LSHG-CT-2004-503464

Regulation of Mitosis by Phosphorylation

- A Combined Functional Genomics, Proteomics and Chemical Biology Approach

(MITOCHECK)

Integrated Project

In

Life sciences, genomics and biotechnology for health

FINAL ACTIVITY REPORT

Start date of project:	April 1, 2004	Duration: 60 months
Project Coordinator:	Dr. Jan-Michael Peters Forschungsinstitut für Molekulare Pathologie GmbH	
Date of preparation:	24 August, 2009	Revision: Draft 1

TABLE OF CONTENTS

Final Activity Report

1. PROJECT EXECUTION

1.1 Project objectives

Objective 1: Identify all human proteins that are required for mitosis

During the past five years, the MitoCheck consortium has carried out a genome-wide RNA interference (RNAi) screen that have led to a systematic analysis of genes and proteins required for chromosome segregation and cell division in human cells.

Objective 2: analyze function of the mitotic proteins by large-scale localization and interaction studies

RNAi screening is a powerful technique for identifying genes required for mitosis and other cellular processes. However, detailed molecular analyses are needed to understand the precise function of these genes, among which protein localization and identification of protein interactions are particularly informative. The MitoCheck consortium has successfully established a pipeline for fast and reliable introduction of genes tagged in bacterial artificial chromosomes (BACs) into human tissue culture cells (Poser *et al.*, *Nat Methods* **5**, 409), using previously developed techniques of recombineering (Zhang *et al.*, *Nat Genet* **20**, 123). These allow the stable expression of genes under their own promoters at physiological levels in mammalian cells (Poser *et al.*, *Nat Methods* **5**, 409). MitoCheck has applied this “BAC TransgeneOmics” approach to characterize proteins complexes required for mitosis by performing localization experiments and by purifying protein complexes and analyzing them by mass spectrometry.

Objective 3: study the functions of the mitotic kinases at a molecular level

It is well established that the protein kinases Cdk1, Plk1 and Aurora kinases are essential form mitosis, but the precise molecular functions of these enzymes remain incompletely understood. MitoCheck has therefore also developed novel approaches to study mitotic kinases.

Objective 4: systematically evaluate the diagnostic or prognostic potentials of the mitotic kinases in tumor pathology

Some mitotic kinases are over-expressed in human tumors. The MitoCheck Pathology Unit has developed assays to systematically evaluate the potential diagnostic or prognostic value of mitotic kinases in tumor pathology.

Objective 5: disseminate and exploit the knowledge and technology generated by the MitoCheck consortium

MitoCheck has taken the following approaches to disseminate the knowledge and technology generated by the MitoCheck consortium to the scientific community.

- publications in peer-reviewed journals;
- presentations at national and international conferences;
- multiple training courses on technology developed by MitoCheck
- a human genome-wide database (*www.mitocheck.org*).

1.2 Contractors involved

Partner #	Partner name	Partner short name	Country
1	Research Institute of Molecular Pathology	IMP	Austria
2	European Molecular Biology Laboratory	EMBL	Germany
3	Deutsches Krebsforschungszentrum	DKFZ	Germany
4	Leica Microsystems CMS GmbH	Leica	Germany
5	Max Planck Institute of Molecular Cell Biology and Genetics	CBG	Germany
6	Gene Bridges GmbH	GB	Germany
7	European Institute of Oncology	EIO	Italy
8	Centre National de la Recherche Scientifique	CNRS	France
9	Clare Hall Laboratories, Cancer Research UK	CHL-CRUK	U. K.
10	University College London	UCL	U. K.
11	Wellcome Trust Sanger Institute	Sanger	U. K.

1.3 Project Logo

1.4 Project website

www.mitocheck.org

1.5 Work performed and end results

During the past five years, the MitoCheck consortium has developed and applied technologies that have enabled a systematic analysis of genes and proteins required for chromosome segregation and cell division in human cells. Human cells, like all eukaryotic cells, pass their genomes from one cell generation to the next by first duplicating their DNA in S-phase and then segregating the resulting copies during mitosis. Chromosome segregation during mitosis is an immensely complex process that remains poorly understood at the molecular level. Mistakes during mitosis contribute to cancer, whereas mistakes during meiosis are the leading cause of infertility and mental retardation. Although many proteins required for mitosis had previously been identified, the entire set of proteins that are needed for this process remained unknown. One major objective of the MitoCheck consortium was therefore to identify all human proteins that are required for mitosis. To achieve this goal, a genome-wide RNA interference (RNAi) screen was performed by the MitoCheck Screening Unit, which was formed by the European Molecular Biology Laboratory (EMBL), the German Cancer Research Center (DKFZ) in Heidelberg and Leica Microsystems CMS in Mannheim. To enable the genome-wide RNAi screen, the MitoCheck Bioinformatics Unit at the Sanger Institute in Hinxton annotated about 22 000 human genes, all of which were then depleted one by one with several siRNAs in a newly developed solid-phase transfection assay by the MitoCheck Screening Unit. All siRNA transfected cells were analyzed by live cell imaging, resulting in about 260 000 movies. These movies were analyzed by automatic image analysis tools that were developed by MitoCheck, and in the future, all movies and their annotation will be displayed on the MitoCheck website (*www.mitocheck.org*).

RNAi screening is a powerful technique for identifying genes required for mitosis and other cellular processes. However, detailed molecular analyses are needed to understand the precise function of these genes, among which protein localization and identification of protein interactions are particularly informative. In principle these secondary analyses should proceed at the same rate as phenotypic screens. Indeed, in yeast efficient intrinsic homologous recombination has enabled the modification of most genes at their endogenous loci with tag-coding sequences and has been valuable in allowing systems wide analysis of

protein function (Ghaemmaghami *et al.*, *Nature* **425**, 737; Huh *et al.*, *Nature* **425**, 686; Gavin *et al.*, *Nature* **440**, 631; Krogan *et al.*, *Nature* **440**, 637). In mammalian cells, large-scale localization and interaction studies of proteins expressed under control of their own regulatory sequences have so far lagged far behind phenotypic analysis. These analyses are therefore performed on an individual gene basis, precluding systems-scale analysis of protein function. To enable the latter approach in mammalian cells, the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (CBG), which forms the core of the MitoCheck Tagging Unit, has developed a pipeline for fast and reliable introduction of genes tagged in bacterial artificial chromosomes (BACs) into human tissue culture cells (Poser *et al.*, *Nat Methods* **5**, 409), using previously developed techniques of recombineering (Zhang *et al.*, *Nat Genet* **20**, 123). These allow the stable expression of genes under their own promoters at physiological levels in mammalian cells, thus avoiding artifacts that are due to over-expression (Poser *et al.*, *Nat Methods* **5**, 409). MitoCheck has applied this “BAC TransgeneOmics” approach to characterize proteins complexes required for mitosis by performing localization experiments in the Tagging Unit, and by purifying protein complexes and analyzing them by mass spectrometry in MitoCheck's Phosphorylation Unit at the Research Institute of Molecular Pathology (IMP) in Vienna. This work has led to the characterization of about 100 protein complexes, many of which had previously not or only incompletely been characterized. In combination with secondary RNA interference assays this work has led to important new molecular insights into centrosome function, spindle assembly and chromosome segregation. The approaches developed by MitoCheck will be generally applicable to high throughput follow up of phenotypic screens in mammalian cells.

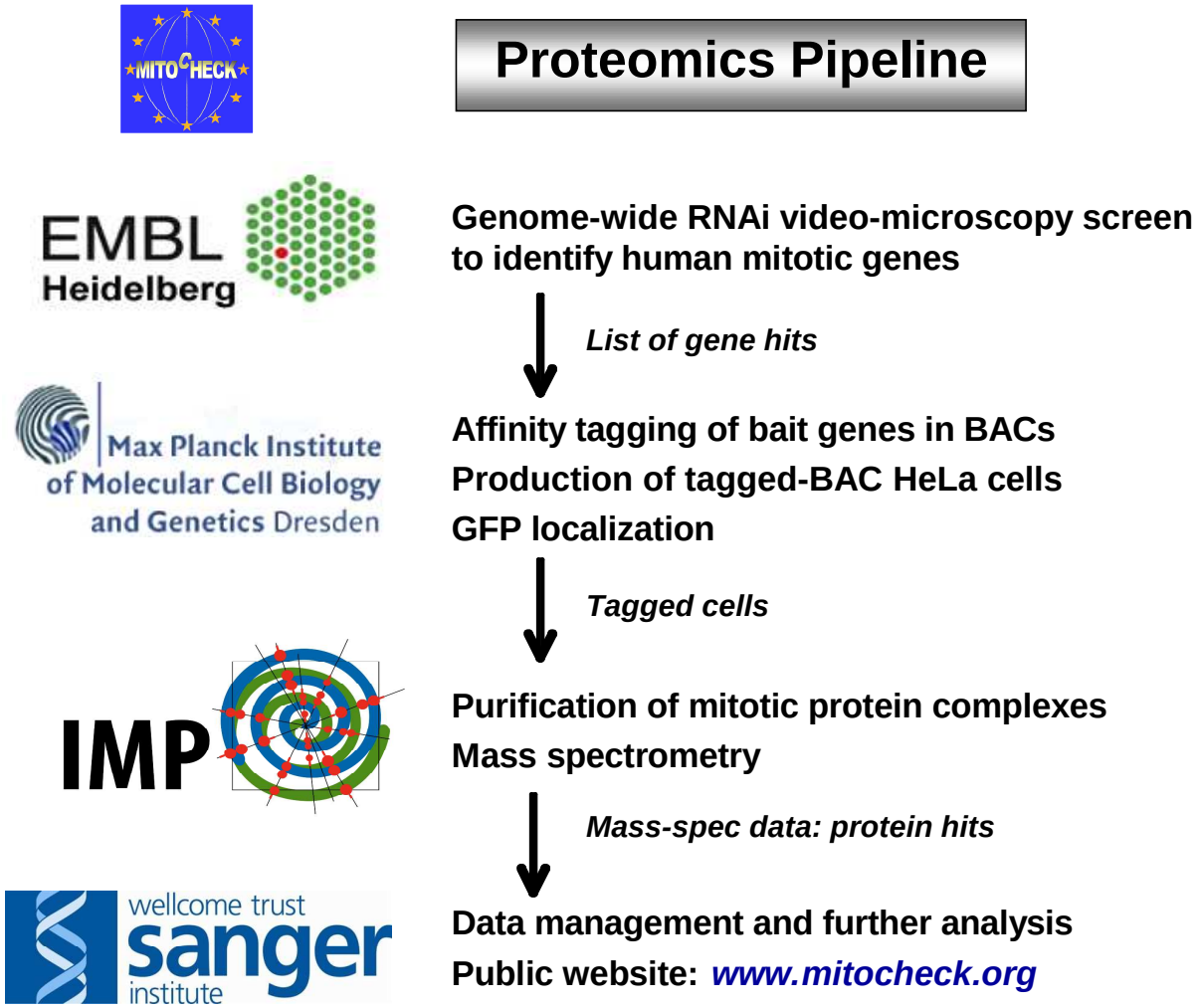

Figure 1: A schematic outline of the major activity flow within the MitoCheck proteomics pipeline.

It is well established that the protein kinases Cdk1, Plk1 and Aurora kinases are essential form mitosis, but the precise molecular functions of these enzymes remain incompletely understood. MitoCheck has therefore also developed novel approaches to study mitotic kinases. To identify substrates of Plk1 and Aurora kinases the Phosphorylation Unit used selective small molecule inhibitors of these enzymes. These compounds were provided by the pharmaceutical company Boehringer Ingelheim. By using these inhibitors previously unknown substrates of Plk1 and Aurora B were identified by mapping phoshorylation sites by mass spectrometry. In parallel, the MitoCheck Kinase Engineering Unit at the European Institute of Oncology in Milan (EIO), the Centre de Recherches de Biochimie macomoleculaire in Montpellier, and the Clare Hall Laboratories of Cancer Research UK in South Mimms used protein crystallography and genetic engineering to create alleles of mitotic kinases whose activities can be controlled by synthetic analogs of ATP (called, after their inventor, Shokat analogs), and to understand how existing kinase inhibitors interact with their target kinases. The Kinase Engineering Unit solved the structure of Aurora B bound to a fragment of its activator INCENP and the small molecule inhibitor Hesperadin (Sessa et al., *Mol. Cell.* **18**, 379).

Some mitotic kinases are over-expressed in human tumors, and the MitoCheck Pathology Unit at the Department of Pathology of the University College London has therefore developed assays to systematically evaluate the potential diagnostic or prognostic value of mitotic kinases in tumor pathology. The Pathology Unit identified antibodies that are suitable for the development of such clinical assays and has performed clinical trials on ovarian and breast cancer patients with these new assays. These studies have led to the identification of new biomarkers which are more reliable predictors of disease-free survival than previously used assays (Kulkarni et al., *Clin Cancer Res*. **15**, 2417; Loddo et al., *Br J Cancer*; **100**: 959).

The MitoCheck project was managed by the IMP in Vienna, which also helped to coordinate scientific exchange among the consortium members. In addition to eleven meetings that were held during the past five years, numerous smaller meetings and visits took place among MitoCheck consortium members to facilitate efficient communication, collaboration and to transfer know-how from

one partner to another. To disseminate MitoCheck technologies within the larger scientific community one practical training course on "High Throughput Microscopy for Systems Biology", three training courses on proteomics and two training courses on BAC tagging technology were held at EMBL, IMP and at MPI-CBG, respectively. In addition, the MitoCheck Pathology Unit co-organized a meeting in June 2008 on diagnostic, prognostic and predictive testing in breast cancer where MitoCheck results were presented to a large audience of clinical cancer pathologists.

To enable the exploitation of MitoCheck data by the scientific community a human genome-wide database (*www.mitocheck.org*) has been generated. This database will contain data generated by the MitoCheck consortium, including information on tagged BACs, immunofluorescence images obtained by GFP localization, silver stained SDS-PAGE gels of all protein samples obtained by tandem affinity purification, and all protein interaction lists obtained by mass spectrometry. In addition, this database will contain all movies from the MitoCheck RNAi screen in which mitosis has been analyzed by live imaging of cells in which all human proteins have been targeted by siRNAs. The database also provides information about gene synonyms used in the literature, orthologs in other species and protein interactions reported in public databases. This collection of localization, interaction and phenotypic data will be a useful resource for understanding the functions of human proteins.

2. DISSEMINATION AND USE

2.1 Exploitable knowledge and its use

Confocal microscopy-based platform for intelligent, large-scale data acquisition

During the course of the MitoCheck project, the Screening Unit (EMBL, Leica Microsystems CMS and DKFZ) have developed advanced, confocal microscopy-based screening platforms allowing high speed screening of different sample types at large scale over an extended period of time. This platform is highly flexible and can be adapted for various applications. Using these platforms, MitoCheck has carried out genome-wide RNA interference (RNAi) screens in human cells searching for genes required for mitosis. This effort has resulted in some 260,000 high quality movies soon available at the MitoCheck website (*www.mitocheck.org*).

New biomarkers with diagnostic or prognostic potential in clinical tumor pathology

Some mitotic kinases are over-expressed in human tumors, and the MitoCheck Pathology Unit at the Department of Pathology, University College London, has therefore developed screens to systematically evaluate the potential diagnostic or prognostic value of mitotic kinases in tumor pathology. The Pathology Unit identified antibodies that are suitable for the development of such clinical tests and has performed clinical trials on ovarian and breast cancer patients with these new assays. These studies have led to the identification of new biomarkers which are more reliable predictors of disease-free survival than currently used clinico-pathological parameters (Kulkarni et al., *Clin Cancer Res*. **15**, 2417; Loddo et al., *Br J Cancer*; **100**: 959). These proof-of-concept studies have generated the rationale for developing a novel cell cycle biomarker test for cancer diagnosis, prognosis and prediction of response to treatment. The Pathology Unit is currently in discussions with commercial parties interested in developing such a theranostics test. The R&D programme involving development of the test and an image analysis platform as well as clinical trials approved by regulatory bodies is foreseen to last at least 4-5 years.

The table below provides an overview of the exploitable knowledge generated by the MitoCheck project and their (potential) applications.

Exploitable Knowledge	Exploitable product	Sectors of application	Timetable for commercial use	Patent	Inventor/Owner
Confocal microscopy-based platform for intelligent, large-scale data acquisition	Confocal MatrixScreener (intelligent mass data acquisition for image-based high content analysis)	research, medical investigation, industrial application	MatrixScreener 2: April 2007; MatrixScreener 3: December 2009	patent applications filed	Frank Sieckmann (Leica Microsystems CMS), Urban Liebel and Siegfried Winkler (EMBL)
New biomarkers with diagnostic or prognostic potential in clinical tumor pathology	a novel cell cycle biomarker test kit for cancer diagnosis, prognosis and prediction of response to treatment	clinical tumor pathology, medical research	currently unclear	patent application filed	Gareth Williams, Kai Stoeber/Department of Pathology, University College London

2.2 Dissemination of knowledge

MitoCheck has taken the following approaches to disseminate the knowledge and technology generated by the MitoCheck consortium to the scientific community:

First, **publications** in peer-reviewed journals. During the past five project years, the MitoCheck consortium has published more than 50 manuscripts in the scientific journals, some of which are top impact journals such as *Nature* and *Cell*.

Second, the consortium members frequently attended the national and international **conferences** to present the MitoCheck results.

Third, MitoCheck has held one practical **training course** on "High Throughput Microscopy for Systems Biology", three training courses on proteomics and two training courses on BAC tagging technology. In addition, the MitoCheck Pathology Unit organized a **workshop** on diagnostic, prognostic and predictive testing in breast cancer where MitoCheck results were presented to a large audience of clinical cancer pathologists.

Fourth, a human genome-wide **database** (*www.mitocheck.org*) has been generated. This database will contain data generated by the MitoCheck consortium, including information on tagged BACs, immunofluorescence images obtained by GFP localization, silver stained SDS-PAGE gels of all protein samples obtained by tandem affinity purification, and all protein interaction lists obtained by mass spectrometry. In addition, this database will contain all movies from the MitoCheck RNAi screen in which mitosis has been analyzed by live imaging of cells in which all human proteins have been targeted by siRNAs. The database also provides information about gene synonyms used in the literature, orthologs in other species and protein interactions reported in public databases. This collection of localization, interaction and phenotypic data will be a useful resource for understanding the functions of human proteins.

The table below provides an overview of all the dissemination activities of the MitoCheck project.

Table 1: Overview of the dissemination activities carried out by MitoCheck

Type	Type of Audience	Countries addressd	Size of audience	Partner responsible/involved
Press release				
Lauch of the project (8.7.2004)	General public	international	unknown	IMP, EMBL, EIO, CRUK, CNRS
MitoCheck project and its achievements (12.8.2008)	General public	international	unknown	EMBL
Conferences	Researchers	international	unknown	all partners
Publications	scientific community	international	unknown	all partners
Project website	General public	international	unknown	Sanger, EMBL, IMP
Training courses and workshop	Researchers, physicians	international	> 320	EMBL,IMP,UCL,GB,Sanger,CBG
Posters	Researchers	international	unknown	all partners
Project flyer	scientific community	international	unknown	all partners

2.3 Publishable results

Confocal microscopy-based platform for intelligent, large-scale data acquisition

During the course of the MitoCheck project, the Screening Unit (EMBL, Leica Microsystems CMS and DKFZ) have developed advanced, confocal microscopy-based screening platforms allowing high speed screening of different sample types at large scale over an extended period of time. This platform is highly flexible and can be adapted for various applications. Using these platforms, MitoCheck has carried out genome-wide RNA interference (RNAi) screens in human cells searching for genes required for mitosis. This effort has resulted in some 260,000 high quality movies soon available at the MitoCheck website (*www.mitocheck.org*).

New biomarkers with diagnostic or prognostic potential in clinical tumor pathology

Some mitotic kinases are over-expressed in human tumors, and the MitoCheck Pathology Unit at the Department of Pathology, University College London, has therefore developed screens to systematically evaluate the potential diagnostic or prognostic value of mitotic kinases in tumor pathology. The Pathology Unit identified antibodies that are suitable for the development of such clinical tests and has performed clinical trials on ovarian and breast cancer patients with these new assays. These studies have led to the identification of new biomarkers which are more reliable predictors of disease-free survival than currently used clinico-pathological parameters (Kulkarni et al., *Clin Cancer Res*. **15**, 2417; Loddo et al., *Br J Cancer*; **100**: 959). These proof-of-concept studies have generated the rationale for developing a novel cell cycle biomarker test for cancer diagnosis, prognosis and prediction of response to treatment. The Pathology Unit is currently in discussions with commercial parties interested in developing such a theranostics test. The R&D programme involving development of the test and an image analysis platform as well as clinical trials approved by regulatory bodies is foreseen to last at least 4-5 years.