EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

Exploratory Workshops Scheme

Standing Committee for Life, Earth and Environmental Sciences (LESC)

ESF Exploratory Workshop on

Heterochromatin structure and function: from repetitive DNA sequences to epigenetics

Donja Stubica (Croatia), 20-23 September 2008

Convened by:

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SCIENTIFIC REPORT

1. Executive summary

The ESF Exploratory Workshop "Heterochromatin Structure and Function: From Repetitive DNA Sequences to Epigenetics" was held in Donja Stubica (Hotel Jezerčica) Croatia, from 20th to 23rd September 2008. The workshop was convened and organized by Miroslav Plohl (Ruđer Bošković Institute, Zagreb, Croatia), and co-convened by Barbara Mantovani (University of Bologna, Italy), Fernando Azorin (Institute of Molecular Biology of Barcelona, Spain), and John S. (Pat) Heslop-Harrison (University of Leicester, United Kingdom). The workshop was supported by a 10,300 € grant from the ESF, of which approx. 9,900 € was utilized. The venue and accommodation in hotel Jezerčica enabled contacts and communications between all participants during the whole day.

Scientists from 12 groups from Universities and research Institutes located in 9 countries including Israel were invited to the workshop. 21 scientist was present out of 24 invitees, three could not come for personal reasons (B. Mantovani, Bologna; G. Kuhn, Leicester; D. Segal, Tel-Aviv), and instead of one participant unable to come (A. Sanches, Jaen) another member of the same group participated to the workshop (J. Marchal). However, all invited groups were represented with at least one participant. The presentations planned to be given by invitees unable to come were held either by their colleagues from the group or in the form of a teleconference by using Internet facilities, also utilized for discussions. In this way, all planned presentations were held without cancellations.

Two scopes of the meeting were of particular importance:

- to create a scientific community focused on different aspects of DNA/protein structure and interactions in heterochromatin in order to build the interdisciplinary environment in which molecular genetics, protein biochemistry and epigenetic data from models and other organisms of either basic or applied interest can be studied
- to explore possibilities for preparation of a European-scale project

In two and a half days, the workshop was organized as a series of oral presentations, followed by extensive discussions. In particular, round table discussions aimed to establish a basis for a joint project application. All sessions were plenary.

The workshop was opened by a foreword given by M. Plohl in which planned goals were emphasized. ESF was presented in the talk given by ESF representative Professor Željko Kućan. Subsequently, representatives of each participating group gave a short review of their interests and ongoing projects. This was followed by two introductory presentations, in which several aspects and ideas related to heterochromatin and underlying DNA sequences were given.

Presentations were grouped into three sessions.

- Session 1: DNA aspects of heterochromatin

Data on satellite DNA diversity and evolution were presented in 7 talks. Two of them referred to the phenomenon of extrachromosomal circular DNA as a possible mechanism of spread and maintenance of satellite DNAs (sequence segments repeated in tandem) that underlie all heterochromatic compartments.

- Session 2: Protein data and epigenetics

Five presentations showed some specific aspects of heterochromatin structure and the role of epigenetic modifications, such as in condensation and maintenance of the heterochromatic state. New results concerning chromatin structure in the functional centromere were presented.

- Session 3: Model organisms and genome projects

In 7 talks, data on large-scale DNA composition of heterochromatic regions were presented. Limitations concerning the outputs of genome projects and heterochromatin DNA sequence composition were highlighted in studies of two recently sequenced invertebrate model organisms. The second generation sequencing technologies (454 sequencing method) proved advantageous in the large-scale satellite DNA analyses and will certainly make a breakthrough in studies of repeated DNA sequences in heterochromatin.

Presentations were thoroughly discussed, discussions extending over the scheduled program. Round table discussions resulted in defining a basis for European-scale joint research project. The most important conclusion was to prepare joint application for a project within the ESF-funding abilities that will stress questions regarding the structure, function, epigenetic control and evolution of heterochromatic regions. Rough draft of ideas relevant for the proposal in the form of possible working packages was worked out.

2. Scientific content of the event

Heterochromatin is the least explored genomic compartment. Composed mostly of sequences repeated in tandem (satellite DNA), it is an epigenetically determined domain of eukaryotic chromosomes located in functionally crucial pericentromeric and telomeric regions, able to affect a number of genes in a coordinated action on a global basis. Recent years brought a number of data concerning composition and mechanisms of heterochromatin formation and maintenance, and the role of the histone code as an epigenetic determinant in heterochromatin-related processes. However, organizational, functional and evolutionary properties of DNA and protein heterochromatin constituents and epigenetic mechanisms acting on them are still poorly understood. In particular, there is a lack of data linking DNA sequence and heterochromatin structure and properties. The purpose of this Exploratory Workshop was to create scientific community able to use an integrative and interdisciplinary approach in focusing on heterochromatin structure and functionality, by linking DNA data (structure, organization and evolution of satellite DNAs) and protein-related information (centromere/heterochromatin assembly and structure, histone code in heterochromatic nucleosomes) in model organisms, but also in other organisms of either basic or applied interest.

The workshop gathered leading experts from Europe and Israel working on different aspects of heterochromatin structure, function and evolution. The scientific presentations were grouped into three consecutive sessions, and talks were followed by extensive discussions. General comments were envisaged at the end of each session.

- Session 1: DNA aspects of heterochromatin, chairs: Sarit Cohen (Israel) and Nevenka Meštrović (Croatia)

Talks in this session presented evolutionary dynamics of satDNAs in insect and rodent species as well as analysis of extrachromosomal circular DNA (eccDNA) in animals and plants. Analysis of satDNA dynamics in insects with non-canonical reproductive systems demonstrated higher intra- than intergroup sequence homogeneity as predicted by the model of concerted evolution only in bisexuals. Exceptionally, also specific biological traits in eusocial termites can lead to the non-concerted patterns of satellite DNA evolution. Comparison of satDNAs in two sibling *Tribolium* species suggests an evolutionary scenario where both DNA sequence and higher-order architecture of a unique basic building element are changed to generate different, species-distinctive centromerically-positioned satDNA families. In Chrysomelidae, interspecific analysis of satDNA shows high heterogeneity in relation to the organization and complexity. Comparative studies of structure and organization of two satDNAs (pBum and DBC-150) in *Drosophila buzzati* species group revealed complex pattern of variation concerning abundance, chromosomal distribution, long-

range organization and homogenization rate. Analysis of Msat-160 satDNA in three rodent genera shows species-specific chromosomal distribution. Studies of eccDNA have demonstrated that it consists of exact multiples of the repeating units. Suggested intra-chromosomal recombination in the formation of eccDNA may contribute to shrinking of satDNA arrays. In addition, evidence for rolling circle replication of eccDNA in *Drosophila* and humans, suggests an efficient mechanism for satellite DNA expansion and homogenization.

- Session 2: Protein data and epigenetics, chairs: Fernando Azorin (Spain) and Gunter Reuter (Germany)

This session focused on different aspects related to the epigenetic control of heterochromatin structure and function. Special emphasis was on the analysis of heterochromatin in two different model organisms, *Drosophila* and *Arabidopsis*; similitude and differences in relation to patterns of histone post-translational modifications as well as on the functional properties of enzymes responsible for these modifications was presented. These studies reflect the variety, and complexity, of the molecular processes controlling heterochromatin formation and maintenance in different organisms, with implications in favor to the “histone code” hypothesis. Studies using different *Arabidopsis* species indicate the versatility of heterochromatin and its ability to response functionally to different types of stress, such as light. Strikingly, light-stress induces a reversible decondensation of heterochromatin. In addition, the structural and functional consequences of a paracentric inversion in *Arabidopsis* also provide insights on the contribution of heterochromatin to the structural organization and functional properties of flanking euchromatin. The structural organization of centromeric chromatin was also analyzed in several *Beta* species, which is composed by different satellites and centromere-specific retrotransposons. Finally, the question of the epigenetic regulation of centromere identity and function was also addressed in *Drosophila*, and, in particular, the molecular mechanisms determining deposition at centromeres of the centromere-specific histone H3 variant, CenH3, and its specific contribution to the kinetochore assembly.

- Session 3: Model organisms and genome projects, chairs: Thomas Schmidt (Germany) and Jiři Macas (Check Republic)

The results of molecular analysis of genomes of diverse species, each representing a model organism in its taxonomic group have been presented in the talks. The species included insects (ants and the beetle *Tribolium castaneum*), molluscs (*Bivalvia*), plant-pathogenic nematodes (*Meloidogyne incognita*) and plants (*Beta vulgaris*, *Pisum sativum* and *Oryza sativa*). Even when genomes are completed in sequencing projects, the annotation of repetitive DNA such as satellite DNA and transposable elements is lagging behind, showing that the molecular analyses of repetitive DNA sequences is critical for the full understanding of the genome structure, function and

evolution. This fact is of importance in a period when next generation sequencing technologies are able to produce genomic data at an ever accelerating speed. The in depth-annotation of satellite DNA requires novel approaches and bioinformatic tools. The application of such bioinformatic tools has been presented using the available genome sequence of rice as an example. Results of particular scientific interest were the presentation of a 540 Myr old satellite DNA in molluscs. This satellite DNA is the oldest tandemly repeated sequence family isolated so far from eukaryotic genomes, raising the question which constraints are acting during the evolution of this repetitive DNA. The intragenomic variability and segmental genome duplication of the nematode *M. incognita*, the formation of higher-order complex satellite DNA repeats of *B. vulgaris* and their conservation across species borders and the impact of the haplo-diploid reproduction system on satellite DNA homogenization in ants are striking examples of the scientific importance and significance of the analysis of repetitive DNA and its linking with the heterochromatin structure.

The idea of future collaborative actions in the field was assessed in round table discussions devoted to the “Defining a basis for European-scale joint research project” (part I and II), chairs John S. Heslop-Harrison (United Kingdom) and Miroslav Plohl (Croatia), and “Conclusions and preparation of joint report”, chairs Miroslav Plohl (Croatia) and Fernando Azorin (Spain). It has been concluded that the emerging research topic is of the highest potential (i) because of the recently accumulated data that link DNA, protein and genome structure and epigenetic modifications as a mechanism that regulates chromatin structure, and (ii) because of recent advances in experimental methods that enable easier assessment to these regions. With this idea, it was decided:

- to prepare a joint project application that will include participating groups (and probably few additional interested in the field)
- to submit project application to one of the ESF collaborative calls
- to open WEB pages to facilitate exchange of ideas, discussions among interested parts and preparation of the project proposal (http://w3lamc.umbr.cas.cz/satnet/)

As the first step, the main points of the future application were discussed and defined on the meeting, stressing the impact of satellite DNAs and heterochromatin on the function and evolution of genomes as the main objective. Six working packages were sketched, going from the structure, organization, regulation and dynamics of heterochromatin compartments to consequences that changes in these genomic regions might cause, such as various diseases including cancers. The planned work will include different model organisms in order to resolve fundamental questions in a complex and diverse system of heterochromatin, epigenetic modifications and gene expression.

3. Assessment of the results, contribution to the future direction of the field

The workshop fulfilled its objectives in gathering leading scientists in the field and in creating scientific community interested in elucidation of heterochromatin composition, structure and dynamics, its functional roles and impacts on the genome and gene regulation. There was a general consensus that establishing a European-scale network is necessary in order to provide a comprehensive and highly competitive platform based on knowledge and experience of all participating groups. Different fields of expertise offered by participants guarantee interdisciplinary character of future collaborative actions. In addition, it was concluded that heterochromatin is far too complex and diverse to rely on just a few model organisms. Although the well-defined established model organisms offer obvious advantages, it is clear that the work on other organisms, particularly on those in taxonomically distinct groups can reveal valuable information. The first draft of objectives and ideas that will be integrated in the future collaborative project proposal was outlined.

It has been observed that scientific community interested in heterochromatin research is often split to scientists working on repetitive DNA sequences and those studying different aspects of protein components in heterochromatin. Integrative approach to the study of heterochromatin structure and function by linking DNA and protein data is needed to obtain comprehensive picture of these complex genomic regions. For example, this will explain the possible role of DNA sequence in heterochromatin compaction and on epigenetic modifications. Significant contribution is also expected in unraveling long-range sequence composition in heterochromatic regions, by filling gaps in outputs of genome projects known to seriously underestimate genomic tandem repetitive fractions. New generation of sequencing technologies is ready for that job. In turn, this will require novel bioinformatic approaches and tools. The long-term output is in obtaining full understanding of heterochromatin structure and function, particularly including the region of functional centromere, in order to understand the eukaryotic genome as a whole.

4. Final programme

Saturday 20 September 2008

Morning *Arrival*

13.00-15.00 *Lunch*

Opening session (Chair: M. Plohl)

16.00-16.30 **Opening and foreword**
Miroslav Plohl

Presentation of the European Science Foundation (ESF)
Željko Kućan (ESF Standing Committee for Life, Earth and Environmental Sciences)

16.30-17.30 **Introduction of each group** *(5 minutes)*
- Pat Heslop-Harrison
- Jiri Macas
- Thomas Schmidt
- Fernando Azorin
- Teresa Palomeque
- Monica Bullejos
- Philippe Castagnone-Sereno
- Gunter Reuter
- Paul Fransz
- Sarit Cohen
- Andrea Luchetti
- Miroslav Plohl

17.30-17.50 *Coffee break*

17.50-18.30 **Satellite DNAs, chromosomes and heterochromatin**
Pat Heslop-Harrison (University of Leicester, UK)

18.30-19.10 **Evolution of satellite DNA sequences: stability versus rapid changes**
Miroslav Plohl (Ruder Boskovic Institute, Zagreb, HR)

20.00 *Dinner*

Sunday 21st September 2008

Session 1: DNA aspects of heterochromatin (Chair: S. Cohen, N. Meštrović)

09.00-09.25 **Satellite DNAs in non-canonical reproducing systems**
Andrea Luchetti (Università degli Studi di Bologna, IT)

09.25-09.50 **Extrachromosomal circular DNA and the plasticity of tandem repeats in eukaryotes**
Sarit Cohen (Tel-Aviv University, IL)

09.50-10.15 **Extrachromosomal circular DNA: Recombination by-product or a key intermediate in evolution of plant satellite repeats?**
Alice Navratilova (Institute of Plant Molecular Biology, Cezke Budejovice, CZ)

10.15-10.40 **Satellite DNAs in closely related species: the case of *Tribolium* beetles**
Brankica Mravinac (Ruder Boskovic Institute, HR)

10.40-11.00 *Coffee break*

11.00-11.25 **Satellite DNAs in Chrysomelidae (Coleoptera)**
Pedro Lorite (Universidad de Jaen, ES)

11.25-11.50 **Satellite DNA organization and evolution in neotropical cactophilic *Drosophila***
Gustavo Kuhn (University of Leicester, UK)

11.50-12.15 **Constitutive heterochromatin blocks in giant sex chromosomes**
Juan Marchal (Universidade de Jaen, ES)

12.15-13.00 **Round table: DNA aspects of heterochromatin (Chair: S. Cohen, N. Meštrović)**

13.00-15.00 *Lunch*

Session 2: Protein data and epigenetics (Chair: F. Azorin, G. Reuter)

15.00-15.40 **Epigenetic processes controlling heterochromatin differentiation in *Drosophila* and *Arabidopsis***
Gunter Reuter (Martin-Luther University Halle-Wittenberg, DE)

15.40-16.20 **Centromere identity and function**
Fernando Azorín (Institute of Molecular Biology of Barcelona, ES)

16.20-16.50 **Genetic and epigenetic consequences of a paracentric inversion in *Arabidopsis thaliana***
Paul Fransz (University of Amsterdam, NL)

16.50-17.10 *Coffee break*

17.10-17.40 **Heterochromatin decondensation in *Arabidopsis* triggered by light stress**
Paul Fransz (University of Amsterdam, NL)

17.40-18.10 **The structural organization of major DNA sequences at *Beta* centromeres**
Thomas Schmidt (Dresden University of Technology, DE)

18.10-19.00 **Round table: Protein data and epigenetics (Chair: F. Azorin, G. Reuter)**

19.00-21.00 *Dinner*

21.00-22.00 **Round table: Defining a basis for European-scale joint research project I (Chair: Pat Heslop-Harrison, M. Plohl)**

Monday 22nd September 2008

Session 3: Model organisms and genome projects (Chair: T. Schmidt, J. Macas)

09.00-09.40 **Genome projects and heterochromatin, nematodes and diffuse centromeres**
Philippe Castagnone-Sereno (INRA/UNSA/CNRS, FR)

09.40-10.05 **Satellite DNAs and insect models – *Tribolium***
Nevenka Meštrović (Ruder Boskovic Institute, HR)

10.05-10.30 **Repetitive DNAs in bivalve molluscs**
Andrea Luchetti (Università degli Studi di Bologna, IT)

10.30-10.55 **Higher order structure of a major centromeric satellite of sugar beet (*Beta vulgaris*)**
Gerhard Menzel (Dresden University of Technology, DE)

10.55-11.15 *Coffee break*

11.15-11.40 **High throughput genome sequencing as a new tool for global characterization of satellite DNA**
Jiri Macas (Institute of Plant Molecular Biology, Ceske Budejovice, CZ)

11.40-12.05	**Satellite DNAs and haplo-diploid insect models** **Teresa Palomeque** (Universidad de Jaen, ES)
12.05-12.30	**Pericentromeric satellite DNA in a rodent family** **Monica Bullejos** (Universidad de Jaen, ES)
12.30-13.05	**Round table: Model organisms and genome projects (Chair: T. Schmidt, J. Macas)**
13.05-15.30	*Lunch*
15.30-17.00	**Round table: defining a basis for European-scale joint research project II (Chair: Pat Heslop-Harrison, M. Plohl)**
17.00-17.30	*Coffee break*
17.30-19.00	**Conclusions and preparation of joint report (Chair: M. Plohl, F. Azorin)**
20.00	*Conference Dinner and informal discussions*

Tuesday 23rd September 2008

Morning	*Departure*

5. Statistical information on participants

Country	Lab/group	# participants
Spain	3	5
France	1	1
United Kingdom	1	2
Italy	1	2
Croatia	1	4
Germany	2	4
The Netherlands	1	1
Check Republic	1	2
Israel	1	1

Gender:	M	13
	F	9

Young researchers	13
Senior researchers	9

6. The final list of participants

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