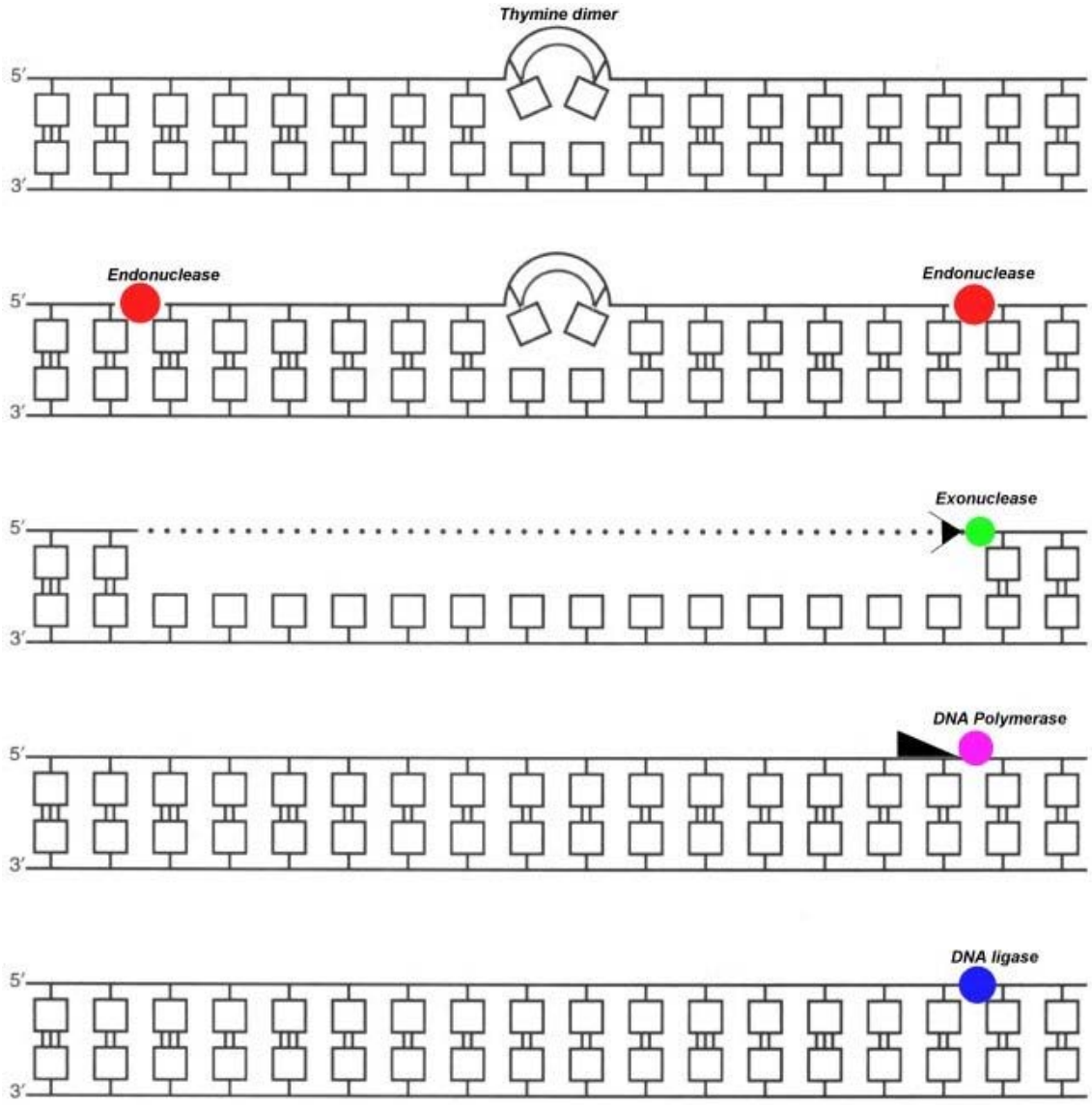

Figure 5. Error-prone DNA repair in prokaryotes

3. Transformation

Prokaryotic genetics revolves around three mechanisms of gene exchange, transformation, transduction and conjugation. Transformation in prokaryotes is genetic exchange by the uptake of naked DNA. In other words, the bacterial cell either naturally or under artificial conditions, moves a foreign DNA molecule across its membrane(s) and then introduces it into its chromosome by recombination. Inherently, the naked DNA is highly vulnerable while floating free in the natural or artificial environment. Equally, *E. coli* cells tend to want to retain their normal gene complement and, therefore, in many cases, only undergo transformation under very specific conditions. The export of nucleases into the environment and the presence of restriction endonucleases in the bacterial cytoplasm both restrict transformation. Not withstanding this, transformation is probably the most important method of horizontal genetic exchange between highly divergent prokaryotes. This is confirmed by the identification of many genes which have

undergone horizontal genetic exchange in a wide range of complete genome sequences. While conjugation, as described in 5.0, probably has had the most impact on humankind in terms of horizontal genetic exchange, transformation requires no mechanism other than DNA.

3.1. Gram -ve Bacteria

The Gram –ve bacteria form a broad group of prokaryotes. Natural transformation in these bacteria is rare, possibly because of their double membrane structure. Artificial transformation in the model organism *E. coli* was first discovered by Chang and Cohen in 1969. Using a calcium chloride treatment and heat shock, it was possible to induce *E. coli* to take up plasmid DNA. Linear DNA is taken up much less efficiently. However, this did not prove to be a general method of transforming Gram –ve bacteria. Electroporation, where an electrical current is used to induce the uptake of DNA has proved more generally applicable, although efficiencies for many species are very low.

3.2. Low G+C% Gram +ve Bacteria

The low G+C% Gram +ve bacteria have proved to be more susceptible to natural transformation. In many cases it is associated with germination of the endospore, although not necessarily. The first case of transformation was the experiments by Griffith which eventually proved that DNA was the genetic principle. It is also possible, because there is only one cell membrane, to remove the cell wall of these bacteria using an enzyme like lysozyme. If the bacterial cell is in an isotonic medium, the cell forms a stable protoplast. If DNA is added in the presence of a chemical such as polyethylene glycol, DNA uptake takes place. Transformation follows, although the cell must be induced to reform its cell wall before it can be plated on a normal medium.

3.3. High G+C% Gram +ve Bacteria

The high G+C Gram +ve bacteria are very different to those described in 3.2, but there are similarities in their mechanisms of transformation. Natural transformation seems to be rare, probably due to the widespread export of nucleases. However, some Actinomycetes can undergo transformation very easily. *Thermoactinomyces vulgaris* will undergo transformation if DNA is incorporated into a Petri dish and the species spores spread on the dish and incubated (Hopwood and Wright). However most Actinomycetes including the *Streptomyces* and the *Mycobacteria* do not transform easily. A method similar to the polyethylene glycol method described in 3.2, needs to be used. Electroporation does not seem to have been very successful

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Biographical Sketch

Ralph Kirby is Professor of Microbiology at Rhodes University, Grahamstown, South Africa. He has held this position for the last ten years. He graduated with his B.A. at Trinity College, Cambridge, UK, in 1972, and completed his Ph.D. at the University of East Anglia, UK, in 1976. Post-doctoral research followed at the University of Bristol, UK, then a lectureship and senior lectureship at the University of Cape Town, South Africa. His major interests are the molecular genetics of Actinomycetes, horizontal gene transfer, molecular population genetics, and the interaction between law and science. He is presently completing a LL.B.

Timeline

Full Professor, Department of Life Science, National Yang-Ming University, Peitou, Taipei, Taiwan. 2003-onwards.

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Full Professor, Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, South Africa. 1990-2003.

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John Innes PhD Studentship, Department of Genetics, John Innes Institute, Norwich, UK. 1972-1975.

Undergraduate, Trinity College, Cambridge, UK. , 1969-1972.

Qualifications

LLB (UNISA)	Intellectual Property and DNA Profiling in Criminal Procedure.
MA (Cantab)	Genetics.
PhD (UEA)	Genetic studies on *Streptomyces coelicolor* Plasmid One.
Pr. Nat. Sci.	Registered Professional Natural Scientist.
LLM (UNISA)	In Intellectual Property. Registered 2004-onwards.

Research Interests

Molecular Population Genetics

1) Molecular Microbial Ecology of Sulfate Reducing Bioreactor System.
2) Molecular Microbial Ecology of thermophilic Actinomycetes.
3) Molecular evolution of lignin degrading enzymes.

Actinomyces

1) Evolution, linear chromosomes and horizontal gene transfer in Actinomycetes.
2) Biotechnology application of lignin degrading enzymes from thermophilic Actinomycetes.
3) Genome scale analysis of various Streptomyces and the Actinomycetales using a *Streptomyces coelicolor* microarray.
4) Use of membrane bioreactors to produce Actinomycetes secondary metabolites.
5) Genome scale analysis of *Streptomyces* and related organisms using microarrays.
6) Development of a differential microarray between *Streptomyces coelicolor* A3(2) and *Streptomyces.*

Other

1) The law and molecular biotechnology.

Although Ralph Kirby has made a career as a full-time academic with research interests in genetics and Actinomycetes from PhD onwards, he have also had a long time interest in the law and is a qualified lawyer, although he does not practice. His legal interests revolve around DNA profiling in both criminal and civil cases as well as biotechnology as intellectual property and have involved both the academic aspects and court work. Also, he have been and am involved in a number of biotechnology start-up companies.

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