EUROPEAN SCIENCE FOUNDATION

ESF EMRC/LESC Exploratory Workshop

Hypermutation of Simple Sequences (Microsatellites) and the Contribution of Hypermutation to Bacterial Pathogenesis

The executive report will discuss the organisation of the meeting, will summarise the scientific content of the meeting, and will highlight the prospects and plans for future interactions between European scientists interested in this field of research. Additional documents will include a scientific report, a financial report, the final programme, a final list of participants and statistical information on participants.

Executive Report

Microsatellites, also termed simple sequence repeats, are hypermutable DNA regions that are found in multiple loci of both human and bacterial genomes. This workshop, largely funded by the European Science Foundation (ESF), was designed to generate interactions between researchers with extensive experience of the mutational mechanisms of microsatellites in eukaryotes and those interested in the bacterial simple sequences, and to explore the contribution to bacterial pathogenesis/adaptive behaviour of elevated mutation rates, hypermutation, in either localised regions of genomes (eg. loci containing microsatellites) or across the whole genome (i.e. global).

The majority of funding for the workshop came from the ESF. Additional funding was provided by the Weatherall Institute for Molecular Medicine, which funded one of the speakers from the USA. The conference was held between the 19th and 21st of March in the Habakkuk Conference Building in Jesus College, an Elizabethan college in the centre of Oxford. The college provided all the conference facilities free and accommodation and meals for all participants at reasonable rates. Administration was mainly undertaken by the Department of Paediatrics, University of Oxford.

Scientific excellence was promoted by having present at the workshop not only a number of senior European scientists but also two senior colleagues from America and one from Israel. Participants were drawn from diverse disciplines - biophysics, biochemistry, eukaryotic and prokaryotic genetics, epidemiology and genomics - and represented a range of fields - DNA structure, DNA replication and repair enzymes/pathways, microsatellites, genetic diseases in humans, microbial population structure and bacterial pathogenesis. European interactions were stimulated by having present representatives of eight European countries. Attendance suffered from the effects of ill-health, with Igor Stojiljkovic, Nora Goosen and Dan Andersson, all having to cancel their participation at the last minute. We were particular grateful however to our American and Israeli colleagues who attended despite the initiation of the conflict in Iraq.

The conference consisted of four sessions, two specialist seminars and four posters. The sessions were enthusiastically chaired by Tom Petes, Erling Seeberg, Richard Moxon and Ben Appelmelk, who led discussion during the course of the talks and at the end of sessions when time was set aside for more general discussions. The

specialist seminars were given by Tom Kunkel and Darren Monckton, the former of these being given in the WIMM, a requisite of their funding of the workshop. The posters were presented by Rosann Farber, Kate Grattan, Crisitine Pourcel, and Wendy Sweetman. Finally, we also had a presentation on the functions of ESF by Hui Wang and a discussion of ESF and European funding initiatives lead by Tone Tonjum and Chris Bayliss.

The four sessions of the workshop covered:- i) *cis*-acting factors that influence mutation rates of microsatellites; ii) *trans*-acting factors that influence mutation rates of microsatellites; iii) influence of *cis*-/*trans*-acting factors on the mutation rates of bacterial microsatellites; and iv) studies of localised or global hypermutation using epidemiological samples or models systems. The next four paragraphs summarise the important points relevant to the aims of the workshop generated by the talks and discussions in each of these sessions and highlights the current extent of our knowledge and some questions that point to future directions for research in this field.

Session 1. A biophysical approach, involving nuclear magnetic resonance, indicated that A-tracts have higher stability than mixed G/C-tracts. Biochemical assays demonstrated that mutation rates of repeat tracts are dependent on repeat number, properties of the DNA polymerase and mismatch repair (MMR). The differential effects of the leading/lagging strands on basal and microsatellite mutation rates were shown in genetic studies. Discussion separated *cis*-acting factors into the properties or context of the repeats. Predictive criteria for some properties were identified but general rules for many *cis*-factors are not yet clear. Interesting questions were raised concerning the position of repeats relative to the origin of DNA replication, the influence on microsatellite mutation rates of chromosome structure and physiological parameters such as nucleotide concentration.

Session 2. Genetic complementation and biochemical analysis enabled the identification of new alkylation repair enzymes. Defined genetic mutants and mutagenic DNA substrates were utilised to understand DNA polymerase switching during an *Escherichia coli* SOS response. The effects of DNA structures formed by triplet repeat tracts in mutants of *sbcCD* nuclease and other genes of *E. coli* were then described. Genetic assays demonstrated the similar mutagenic effects of MMR mutants on microsatellites in yeast and nematode worms and genes involved in the stability of telomere repeats in yeast were also identified. Factors influencing the expansion of human disease derived triplet repeat tracts were generated by analysis of sperm, other tissues and cultured cells. The discussion enumerated the number of DNA repair pathways and types of DNA damage and questions were formulated on the roles in microsatellite stability of these varying types of DNA repair/damage particularly during non-replicative growth.

Session 3. Evolution of localised and global hypermutation and the key role of genome sequencing in identification of bacterial microsatellites was highlighted. Through phenotypic analyses and comparative sequencing of *Neisseria meningitidis* strains, it was observed that many serogroup A strains have elevated basal and/or DNA repeat tract mutation rates, that allelic variations in *recB* are associated with variations in transformation frequency, and that there are associations between repeat number and potential for phenotypic variation for particular loci. Assays of phenotypic switching using defined mutants also identified *mutS*, *mutL*, *dinB* and a Rho-factor inhibitor as factors that destabilise *N. meningitidis* repeat tracts. Similarly, tract length and lagging strand DNA synthesis were shown to be major factors driving tetranucleotide repeat mutation rates in *Haemophilus influenzae*. Genomic analyses identified an overabundance of uptake sequences in the DNA maintenance genes of *N. meningitidis*. Discussion focussed on the areas of research that need to be addressed in this field. The

major questions relate to the influence on microsatellite stability of different types of DNA damage and to establishing whether there are correlations for particular species between the types of repeats and DNA maintenance pathways.

Session 4. Variations in tetranucleotide repeat tracts were used to generate phylogenetic trees of non-diverse bacterial species (eg. *Mycobacterium tuberculosis*) or to differentiate isolates of phase variable species (eg. *H. influenzae*) during the course of infections. Epidemiological investigations of basal mutation rates indicated that mutators of different strengths are present among isolates of many bacterial agents of infectious disease. Analysis using antibodies of antigenic variants of *Mycoplasma hyorhinis* has led to the identification of the mechanisms of switching. Genome sequencing of *Campylobacter jejuni* identified multiple loci containing repeats and the functions of some loci were characterised by genetic and biochemical analyses. Repeat-associated loci were shown to control LPS variation in *H. influenzae* and *N. meningitidis* and loci mediating sialic acid addition were required for survival in animal models of infection. The Lewis antigen of *Helicobacter pylori*, subject to repeat tract-mediated alterations in expression, mediated survival in animal models of infection and also bound to DCSIGN, an immunomodulatory molecule. In discussions it was noted that most bacterial microsatellites control expression of surface molecules. Speculation as to the reasons for repeat tract controlled switches in gene expression thus focussed on functional consequences, immune escape and infection by bacteriophages, whose receptors are surface molecules. Questions also centred on whether organisms have evolved an optimum mutability for their life-style and on the relationship between alterations in mutation rate and disease.

Many laboratories across Europe and the world are working on related ideas and concepts in isolation. The intense learning curves experienced by all participants of this workshop indicated that the field of microsatellite and hypermutation research is no exception. The dissemination of ideas is, therefore, critical. An initial idea for future interactions was a network focussed on elevated mutation rates, thus including cancer biology, somatic hypermutation, microsatellite repeat diseases in humans and the role of hypermutation in bacteria. This field was felt to be too large and many of these research fields already have networking facilities. The infectious diseases field is the least developed of these research areas. A network in this area is proposed with the title Generators of Genetic Diversity in Bacterial Agents of Infectious Disease. The network would focus on understanding the molecular mechanisms of and the biological consequences resulting from the genetic diversity generated by the processes of transformation, localised and global hypermutation. This network would intend to generate interactions leading to both dissemination of knowledge and collaborative research projects. The network would be multidisciplinary requiring laboratories specialised in biophysics, biochemistry, genetics, genomics, theoretical modelling, and epidemiology. The network would influence clinical medicine by driving Pan-European sampling of bacterial populations for knowledge of the role of hypermutation in commensalism/disease and would facilitate this process by the generation of Web-based devices for information comparison. In the first instance it was decided to submit a proposal for an ESF Network as means to evaluate the need for such a network and to expand the number of participants.

Scientific Report

Mutation rates are the two key words that permeated this workshop and were either explicit or implicit in each of the talks. Thus, the intention of the workshop was to explore the mechanisms that control the mutation rates of microsatellites in various organisms and the contribution to bacterial pathogenesis/adaptive behaviour of elevated mutation rates in either localised regions of genomes (eg. loci containing microsatellites) or across the whole genome. The important points of each of the talks relevant to these aims or to other general scientific problems are highlighted in the first section of this report. Through discussions in each of the sessions, the workshop also revealed the current extent of our knowledge of this field and contributed some questions that may point to the future directions which research in this field could take. These are summarised in the second section of the report.

Talks

Session 1. *Cis*-acting factors for eukaryotic microsatellites

DNA structure is a field poorly understood by many non-specialists. Mikael Leijon described a feature of DNA, "the propeller twist", that increases the interactions in A-tracts, but not mixed G/C-tracts. The effects of this structure and of other properties of nucleotides (eg. hydration and methylation) were measured by nuclear magnetic resonance (NMR) of specific oligonucleotides. A-tracts were found to have lower disassociation constants and slower base pair opening dynamics than G/C-tracts. It was discussed, however, that this higher stability of A-tracts in internal positions may be countered by the higher "end-fraying" of A-tracts.

Using a model of DNA replication fidelity, involving gapped-DNA templates and purified proteins, and detailed structural models, Tom Kunkel, in two talks, explained the intricacies of the interactions occurring during DNA polymerisation and the impact of DNA composition and DNA polymerase properties on mutation rates. Repeat tract length and sequence were major *cis*-acting factors for generation of frameshift mutations while the *trans*-acting factors for missense and/or frameshift mutations included the processivity/proof-reading capacities of the DNA polymerase, presence of accessory factors and mismatch repair (MMR). Of particular interest were the differing activities of the multiple DNA polymerases of *E. coli* (five) and humans (~14) and the elucidation from studies of genetic diseases of biological roles (eg. in somatic hypermutation) for some of the human enzymes. An exciting speculation concerned a demonstration of the molecular mechanism of damage caused by an environmental carcinogen and the potential to modify these effects.

The differing mutagenic effects of leading and lagging strand DNA synthesis have been investigated using chromosomally-located reporter constructs in both orientations in *E. coli* by Iwona Fijalkowska. These studies indicate that there are orientation effects for frameshifts in microsatellites and that G-tracts are more mutagenic than A-tracts. For missense mutations the leading strand is more mutagenic during normal growth, this phenomenon being reversed by SOS induction, overexpression of Pol IV or mutations in *dnaX*. These studies provide one approach towards an understanding of the complex problem of DNA polymerase switching at the replication fork.

Session 2. *Trans*-acting factors for eukaryotic microsatellites

Organisms have multiple pathways of DNA repair and new activities are still being found. Erling Seeberg described some new alkylation repair enzymes whose identification began with complementation of alkylation-sensitive *E. coli* mutants. The Ada protein is active on O^6-methyl(me)-guanine and O^4-me-thymine and is also in *E. coli*, but not *Mycobacterium tuberculosis*, a transcriptional regulator. AlkA is active on 7-me-guanine and 3-me-adenine and whose over-expression increases mutation rate by introducing abasic sites into the genome. AlkB is active on 1-me-adenine and 3-me-cytosine and on methylated nucleotides in ssRNA, i.e. it is an RNA damage repair enzyme. Serendipitously, these studies led to the identification of non-coding RNAs that are induced during, and may down-regulate, the SOS response. An exciting prospect is that there are many more non-coding RNAs and that these genes may modulate the responses of pathogens.

The similar mutagenic effects on microsatellites, measured using reporter assays, of mutations in MMR-genes in both yeast and *C. elegans* were described by Thomas Petes. Intriguingly, MMR mutations in nematodes lead to extinction during serial passage, presumably due to the accumulation of mutations, likely through effects on fecundity and senescence. The destabilising effects of mutations in protein kinases on the lengths of the telomeres, a specialised type of microsatellite, were also elaborated. These mutations increase deletions in the telomeres, chromosome loss and telomere-telomere fusions. These results may have implications for antigenic variation in trypanosomes and malaria and for certain genetic diseases.

Translesion DNA synthesis (TLS) is the process whereby DNA replication proceeds through DNA damage with the trade-off of introducing mutations into the genome. Jerome Wagner discussed research using defined substrates and genetic mutants in *E. coli* that has permitted elucidation of the types of damage by-passed by the three SOS-induced DNA polymerases (II, IV and V) and the key role of the β-clamp in polymerase switching during TLS. The potential for alternate forms of the β-clamp indicate that these interactions could be a point at which genome instability is regulated.

The contribution of misfolding of simple sequence repeats to instability of microsatellites is a daunting issue. David Leach has tackled this issue for the human triplet repeat tracts using assays of phage λ plaque size or of alterations in size of plasmid-borne repeat tracts in *E. coli*. Repeat tracts were destabilised by formation of "tight" hairpin loops or particular orientations. These effects were dependent on the complex interactions between SbcCD nuclease, MutS and RecA. Furthermore, repeat array length changes could not be correlated with folding preferences arguing that expansion/contraction of these tracts in *E. coli* is insensitive to misfolding preferences.

Session 3. *Cis*-/*Trans*-acting factors for bacterial microsatellites

Theoretical considerations for the evolution of global and localised hypermutation were the corner-stone of Richard Moxon's introduction to this session. The role of contingency loci as a powerful system for generating stochastic diversity in contrast to gene regulation was reviewed and the key role of genome sequencing in identifying multiple DNA repeat tracts in bacterial genomes was identified as a significant step forward. This overview also summarised Igor Stojiljkovic's research, i.e. the widespread occurrence of mutator strains in *Neisseria meningitidis* group A strains, identification of mutator alleles and the influence of this mutator phenotype on microsatellite mutation rates. The potential synergy of the two modes of hypermutation suggested by these results underlines the importance of current interest in the impact of hypermutation on bacterial pathogenesis.

Capsule switching rates of *N. meningitidis* are not altered by *dam* inactivation but are increased by bicyclomicin, a Rho-inhibitor, were the initial points of Pietro Alifano. Through linkage analysis of different strains, allelic variations in *recB* were identified and shown to alter UV sensitivity, transformation rates and pilin phase variation. Repeated elements in *mfd* of some of *N. meningitidis* strains were also observed.

The genome of *N. meningitidis* contains many repeat tracts. Patricia Martin first revealed how predictive criteria for particular microsatellites being unstable were drawn from comparative sequencing of a sub-set of microsatellites from divergent *N. meningitidis* strains. Then the analysis of switching rates in defined mutants were used to show that inactivation of *mutS* or over-expression of Pol IV destabilised, to varying extents, mononucleotide but not tetranucleotide repeat tracts. Finally, a novel mechanism of tetranucleotide repeat tract-mediated alterations in gene expression was elaborated.

The *Haemophilus influenzae* genome contains multiple tetranucleotide repeat tracts and Chris Bayliss reviewed the research into the *cis*-/*trans*-acting factors controlling the mutation rates of these and other repeats in this bacterial species. Analysis of mutation rates using reporter constructs and defined mutants showed that tract length, Pol I polymerase activity or RnaseH are major factors controlling tetranucleotide repeat tract stability in *H. influenzae* whilst MMR and some other repair pathways have no effect. In contrast, dinucleotide repeat-mediated switching of reporter constructs, but not surprisingly pilus, were shown to be elevated by MMR mutations.

Transformation in *N. meningitidis* and *H. influenzae* is enhanced by a particular DNA sequence, the uptake sequence. Tone Tonjum presented a bioinformatic analysis of these genomes that has led to the observation of the overabundance of the uptake sequence in DNA maintenance genes, potentially indicating that these genes are frequently subject to horizontal transfer. Biochemical analysis has resulted in the potential identification of a binding protein for this uptake sequence.

Specialised seminar. Unstable DNA and inherited human disease.

Many human disorders are associated with the expansion of simple sequence repeats and this phenomenon was the subject of Darren Monckton's presentation. Analysis of human sperm, blood and *ex vivo* cultured cells, has demonstrated that mutation rates can be very high, that there are large biases towards expansions and that tract length and high G+C% content of the flanking DNA increase the mutation rates, whilst loss of MMR generally decreases mutation rates. Notably, expansions seem to result from small changes over many divisions. Observations of expansions in non-dividing cells and varying effects of MMR mutations indicates that inappropriate mismatch repair of unusual structures, and not replication slippage, may be a major mechanism of repeat instability.

Session 4. Epidemiology and Functional effects of elevated mutation rates

Campylobacter jejuni is a commensal of chickens that causes a wide-spectrum of diseases in humans ranging from mild gastoentritis to Guillain Barre syndrome. Brendan Wren highlighted the critical role of genome sequencing in advancing studies of localised hypermutation in *C. jejuni*. Analysis of the genome sequence enabled the rapid identification of loci containing microsatellites. Subsequent genetic and biochemical analysis demonstrated that some of these microsatellites control expression of loci involved in biosynthesis of capsule, addition of terminal sugar residues to lipopolysaccharide (LPS) and motility. It was speculated that escape of phage infection is major factor driving localised hypermutation in this bacterial species.

Mycoplasmas are wall-less microorganisms with small genomes. David Yogev introduced us to the extensive antigenic variation of lipoproteins that occurs in many mycoplasma species. Sectoring was an obvious, common and noteworthy phenomenon in these studies. A specific analysis was described of alterations in expression and size of *M. hyorhinis* Vlp and *M. bovis* Vsp proteins. In the former case variation was mediated by alterations in the numbers of micro- and mini- satellites. In the latter case, variations resulted from intragenic recombination and inversion of fragments mediated by a site-specific recombinase homologous to the *E. coli* Xer protein.

The occurrence of mutators in clinical populations of bacteria has been investigated by measuring the rate of rifampicin resistance. Fernando Baquero reminded us of this methodology and then described the findings. Mutators of different strengths were observed to occur in both carriage and disease-causing isolates of *E. coli*, *Klebsiella* sp., *Pseudomonas aeroginosa*, and *Streptococcus pneumoniae*. In some cases (eg. cystic fibrosis patients) mutators persist for many years. Interesting points concern the potential contribution to genetic variation of "weak" mutators, the possibility of an optimum mutation rate for a particular life-style and the implications of mutators for development of antibiotic resistance.

Highly monomorphic bacterial species are difficult to type and this new field of bacterial forensics is the provenance of Gilles Vergnaud. Isolates were separated based on polymorphisms in tetranucleotide repeat tracts for *M. tuberculosis*, *Yersinia pestis* and other species. The validity of this method was tested by construction of phylogenetic trees and comparison to other typing schemes. A database and website was developed for analysis of tetranucleotide repeats in bacterial genomes and for comparison of results between laboratories.

Clinical isolates of bacteria from similar isolates of a bacterial species are also difficult to type reliably and rapidly. Alex van Belkum demonstrated that variations in microsatellites has utility for differentiation of isolates during the course of infections by *H. influenzae* and *Staphylococcus aureus*. In some persistent infections alterations in particular loci were observed suggesting adaptation. This analysis has also been applied to *Candida albicans* isolates from HIV-infected patients and provides an alternative to schemes such as Amplified Fragment Length Polymorphism.

LPS is the major surface glycolipid of *Haemophilus* and *Neisseria* species. Derek Hood presented the molecular analyses that have shown how variations in structural epitopes are key to virulence and commensal behaviour in these bacteria. Genome analysis, construction of defined mutants and biochemical analysis of LPS molecules by both gel electrophoresis and mass spectrometry has identified the functions of many repeat-associated loci of *H. influenzae* and *N. meningitidis* whose products mediate biosynthesis of LPS epitopes. Use of this knowledge to design biological assays, has resulted in the demonstration that expression of *H. influenze* loci involved in addition of sialic acid is correlated with survival of complement in *in vitro* bactericidal assays and with persistence in animal models.

Helicobacter pylori is responsible for gastritis, ulcers and gastric cancers/lymphomas. Strains of this bacteria expresses Lewis blood group antigens on their LPS molecules and Ben Appelmelk's presentation discussed the biological roles of these antigens. Phase variation of the Lewis antigens in *H. pylori* is mediated by C-tracts in genes encoding glycosyltransferases. Expression of these antigens is required for survival of *H. pylori* in a mouse model of infection and for adherence of *H. pylori* LPS to stomach tissues and to a dendritic cell marker, DCSIGN. Binding to DCSIGN altered the intracellular cytokine profile which is an important modifier of *H. pylori* infection, thus Lewis antigen phase variation could critically influence host adaptation and persistence of this bacterial species.

Assessment of Results and Contribution to Future Directions of Research

Microsatellites (General)

In the first two sessions the talks presented some of the current knowledge of the factors controlling the mutation rates of microsatellites that has been found from studies in biophysical/biochemical assays and genetic systems in humans, *C. elegans*, yeast and *E. coli*. The discussions in these sessions provided a further assessment of this knowledge. Thus, in session one, the discussion split the *cis*-acting factors into those pertaining either to the properties or the context of the repeat tracts. Then it was observed that predictive criteria relating to some properties have been identified, eg. tract length and the interplay between repeat unit size and MMR, but that the detailed molecular mechanisms still remain to be elucidated and that for other properties and for contextual characteristics, factors have been examined but general rules are not yet clear. Discussion in the second session firstly indicated that many types of DNA damage can occur and that there are a large number of DNA pathways for dealing with this DNA damage. The talks had indicated the key role of MMR repair in all systems. No clear rules emerged concerning the influence, across microsatellites and organisms, of any other DNA replication or DNA repair pathways. The general consensus is that there is not yet enough detailed knowledge to make general conclusions and that the mutation rate of each microsatellite may have unique characteristics.

These discussions did, however, raise some interesting questions that may indicate the directions research in this field could take. Some of these questions are:- Does tract length increase mutation rate only through re-alignment or is disassociation of the DNA polymerase also important? How is the DNA strand transferred from the polymerase to the exonuclease domain in DNA polymerases and does this increase repeat instability? What are the effects on microsatellite stability of position relative to the origin of DNA replication and of chromosome structure? Do physiological parameters such as nucleotide concentration influence mutation rate? What is the origin of and frequency of exposure to spontaneous DNA damage for different organisms? What is the influence of spontaneous DNA damage on microsatellite stability? A role for oxidative stress has been observed but what is the mechanism? To what degree in different organisms are microsatellites destabilised during non-replicative growth?

Two key areas of interaction were suggested by these discussions. Firstly, there needs to be established Web-based databases that identify all of the microsatellites (i.e. from all different organisms) and their contexts. Dawn Field reported that such a database for microbial genomes is currently being developed. Secondly, a mutation rate database is required to standardise methodology and to permit comparisons of mutation rates from different organisms and systems.

Bacterial Microsatellites and Mutation Rates

The talks in the third session indicated that there is a paucity of knowledge relating to the *cis*- and *trans*-acting factors that control mutation rates in the bacteria that use these repeats to mediate alterations in gene expression. Discussion, therefore, focussed on the areas of research that need to be addressed in this field and some questions were raised. What influence does different types of DNA damage have on microsatellite stability in the different bacteria using repeats to modulate gene expression? Is there a correlation between the presence of repeats and natural competence? Is there a correlation between the types of repeats utilised and the DNA repair pathways that a bacteria encodes? Are repeats orientated in a particular direction relative to DNA replication or transcription?

In the fourth session, two major topics were covered:- the epidemiology of localised and global mutation rates and the functional implications of altered mutation rates. Epidemiology. This is an area of intense speculation with some provocative findings but many questions. Thus, global mutators exist in natural populations but what effect, if anything, are they having? Similarly, variations in rates of localised hypermutation exist, do they effect pathogenesis? Has mutability evolved to an optimum consistent with the life-style of an organism? Is mutability associated with an increased risk of disease? More epidemiological data is required and has to be combined with experimental and theoretical approaches to provide some answers. Function. General conclusions related to this topic are that the majority of bacterial microsatellites control expression of surface determinants and that alterations in expression may have both functional consequences and permit escape of immune responses. General rules are once again, however, not readily apparent. The workshop did highlight the fact that many surface molecules are receptors for bacteriophages which suggests alterations in expression of these molecules may permit escape of infection but there was no time to discuss the repeat associated restriction-modification systems and many questions remain. The burning questions related to this section are:- To what extent is escape of immune responses important? Is escape of bacteriophage infection mediated by switches in expression of surface molecules and/or restriction-modification systems? This section also raised interest in the possibility that eukaryotic contingency loci exist with analyses of yeast microsatellites being proposed as an initial starting point.

European Science Foundation

Standing Committee for the European Medical Research Councils (EMRC)
Standing Committee for Life and Environmental Sciences (LESC)

ESF EMRC/LESC EXPLORATORY WORKSHOP

Hypermutation of Simple Sequences (Microsatellites) and the Contribution of Hypermutation to Bacterial Pathogenesis

Oxford, United Kingdom
19-22 March 2003

Convened by: Christopher D. Bayliss and E. Richard Moxon
Molecular Infectious Diseases Group, Dept. of Paediatrics,
University of Oxford, United Kingdom
Additional support is generously provided by the
Weatherall Institute for Molecular Medicine.

FINAL PROGRAMME

Wednesday 19th March 2003

20:00 *Welcome*

Introduction to the European Science Foundation
Dr. Hui Wang (ESF EMRC)

Thursday 20 th March 2003

09:00 **1. Introductory Comments** {15 min}

2. Influence of cis-acting factors on the mutation rates of simple sequences/microsatellites

2.1 Sequence effects on spontaneous Base-Pair Opening Fluctuations in Double-Stranded DNA
09:15 Mikael Leijon (Sweden) {45 min}

2.2 Nucleotide Deletions and Additions Due to substrate Misalignment During DNA synthesis
10:00 Tom Kunkel (United States) {45 min}

* *Break (10:45 - 11:00)* *

11:00 **2.3 Unequal fidelity of leading and lagging strand DNA replication on the *E. coli* chromosome**
Iwona Fijalkowska (Poland) {45 min}

11:45 **2.4 Discussion** {45 min}

* *Lunch (12:30 - 13:30)* *

3. Influence of trans-acting factors on the mutation rates of microsatellites

3.1 Overview of DNA replication/DNA repair

13:30 **3.1.1 Inducible responses to DNA alkylation damage in bacterial cells**
Erling Seeberg (Norway) {60 min}

3.2 Genetic regulation of microsatellite stability in yeast and worms

14:30 Thomas Petes (United States) {45 min}

* *Break (15:15 - 15:30)* *

3.3 SOS induced DNA polymerases and Translesion DNA Synthesis within *Escherichia coli* cells

15:30 Jerome Wagner (France) {45 min}

3.4 Role of DNA replication / DNA repair in *Escherichia coli*

16:15 David Leach (UK) {45 min}

17:00 **3.5 Discussion** {45 min}

* *Adjourn (17:45)* *
* *Banquet (19:30)* *

Friday 21 st March 2003

09:00 **4. Influence of trans-acting factors on the mutation rates of simple sequences**

4.1 Overview of localized hypermutation in bacteria
4.1.1 Adaptive strategies of bacterial pathogens: the role of phase and antigenic variation
Richard Moxon (UK) {60 min}

4.2 Role of trans-acting factors in *Neisseria meningitidis*

10:00 **4.2.1 Functional allelic variation of DNA repair and recombination genes in *Neisseria meningitidis* strains**
Pietro Alifano (Italy) {30 min}

* *Break (10:30 - 11:00)* *

10:00 **4.2.2 Involvement of dam, mutS and dinP in the regulation of phase variation frequencies in *Neisseria meningitidis***
Patricia Martin (France) {45 min}

* *Lunch (11:45)* *

Transfer to Weatherall Institute for Molecular Medicine

13:00 **Human DNA Polymerases:Structure-function Studies and Health Implications**
Institute Seminar by Tom Kunkel {60 min}

Return to Jesus College for continuation of workshop

4.3 Role of trans-acting factors in *Haemophilus influenzae*

15:00 **4.3.1 Influcence of repeat tract length and trans-acting factors on simple sequence-mediated phase variation rate in *Haemophilus influenzae***
Chris Bayliss (UK) {45 min}

4.4 Genome dynamics in *Neisseria meningitidis*

15:45 Tone Tonjum (Norway) {30 min}

16:15 **4.5 Discussion** {45 min}

17:00 **5. Unstable DNA and inherited human disease**
5.1 Darren Monckton (UK) {45 min}

* *Adjourn (17:45)* *
* *Dinner (19:00)* *

20:00 *Discussion: initiatives for European-funded collaborative research projects*

Saturday 22nd March 2003

09:00 **6. Studies of switching in simple sequence contingency loci or global hypermutation using epidemiological samples or models systems**

6.1 Overviews of contingency loci in specific bacteria

09:00 **6.1.1 Chameleonbacter jejuni – the changing polysaccharide coat of a nasty food-borne pathogen**
Brendan Wren (UK) {30 min}

09:30 **6.1.2 Highly Mutable Genomic Modules for the Generation of Surface Antigenic Variation in Pathogenic Mycoplasmas**
David Yogev (Israel) {45 min}

6.2 Epidemiological studies

10:15 **6.2.1 Variability in Rifampicin Mutation Frequency of *Escherichia coli*, *Pseudomonas aeruginosa* and *Sptreptococcus pneumoniae* Clinical Isolates**
Fernando Baquero (Spain) {30 min}

* *Break (10:45-11.00)* *

11:00 **6.2.2 The use of tandem repeats for the molecular epidemiology of bacterial pathogens including *Y. pestis*, *B. anthracis*, *P. aeruginosa*, and *M. tuberculosis***
Gilles Vergnaud (France) {45 min}

11:45 **6.2.3 Short sequence repeats as target motifs for epidemiological typing of pathogenic microorganisms**
Alex van Belkum (Netherlands) {45 min}

* *Lunch (12:30 - 13:45)* *

6.3 Studies in model systems

13:45 6.3.1 **Phase variable expression of lipopolysaccharide epitopes in the pathogenic bacteria *Haemophilus influenzae* and *Neisseria meningitidis***
Derek Hood (UK) {30 min}

14:15 6.3.2 ***Helicobacter pylori* colonizes the gastric mucosa of one third of mankind for a lifetime. How is the organism able to chronically persist in so many people?**
Ben Appelmelk (Netherlands) {30 min}

14:45 **6.4 Final discussion** {45 min}

15:30 **7. Concluding remarks** {15 min}

* End (15:45)

Final List of Participants

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Statistical Information on Participants

i) Age structure.
   Invited Speakers:- 8 30-39, 10 40-49, 3 50+
   Other Participants:- 2 20-29, 3 30-39, 1 40-49.

ii) Country of Origin
   Invited Speakers:-3 France, 1 Italy, 1 Israel, 2 Netherlands, 2 Norway, 1 Poland, 2 Spain, 1 Sweden, 6 United Kingdom, 2 United States of America.
   Other Participants:- 1 France, 3 United Kingdom, 2 United States of America.

iii) Current Country of Work
   Invited Speakers:- 2 France, 1 Italy, 1 Israel, 2 Netherlands, 2 Norway, 1 Poland, 2 Spain, 1 Sweden, 7 United Kingdom, 2 United States of America.
   Other Participants:- 1 France, 4 United Kingdom, 1 United States of America.