# Comparative Functional Genomics and Disease Association of Chromatin Proteins



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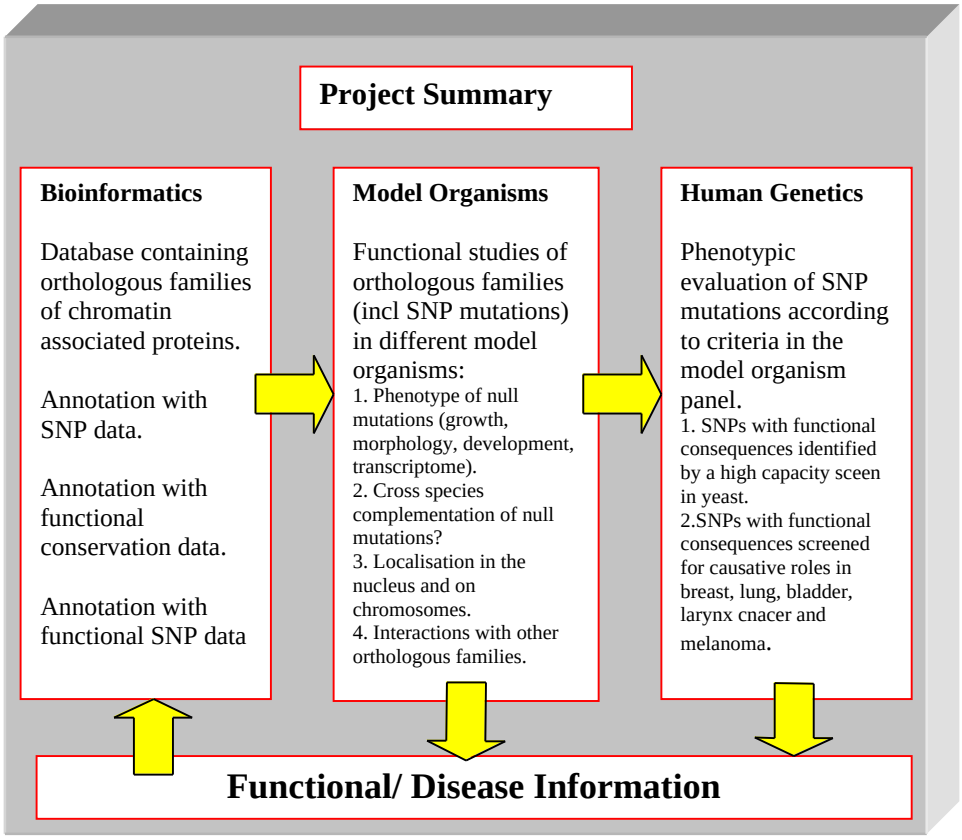
Plant Biology, Swedish University of Agricultural Sciences, Uppsala

**CONTENTS**





## SPECIFIC AIMS

1. To determine the extent of functional conservation within orthologous families of chromatin-associated proteins using different model organisms.
2. To develop a model organism based approach for identifying human single nucleotide polymorphisms (SNPs) that cause defects in the function of conserved chromatin associated proteins.
3. To perform parallel studies on selected SNPs to assess their effects in cancer using a case-control approach.
4. To make the results widely available to scientists and clinicians via a regularly updated database.

## BACKGROUND

Genome sequencing has shown that at least 30% of human proteins have structural orthologues, even in simple eukaryotes such as yeast. Furthermore, complete signalling pathways discovered in fruit flies and nematodes are functionally conserved in humans (eg. wnt, hedgehog, tgf-beta, notch etc). To understand the differences between organisms it is therefore important to understand the balance between functional conservation and divergence within structurally orthologous protein families. This area has not been widely addressed but complete genome sequences for a number of eukaryotes now make such a comparative functional genomics analysis possible using a divergent range of genetically powerful model organisms.

Genetic variability between human individuals is in part due to SNPs. SNPs can be associated with human disease either accidentally, due to genetic linkage to a disease gene, or causally, in which case they directly cause a defect by changing the amino acid sequence of a disease gene. Most applications of SNPs use them as genetic markers in disease gene identification by genetic linkage. It is therefore of obvious interest, both in basic and applied research, to develop approaches for rapid identification and functional characterisation of the second class of disease-causing SNPs.

Chromatin and associated transcription, replication, recombination and repair proteins, is the structure in the cell at which a complex and diverse network of extra- and intra- cellular signals is integrated and converted into a physiological response at the level of regulated transcription of appropriate target genes. The important regulatory role of chromatin associated proteins is reflected in the increasing number of proteins in this class that have been associated to specific disease indications (mainly cancers) in humans. Many chromatin-associated proteins are evolutionarily conserved in humans and model organisms. Furthermore, these proteins are good candidates for functional studies because their precise function can be closely correlated to association with specific subsets of genomic DNA sequences. Taken together these aspects make chromatin associated proteins very attractive targets for a functional genomics project.

## OUTLINE

The project actively combines basic and clinical genomics to develop a new approach for identification and functional characterisation of proteins important for human disease. The key steps in this approach are:

1. Identification of orthologous families of chromatin associated proteins. Database construction and annotation with information about protein function and SNPs.
2. Selection of orthologous families for further study in which at least one known SNP changes the amino acid sequence of the human family member.

3. High capacity screen to identify SNPs with functional consequences (assayed by cross species complementation of phenotypes resulting from deletion of the orthologous gene in yeast).
4. Screening SNPs that alter protein function (from 3) for disease association.
5. Comparative functional genomics studies in model organisms (fruit fly, Drosophila melanogaster, Dm; nematode, Caenorhabditis elegans, Ce; fission yeast, Schizosaccharomyces pombe, Sp; budding yeast Saccharomyces cerevisiae, Sc) of protein families identified in (4) to understand the mechanisms involved in the associated diseases.

The proposal involves parallel activities at the different levels of this process with the aim of achieving "proof-of-principle" status within two years. This will lead to results from all levels of the project including data on associations of selected SNPs to certain types of cancer. In addition it is important to create a database that will allow continuous identification of relevant new SNPs for analysis and which will make the results widely available to scientists and clinicians.

The proposed project is intrinsically multidisciplinary and spans the fields of bioinformatics, model organism genetics and human genetics. At the same time the network of scientists representing all nodes of WCN provides a unique competence and focus in the area of chromatin associated proteins. Almost all the PIs are young scientists (30s and 40s) who share the conviction that the use of multidisciplinary technology directed at a focused biological area is the most effective way to extract useful information from the human genome sequence. The scientists in the network have extensive connections to relevant external networks. In particular, the project will rely heavily on the DNA/RNA and model organism platforms within WCN.

## PROJECT PLAN

The project consists of three main activities, (A) bioinformatics and database construction, (B) functional analysis of orthologous protein families in model organisms and (C) identification of functionally relevant SNPs and disease association studies.

### A. Bioinformatics

Publicly available eukaryotic genome sequences will be used to construct a database containing orthologous chromatin associated protein families. The database will contain aligned protein sequences from humans and model organisms (Dm, Ce, Sp, Sc). Mouse sequences may also be included although experimental work in mice is not included in the proposal. The HGBASE SNP database will then be searched for matches to the human family members. Due to the nature of the SNP database (which is limited to 25 nucleotides flanking the SNP), additional in silico processing of the resulting SNPs will be necessary. Those SNPs that change protein sequences will be selected and subdivided into 3 categories for each model organism sequence, depending on the primary structure and sequence conservation of each orthologous gene family.

(i) The SNP causes a change in a part of the protein sequence that is not conserved over long evolutionary distances.
(ii) The SNP causes a change in a conserved domain of the protein, but the changed residue is not conserved.
(iii) The SNP changes a conserved resude within a conserved domain.

This SNP information will be used to annotate the orthologous family alignments in the database. SNPs classifying in categories (ii) and (iii) are of interest for further study. It will be possible to search the database for SNPs of either category for all or

subsets of the model organisms. Cursory analysis indicates that there are at least 100 such families. Families conserved in all the organisms will be prioritised for functional studies but we may also study some metazoa-specific families. The database will then be annotated with information regarding functional conservation within each orthologous protein family.

Development of this kind of database, combining orthologous sequence alignments, basic biological information and human genetic information, is important because new SNPs are being discovered all the time. It is therefore important that the database can be frequently and automatically updated with new SNP information, which can be categorised as described above and analysed as described below, on a continuous basis.

**B. Comparative Studies in Model Organisms**

Phenotypes Associated with Null Mutations

The phenotype associated with loss of protein function needs to be determined for each model organism. This will be done where necessary by gene deletion (Sc, Sp, Ce, Dm) and/or RNAi (Ce, Dm-cell-lines). In some cases, especially for essential genes, conditional (temperature sensitive) mutations may be used. In Dm the germline clone technique will be used to reveal phenotypes associated with essential proteins required during early embryogenesis. Many resources for these studies are already available, for example almost all Sc genes have been deleted and these strains are available at the WCN model organism platform in Uppsala. This platform will also provide facilities in Stockholm for making deletions in Sp, Dm and Ce. Comparison of mutant orthologues between organisms may not always be easy due to the different appearance and life style of the different organisms. We will score growth, morphology and development and anticipate that common molecular defects (e.g. in the cell cycle) would anyway be detectable in yeast and Ce/Dm, albeit with different phenotypic outputs. Sc and Sp are physiologically more comparable even though they are more evolutionarily diverged from each than either Ce or Dm are from man. We also intend to use transcriptome arrays as a fine measure of mutant phenotypes. This will be tested for wild type and mutant Sc and Sp as well as for Dm cell lines ± RNAi (Affimetrix arrays for Sc and Dm and spotted arrays for Sp available in association with the DNA/RNA platform within WCN).

Cross Species Complementation

Rescue of the null phenotypes, established above, by expressed putative orthologues from other species gives the strongest possible evidence of functional conservation, although failure to cross complement does not prove that two proteins are not orthologues. This is most easily performed in Sc and Sp but it is also possible to introduce such transgenes into Ce and Dm if deletion strains exist. The aim with these studies would be to study a sufficient number of cross species pairs to allow attempts to correlate complementation ability with complementarity of the respective protein sequences. Cross complementation also provides the best method for assessing the functional consequences of human SNPs. An alternative is to introduce the SNP mutations in the context of model organism genes and to test for phenotypic changes. In cases where cross-species complementation does not work it is possible to study other manifestations of protein function such as localisation and interactions with other proteins. It is also possible to use in vitro assays, when available, to perform cross-species complementation studies (e.g. cell-free transcription systems). Such assays can also be used to measure the efficiency of cross-species complementation.

Protein Localisation

Chromatin associated proteins are attractive candidates for this study because they are often located at specific spots in the nucleus. These spots either coincide or not with spots associated with other protein families. Thus, using double labelling immunofluorescence microscopy (IF) approaches, it is possible to classify the locations of different protein families in the cells of different model organisms. To allow the establishment of robust, high capacity technologies we will use epitope tagged proteins (HA, myc, FLAG, GFP, GST) in these studies. In Sc and Sp, C-terminal epitope tags can be efficiently added to proteins which continue to be expressed from their natural promoters using homologous recombination approaches. Alternatively, epitope tagged transgenes can be added (Sc, Sp, Ce, Dm), allowing for temporal or tissue specific conditional expression at different levels (over-expression of proteins sometimes also causes phenotypes that can provide useful indicators of protein function). In this respect we will exploit existing GST fusions which have been made to almost all Sc proteins.

IF establishes the sub-nuclear localisation of proteins with a resolution of ca. 100 nm and without reference to specific DNA sequences. Chromatin immunoprecipitation , on the other hand, can be used to specifically precipitate DNA sequences that are associated with particular proteins. The precipitated DNA can subsequently be used as a quantitative probe to detect the homologous chromosomal regions at high resolution in particular regions using a Southern blot approach. Recent reports show that DNA arrays containing genomic DNA sequences can be used on a whole genome scale to detect protein binding sequences with very high resolution. We intend to make a major effort to develop the technology required to allow such analysis of a large number of “orthologous” promoter sequences in Sc and Sp. We will design these new DNA microarrays but production will be out-sourced to Eurogentec AS, a specialised company with which we already have extensive interactions. The arrays will be analysed in association with the DNA/RNA platform of WCN. Similar information can be obtained for Dm by immuno-staining of polytene chromosomes. When combined, these technologies will allow us to (i) compare the binding patterns and target sequences of chromatin associated proteins in Sc, Sp and Dm and (ii) model how chromosomes with their associated proteins are packaged into nuclei in which the associated proteins are clustered in the form of spots.

Protein Interactions

Many chromatin-associated proteins carry out their functions in physical association with other proteins. Another way to validate true orthologues is therefore to determine whether they associate with the same orthologous partner proteins. This will be done by co-immunoprecipitation using the epitope-tagged proteins discussed in the previous section. We may also use yeast two hybrid approaches as a complement or alternative (much data already available at http://portal.curagen.com/).

**C. Human Genetics**

Correlation of genetic diversity in the human population with disease phenotypes is the main approach available for linking defects in individual proteins to disease indications. By focusing on the subclass of SNPs that alter the amino acid sequence and function of encoded proteins, we will develop an approach for identification of those SNPs that actually cause a specific disease rather than just being linked to the disease gene. A high capacity screen in Sc (using gene deletion

strains at the WCN model organism platform in Uppsala) will be used to identify human cDNAs that can complement defects associated with loss of the cognate yeast orthologues for the ca. 100 orthologous families of interest. The complementing activity of positive cDNAs can then be compared with the complementing activity of cDNAs containing SNP mutations. Functionally relevant SNPs are likely to affect the complementation ability. Based on current knowledge, some SNPs that are likely to cause functional defects can already be predicted by bioinformatic means.

We will further evaluate the potential role of the selected SNPs by looking for them in human cancers (e.g. breast, lung, bladder and larynx cancer and melanoma, each >200 cases) which differ in environmental and genetic risk factors. The cases have been identified from the Swedish Family-Cancer Database and other sources, and samples from matched controls are available. The selected SNPs are likely to affect chromatin associated proteins involved in cell cycle and transcriptional control, DNA repair, and in breast cancer those interacting with the BRCA1 and BRCA2 proteins. The aim is to assess the risks associated with the putative minor alleles in a case-control fashion. SNP assays will be carried out initially using RT-PCR assays, and later with a larger number of samples, using a high-throughput technique.

**PROJECT FEASIBILITY**

Most of the knowledge base and methodology required is already established within the network or is available to us via interactions with WCN platforms or external collaborators. The only major exception is development of array-based methods to determine the localisation of chromatin associated proteins in relation to DNA sequence on a genome-wide scale. This represents a major technology development aspect of the proposal but a couple of recent literature reports indicate that it is achievable. The main challenge will be to study a sufficiently large number of families at a meaningful level of functional detail. The mix of model organisms will help to achieve this, such that rapid high capacity yeast approaches will help to provide better focus for the other model organisms and human SNP studies. Considerable functional information is available, at least for some proteins and in some organisms. Our aim is not to recapitulate this work but to ensure that comparable information is available for each of the organisms studied. Two of the PIs (HR & AW) have extensive experience of genome-scale functional genomics from the European Sc project (1996-99). Very importantly we will hold regular meetings of the network in order to ensure good spreading of information and to allow early identification and solution of problems and bottlenecks.

**TIMETABLE**

Year 1: (i) Initial database containing all known orthologous chromatin families annotated with information about functional conservation and SNPs. (ii) Rapid cross complementation screen to identify “functional” SNPs complete. (iii) Functional studies of targeted protein families initiated in all model organisms. (iv) SNP-cancer association studies initiated. (v) Arrays for genome-wide DNA localisation studies produced and tested.

Year 2: (i) Final, automatically updateable, database available. (ii) Disease association tests of identified “functional” SNPs complete. (iii) Functional studies of targeted protein families complete in all model organisms (including array-based genome-wide analysis of their locations on the DNA).

**BUDGET (KSEK)**

**Equipment Budget** (Year 1 / Year 2)

Equipment >150 KSEK

| | | |
|---|---|---|
| Inverted fluorescence microscope for GFP (Dm) | Y2 | 200 |
| Stereo microscope with fluorescence for GFP (Dm) | Y2 | 200 |
| Microinjection system for Dm | Y2 | 300 |

Equipment <150 KSEK

| | | |
|---|---|---|
| Sterile hood and incubator for Dm cell work | Y1 | 150 |
| Computers and software for bioinformatics | Y1 | 100 |
| PCR apparatus | Y1 | 100 |
| -80 freezer for storage of yeast strains | Y2 | 100 |
| Shaking incubator for yeast | Y1 | 120 |
| Incubator for yeast with cooling | Y1 | 30 |

**Personnel Support, Other Expenses and Consumables**

| **ACTIVITY** | **YEAR 1** | **YEAR 2** |
|---|---|---|
| Bioinformatics | | |
| 2 x postdoc/ research enginee | 700 | 700 |
| Consumables | 100 | 100 |
| Model organisms (Dm, Ce, Sp, Sc) | | |
| 4 x 2 postdoc/ research engineer | 2800 | 2800 |
| Consumables | 800 | 800 |
| GST-fusion Sc strains | 75 | |
| Chips and processing for genome-wide expression analysis | 500 | 1000 |
| Genome-wide localisation on DNA | | |
| 2 x postdoc/ research engineer | 700 | 700 |
| Consumables | 200 | 200 |
| Array production cost | 2000 | 1000 |
| Human Genetics | | |
| 2 x postdoc/ research engineer | 700 | 700 |
| Consumables | 300 | 300 |
| Network meetings and reserve | 500 | 500 |
| VAT (8%), overhead (18%), rent (10%) | 3165 | 2970 |

**Total Costs**

| | | |
|---|---|---|
| Equipment | 500 | 800 |
| Personnel, consumables etc | 12540 | 11770 |
| **Total** | **13040** | **12570** |

**Justification of Budget Costs**

The main part of the budget is dedicated to personnel and consumables costs required to generate results within the project. This is partly because the project rests heavily on infrastructure that is already present in the participant laboratories and partly because other specialist needs will be obtained by interaction with the WCN technology platforms. The only major exception to this is the equipment we request for work with Drosophila melanogaster. Matthias Mannervik, who is responsible for this activity, is a young investigator of high potential who is still in the process of building up his laboratory.

In terms of personnel we have opted for postdoctoral positions and senior technical positions to ensure maximum flexibility with regard to allocating different work tasks at different times (difficult with grad students) and because of the high level of competence and experience required to make this multidisciplinary network function efficiency. For example it would be highly advantageous to employ postdocs with experience in 2 or more model organisms. The consumables cost is based on an annual cost of 100 KSEK per person required for molecular biology work. The consumables budgeted is lower for bioinformatics and higher for human genotyping personnel. The budget for genome-wide expression analysis is based on the cost for Affymetrix chips and their processing by the WCN platform, 30 experiments year 1 and 60 year 2 (2 chips per experiment).

The budget for production of a new array to determine the location of chromatin associated proteins is based on current costs of array production that we are paying to Eurogentec SA. Two arrays would be produced with probes for 1000 orthologous genes from Sc and Sp. Four probes per gene will be present on the array allowing identification of proteins associated with different parts of each gene.

The resources in the budget will create a dynamic network in which researchers form different universities and disciplines will participate in the different activities as shown below.

| **ACTIVITY** | **PI** | **AFFILIATION** |
|---|---|---|
| Bioinformatics | TB | KI |
| | CG | KI |
| Model organisms | | |
| D. melanogaster | MM | SU |
| C. elegans | TB | KI |
| S. pombe | KE | SH |
| | CG | KI |
| | AW | SH |
| S. cerevisiae | SB | UmU |
| | CG | KI |
| | HR | SLU-Uppsala |
| | AW | SH |
| Genome-wide localisation on DNA | SB | UmU |
| | KE | SH |
| | MM | SU |
| | AW | SH |
| Human genetics | KH | KI |
| | HR | SLU-Uppsala |
| | AW | SH |

In our view this is the budget required to pursue this project in the most efficient way. At a cost of reduced efficiency and volume it would be possible to reduce the budget across the board. A severe pain threshold would be reached at around 60-70% of the present budget and the project could no longer be pursued as an effective network below that threshold. The budget could also be reduced by decreasing the scope of the project. The genome-wide localisation on DNA activity could be removed at the cost of reducing the value of the basic science in the project and one of its most novel and exciting aspects. Alternatively, the project's ambition to link basic genomic research to clinical research could be sacrificed by removing the human genetics activity at the price of loosing the most exciting multidisciplinary aspect of the project.

**PARTNERS AND OTHER COLLABORATORS**

As shown earlier in the application we do not have direct support for this project from any other source. However, the project is well integrated with current activities within or laboratories and rests heavily on intellectual and physical infrastructure resources from our on-going activities. Furthermore, the project utilises the WCN model organisms technology platforms for genetic manipulation of Dm, Ce, Sp and Sc as well as the WCN DNA/RNA platform for conducting microarray experiments. In addition to these contacts our laboratories are part of several important national and international networks with contacts as listed below.

| | |
|---|---|
| EU project (AW) | Improvement and applications of Sc microarrays |
| Eurofan II (HR) | Functional analysis of genes in Sc |
| Eurogentec SA (AW, KE, CG) | Production of first transcriptome array for Sp |
| Center for Genome Research, KI (TB) | Bioinformatics/ SNP Database |
| Tore Samuelsson, GU (CG) | Bioinformatics/ Database/ orthologous protein families |
| Günter Reuter, Halle, FRG (KE) | Cross-species chromatin studies (Sp, Dm) |
| Sp functional genomics initiative (KE) | Access to internationally available resources |
| Örjan Wrange, KI (AW) | In vitro chromatin analysis in Xenopus oocytes |
| R Kornberg, L Myres, F Asturias, Y-J Kim (S.B, CG) | Analysis of orthologous mediator subunits in vitro |

**PROJECT DESCRIPTION FOR INTERNET PRESENTATION** (max 1000 characters)

Genetic differences between individuals are an important factor in human disease susceptibility. Many such differences, so-called Single Nucleotide Polymorphisms (SNPs), are being discovered as a result of the human genome project. A major objective of this project is to identify SNPs which cause disease susceptibility by changing the amino acid sequence and thereby the function of important proteins. The project focuses on the class of proteins that are associated with chromatin in the nucleus of cells because of their pivotal role in connecting cellular signalling systems to the physiological response of cells. Comparative functional genomics in a range of model organisms (fruit flies, nematodes and yeast) is used to characterise the evolutionary conservation/ divergence of protein functions and to assess the functional significance of amino acid changes associated with human SNP mutations.

**Curriculum Vitae – Stefan Björklund (640515-7815)**

## APPOINTMENTS

2000: Lecturer (högskolelektor) in Medical Biochemistry at Umeå University.

1997: Lecturer (50%) with responsibility for the Graduate school in Biomedicine at Umeå University.

1997: Assistant professor (Forskarassistent) in Medical Biochemistry at Umeå University

1996: Temporary lecturer with responsibility for the Graduate school in Biomedicine at Umeå University.

1995: Temporary lecturer in Medical Biochemistry at Umeå University

## EDUCATION & POSTDOCTORAL STUDIES

Umeå University. Medicine: Med. Kand. 1988

Umeå University. Regulation of ribonucleotide reductase expression during the mammalian cell cycle. Doct. Medicine. 1993

Stanford University School of Medicine. EMBO long-term postdoc fellowship working on transcriptional regulation in yeast. 1993-1994.

## RESEARCH INTERESTS

My research focuses at transcriptional regulation in eukaryotes. In one project we use yeast *S.Cerevisiae* as a model organism to reveal the mechanisms for transcriptional regulation at the molecular level. During my postdoc period i participated in the identification of the mediator complex. It is a 20 subunit complex that interacts with the RNA polymerase and is required for regulated transcription of most eukaryotic genes. We have recently focused on one of the mediator subunits called Med1 and are using it as a starting point to understand both how transcriptional regulatory proteins transfer signals to the basal transcription machinery, but also to study protein-protein interactions within the mediator. In a separate project we use mouse as a model to study S phase-specific transcriptional activation. We have now cloned cDNAs encoding mouse TBP, TFIIB, both subunits of TFIIE and both subunits of TFIIF and expressed and purified the corresponding recombinant proteins to homogeneity. We have also purified mouse RNA polymerase II and TFIIH from tissue-cultured mouse cells and have shown that when combined, these factors are both sufficient and required for transcription of several promoters *in vitro*. We have so far used the mouse *in vitro* transcription system to study expression of the S phase-specific mouse ribonucleotide reductase promoter and have shown that the results obtained using the *in vitro* transcription system correlate well to results obtained *in vivo* using R2 promoter-reporter genes.

**PUBLICATIONS (5 SELECTED)**

Kim, Y-J., **Björklund, S.,** Li, Y., Sayre, M., and Kornberg, R.D (1994) A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell* 77:599-608.

Svejstrup, J.Q., Li, Y., Fellows, J., Gnatt, A., **Björklund, S.,** and Kornberg, R.D. (1997) Evidence for a mediator cycle at the initiation of transcription. *Proc. Natl. Acad. Sci. U.S.A.* 94:6075-6078.

Balciunas, D., Gälman, C., Ronne, H., and **Björklund, S.** (1999) The Med1 subunit of the yeast mediator complex is involved in both transcriptional activation and repression. *Proc. Natl. Acad. Sci. U.S.A.* 94:376-381.

**Björklund, S.,** Almouzni, G., Davidson, I., Nightingale, K.P., and Weiss, K. (1999) Global transcriptional regulators of eukaryots. *Cell* 96:759-767.

Kotova, I., Hofslagare, A L., Segerman, B., Flodell, S., Thelander, L., and **Björklund, S.** (2000) A mouse *in vitro* transcription system reconstituted from highly purified RNA polymerase II, TFIIH and recombinant TBP, TFIIB, TFIIE and TFIIF. Submitted

**NATIONAL AND INTERNATIONAL NETWORK (active contacts during 2000)**

Roger Kornberg, Stanford University School of Medicine, USA
Hans Ronne, SLU, Uppsala
Claes Gustafsson, Karolinska Institute
Francisco Asturias, The Scripps Research Institute, USA
Young-Joon Kim, Genome Regulation Center, Seoul, Korea
Lars Thelander, Umeå University
Uwe Sauer, Umeå University

**COMPETETIVE GRANT INCOME 2001 (main applicant)**

| | | |
|---|---|---|
| | Swedish Cancer Society | 400.000 SEK |
| | Swedish Natural Science Council | 272.000 SEK |
| | Human Frontiers Science Programme | 41.666 USD |
| * | Swedish Foundation for Strategic Research | 150.000 SEK |

**Curriculum Vitae – Thomas R. Bürglin**

## APPOINTMENTS

2001: Lecturer, Dept. of Biosciences, Karolinska Institutet.

1994: Start Fellow (Assistant Professor equivalent, non-tenure track), funded by the Swiss National Science Foundation.

## EDUCATION & POSTDOCTORAL STUDIES

University of Basel, Switzerland: Diploma in Biology II, speciality microbiology: 1982

University of Basel, Switzerland: Ph.D. in Cell Biology: Nuclear transport in *Xenopus laevis*: 1987

University of California, Los Angeles, USA: Postdoctoral fellow, continuing work on nuclear transport in *Xenopus laevis* with Ph.D. adviser: 1987

Mass. General Hospital & Harvard University, Boston, USA: Postdoctoral fellow working on heterochronic and homeobox genes in *C. elegans*: 1988 - 1994

## RESEARCH INTERESTS

My group's research has been focusing on the role of the transcription factor family of homeobox genes in the development of the model organism *Caenorhabditis elegans*. We have now studied several genes in detail using knockout techniques. In particular, we have found two genes, *ceh-2* and *ceh-14*, which are required for the terminal differentiation of a small subset of neurons in *C. elegans*. Further research will try to elucidate regulators and targets of these homeobox genes. More recently, we have identified a large gene family in *C. elegans* related to the Hedgehog signalling molecule. Functional studies have shown that some of these molecules play an important role in development. All the biological work has always been complemented by bioinformatics: I have developed specialised software to maintain a database of homeobox genes as well as other software for biological research. Further, I have been actively engaged in "genome mining" to exploit the full genome sequence of *C. elegans*.

## PUBLICATIONS (5 SELECTED)

- **Bürglin, T.R.** (1998). PPCMatrix, a PowerPC dotmatrix program to compare large genomic sequences against protein sequences. Bioinformatics, 14, 751-752.

- Aspöck, G., Kagoshima, H., Niklaus, G., and **Bürglin, T.R.** (1999). *Caenorhabditis elegans* has scores of *hedgehog*-related genes: sequence and expression analysis. Genome Res., 9, 909-923.

- Cassata, G., Kagoshima, H., Andachi, Y., Kohara, Y., Dürrenberger, M.B., Hall, D.H. and **Bürglin, T.R.** (2000). The LIM homeobox gene *ceh-14* confers thermosensory function to the AFD neurons in *Caenorhabditis elegans*. Neuron, 25,587–597.

- Cassata, G., Röhrig, S., Kuhn, F., Hauri, H.-P., Baumeister, R., **Bürglin, T.R.** (2000). The *Caenorhabditis elegans* Ldb/NLI/Clim orthologue *ldb-1* is required for neuronal function. Dev. Biology, 226, 45-56.

- **Bürglin, T.R.**, and Ruvkun, G. (2000). Regulation of ectodermal and excretory function by the *C. elegans* POU homeobox gene *ceh-6*. Development, in press.

## NATIONAL AND INTERNATIONAL NETWORK (active contacts during 2000)

Ralf Baumeister, Genzentrum, Universität München, Germany
Ralf Sommer, Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany
Gary Ruvkun, Harvard University, Boston, USA
David H. Hall, Albert Einstein College of Medicine, New York, USA
Yuji Kohara, National Institute of Genetics, Mishima, Japan
Hans-Peter Hauri, Biozentrum, University of Basel, Switzerland
*C. elegans* research community in general

## COMPETETIVE GRANT INCOME 2001

Swedish Foundation for Strategic Research 500'000 SEK

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**Appointments**

2000 Appointed as Associate Professor (Högskolelektor) at University College Sodertorn, Huddinge

1999 Appointed as 'Docent' (Associate Professor) in Molecular Genetics at Karolinska Institutet, April 21, 1999

1997 Appointed as Assistant Professor at Karolinska Institutet, Stockholm (Medical Research Council, MFR)

**Professional preparation**

1988 Bachelor of Microbiology Degree (Examen biologlinjen, mikrobiologisk variant, 120 p) at Uppsala University, Sweden

1988-89 Exchange student at Centre de Genetique Moleculaire, CNRS Univ. de Pierre et Marie Curie, France
supervisors: Prof. Piotr. P Slonimski, Dr Olga Groudinsky

1989-94 Ph D studies at Dept of Molecular Biology , Uppsala University
Obtained a Ph D degree in Molecular Biology in February 1994
supervisors: Docent Tarmo Ruusala, Prof. Charles G Kurland

1994-98 Postdoctoral research at MRC Human Genetics Unit, Edinburgh
in the laboratory of Dr Robin Allshire, Head of Unit: Prof. Nick Hastie
EMBO fellow, March-Sept 1994, HFSP fellow LT0364/1994-M, from Sept. 1994- 1996, EU TMR fellow, from October 1996- March 1997

**Scientific Awards**

Prince Bertil Scholarship, Commonwealth Office, Tetra Pak (1994)

Junior Individual Grant Award, Swedish Strategic Research Foundation (1999) ranked among top ten of young Swedish researchers, including all disciplins

**Current research interests:**

1/ Centromeres structure and function, position effects, silencing
2/ Histone deacetylase (HDAC) enzymes and co-repressor proteins
3/ Chromatin remodelling enzymes, gene repression

**Relevant Publications (5 selected)**

Ekwall, K., J. P. Javerzat, A. Lorentz, H. Schmidt, G. Cranston and R. Allshire (1995). "The chromodomain protein Swi6: a key component at fission yeast centromeres." Science **269**(5229): 1429-31**.**

Ekwall, K., T. Olsson, B. M. Turner, G. Cranston and R. C. Allshire (1997). "Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres." Cell **91**(7): 1021-32**.**

Olsson, T. G. S., K. Ekwall, R. C. Allshire, P. Sunnerhagen, J. F. Partridge and W. A. Richardson (1998). "Genetic characterisation of hda1+, a putative fission yeast histone deacetylase gene." Nucleic Acids Res **26**(13): 3247-54**.**

Ekwall, K., G. Cranston and R. C. Allshire (1999) "Novel fission yeast mutants which alleviate transcriptional silencing in centromeric flanking repeats and disrupt chromosome segregation." Genetics **153:**1153-69.

Yoo J. Y., Y. Jin, Y. Jang, P. Bjerling, M. Tabish, S. Hong. K. Ekwall and S. D. Park (2000) "Fission yeast Hrp1, a chromodomain ATPase, is required for proper chromosome segregation and its overexpression interferes with chromatin condensation" Nucleic Acids Res 28: 2004-11.

**National and international Network (contacts during 2000)**

- Anthony Wright (SH Huddinge)
- Eileen O'Toole, Dick McIntosh (Boulder)
- Robin Allshire (Edinburgh)
- S-D Park (Seoul)
- Anna Okorokova-Facanha Lev Okorokov (Brasil)
- Shiv Grewal (CSH)
- Genevieve Thon ( Copenhagen)
- Henry Levin (NIH)
- Kjell Hultenby, Silwa Mengarelli (KFC/EM unit Huddinge)

**Competetive grant income 2001 (main applicant)**

- Swedish Medical Reseach Council (200.000 SEK)
- Swedish Cancer Society (300.000 SEK)
- Swedish Strategic Research Foundation (822.000 SEK)

**Curriculum Vitae – Claes Gustafsson (660102-5577)**

## APPOINTMENTS

2000 – 2003
Assistant Professor (Cancerforskartjänst) with the Swedish Cancer Foundation. The position is located at Department of Medical Nutrition. Karolinska Institute.

1998 – 1999
Independent postdoctoral scientist with the Swedish Society for Medical Research. Located at the Dept. of Medical Biochemistry, Göteborg University

1995 – 1997
Postdoctoral Scientist with the Swedish Cancer Foundation. Located at Department of Structural Biology, Stanford University, CA

1992 – 1995
Ph.D. student at the Dept. of Medical Biochemistry, Göteborg University

## EDUCATION & POSTDOCTORAL STUDIES

| | |
|---|---|
| *Undergraduate university:* | Göteborg University |
| *Area:* | Medicine |
| *Degree and year:* | M.D. (examinerad läkare), 1992 |
| *Graduate university:* | Göteborg University, Department of Medical Biochemistry |
| *Area:* | Molecular mechanisms of DNA replication in Herpes Simplex virus type 1. |
| *Supervisor:* | Professor Per Elias |
| *Degree and year:* | Ph.D. (Med. Dr.), 1995 |
| *Postdoc university:* | Stanford University, CA Department of Structural Biology. |
| *Supervisor*: | Professor Roger D. Kornberg. |
| *Area:* | Mechanisms of transcriptional regulation in eukaryotic cells. |
| *Inclusive dates:* | August 1, 1995 – December 1, 1997. |

## RESEARCH INTERESTS

My group studies the molecular mechanisms of transcriptional activation in eukaryotic cells. Our focus of interest is the Mediator, a 20-subunit complex, which transduces regulatory information from enhancers and other control elements to promoters, by connecting regulatory proteins with the RNA polymerase II (pol II) transcription machinery in all eukaryotes from yeast to man. The human Mediator is known under many different names, e.g. DRIP, TRAP, SMCC, ARC, and CRISP

complexes. Despite the confusing nomenclature, all these complexes are nearly identical in their subunit composition and seem to correspond to one single protein complex. The broad objectives of our work are to determine the molecular mechanism of transcriptional regulation by Mediator, and to assess the functional significance of the Mediator mechanism in living cells. These objectives are accomplished by a multidisciplinary approach. The strength of the laboratory is within the field of protein biochemistry/proteomics, where we have an extensive experience of identification, purification, and characterisation of large multi-protein complexes. We have also successfully employed DNA microarray technology to study the role of individual Mediator subunits in global gene regulation. Our experimental organisms have mainly been *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, but lately we have also begun to study transcriptional regulation in human cells.

## PUBLICATIONS (5 SELECTED)

1. Gustafsson CM, Myers LC, Beve J, Spåhr H, Lui M, Erdjument-Bromage H, Tempst P, Kornberg RD. Identifcation of new subunits of the mediator complex from Saccharomyces Cerevisiae.
**J Biol Chem** 1998 Nov 20;273(45):30851-
2. Asturias FJ, Jiang YW, Myers LC, Gustafsson CM, Kornberg RD.
Conserved Structures of Mediator and RNA Polymerase II Holoenzyme
**Science** 1999, Feb 12; 283:985-
3. Myers LC*****, Gustafsson CM*****, Hayashibara KC, Brown PO, Kornberg RD.
Mediator protein mutations that selectively abolish activated transcription.
**Proc Natl Acad Sci U S A** 1999 Jan 5;96(1):67-72
*****Equal contribution
4. Spåhr H, Bève J, Larsson T, Bergström J, Karlsson KA, Gustafsson CM.
Purification and partial characterization of the RNA polymerase II holoenzyme from Schizosaccharomyces pombe.
**J Biol Chem**, 2000 Jan 14;275(2):1351-
5. Lorch Y, Beve J, Gustafsson CM, Myers LC, Kornberg RD.
Mediator – Nucleosome interaction.
**Molecular Cell** 2000 Jul;6(1):197-

## NATIONAL AND INTERNATIONAL NETWORK (active contacts during 2000)

Roger D. Kornberg, Stanford University, USA
Francisco J. Asturias, Scripps Institute, USA
Lawrence C. Myers, Dartmouth College, USA
Steen Holmberg, Copenhagen University, Denmark
Jacques Remacle, Belgium
Jesper Q. Svejstrup, ICRF, UK
Jan-Åke Gustafsson, Karolinska Institute
Karl Ekwall, Södertörns University College
Stefan Björklund, Umeå universitet
Anthony Wright, Södertörns University College

## COMPETETIVE GRANT INCOME 2001 (main applicant)

| | |
|---|---|
| SSF | 150.000 SEK |
| Swedish Society for Medical Research | 150.000 SEK |
| Swedish Cancer Society | 450.000 SEK |
| HFSP Network | 425.000 SEK |
| A number of smaller grants | 280.000 SEK |

## Curriculum vitae - Kari Hemminki

Born 1947 in Finland, male.

**Education:**
MD University of Helsinki, 1973.
Ph.D in medical chemistry University of Helsinki, 1973.
Docent (~ associate professor) in medical chemistry, University of Helsinki, 1975.

**Empoyment:**
Research assisstant and fellow, Department of Medical Chemistry, University of Helsinki, 1973-8.
Scientist 1978-87, research professor 1987-92 and temporary chief physician 1993-95, Institute of Occupational Health, Helsinki, Finland.
Current: Professor in epidemiology with a special reference to chemical health risks (molecular epidemiology) at Department of Biosciences, Karolinska Institute, Huddinge, Sweden 1989-present.

**Visiting scientist:**
Postdoctoral fellow, Department of Biology, Johns Hopkins University, USA, 1976-8.
Frederick Cancer Research Facility (NCI), Frederick, USA, 1984-5.
Massachusetts Institute of Technology, USA 1987.
Institute of Public Health, University of Cambridge, UK 1999.

**Advisory and consultative activities:**
WHO/IPCS Geneva/Copenhagen working groups, 9 occasions, 1980-2000.
IARC working groups, 13 occasions, 1984-2000.
Current: On expert pannels on toxicology and reproductive hazards, and on carcinogens, Chemical Inspectorate, Sweden 1999-.
Scientific Council, IARC 1999-.
Europe aginst Cancer, 1997-.
Editorial boards of 5 scientific journals.
Co-editor of 5 international books.

**Scientific societies:**
European Association for Cancer Research, American Association for Cancer Research, Genetic Epidemiology Society.

**Publications:**
Over 500 publication, some 400 original work on DNA damage, DNA repair, mutations, biomarkers, genotyping, and genetic and environmental epidemiology.

Selected publications:
Hemminki K, Vaittinen P. Familial cancers in a nation-wide Family-Cancer Database:age distribution and prevalence. Eur J Cancer 1999; 35:1109-17.

Sauroja I, Smeds J, Vlaykova T, Kumar R, Talve L, Hahka-Kemppinen M,

Punnonen K, Jansen CT, Hemminki K, Pyrhönen S. Analysis of G1/S check point regulators in metastatic melanoma. Genes Chromosomes Cancer 2000; 28:404-14.

Lichtenstein P, Holm N, Verkasalo PK, Illiado A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K. Environmental and heritable factors in the causation of cancer. Analysis of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000;343: 78-85.

Ma X, Jin Q, Försti A, Hemminki K, Kumar, R. Single nucleotide polymorphism analysis of the human proliferating cell nuclear antigen (PCNA) and flap endonuclease 1 (FEN1) genes. Int J Cancer 2000; 88:938-42.

Försti A, Luo L, Vorechovsky I, Söderberg M, Lichtenstein P, Hemminki K. Allelic imbalance on chromosomes 13 and 17 and mutation analysis of BRCA1 and BRCA2 genes in monozytic twins concordant for breast cancer. Carcinogenesis 2001; in press.

**Curriculum vitae - Mattias Mannervik (670816-0111)**

## APPOINTMENTS

2000: Assistant Professor (Forskarassistent) funded by the Swedish Natural Science Research Council (NFR). Located at Stockholm University.

## EDUCATION & POSTDOCTORAL STUDIES

Stockhom University: Bachelor of Science in Chemistry and Microbiology: 1991

Uppsala University: Transcriptional Regulation by Adenovirus Early Region 1 and 4 Proteins: Doctor of Medical Science, 1997

University of California, Berkeley, U.S.A. Postdoc working on transcriptional regulation during *Drosophila* development: 1998–1999

## RESEARCH INTERESTS

My research aims at understanding how regulation of gene expression controls cell fate, differentiation and development. The *Drosophila* embryo constitutes a particularly well-characterized system in terms of both the cis-regulatory sequences driving gene expression and the trans-acting factors involved in controlling transcription. This knowledge can be exploited to study the mechanisms for regulating transcription that are used *in vivo*, with important implications for both normal development and disease.

The focus of my groups's research is on transcriptional coregulators, proteins that themselves do not bind to DNA, but that facilitate communication between sequence-specific transcription factors and the basal RNA polymerase machinery. One important function of coregulators is to modify the structure of chromatin by, for example, acetylation (coactivators) or deacetylation (corepressors) of histones. We have found that histone acetyltransferases and deacetylases are critical for normal development of the *Drosophila* embryo. The functions of coregulators are determined in mutant embryos by whole-mount *in situ* hybridization, where altered gene expression patterns can be correlated to defects in specific transcription factors. Functional properties of coregulators are also investigated by expressing them as transgenes in the embryo.

## PUBLICATIONS (5 SELECTED)

**M. Mannervik** and G. Akusjärvi (1997). The transcriptional co-activator proteins p300 and CBP stimulate adenovirus E1A conserved region 1 transactivation independent of a direct interaction. *FEBS Lett*. 414, 111-116.

**M. Mannervik**, Y. Nibu, H. Zhang and M. Levine (1999). Transcriptional coregulators in development. *Science* 284, 606-609.

**M. Mannervik** and M. Levine (1999). The Rpd3 histone deacetylase is required for segmentation of the *Drosophila* embryo. *Proc. Natl. Acad. Sci. U.S.A.* 96, 6797-6801.

H. Ashe, **M. Mannervik** and M. Levine (2000). Dpp signaling thresholds in the dorsal ectoderm of the *Drosophila* embryo. *Development* 127, 3305-3312.

**M. Mannervik** (2000). Corepressor proteins in *Drosophila* development. *Current Topics in Microbiology and Immunology* 254, 79-100.

## NATIONAL AND INTERNATIONAL NETWORK (active contacts during 2000)

Daniel A. Chamovitz, Tel Aviv University, Israel
Ylva Engström, Stockholm University
Uli Theopold, Stockholm University

## COMPETETIVE GRANT INCOME 2001 (main applicant)

| | |
|---|---|
| Swedish Natural Science Research Council | 200.000 SEK |
| Swedish Cancer Society | 200.000 SEK |
| Jeanssons Foundation | 150.000 SEK |

**Curriculum Vitae - Hans Ronne**

**DATE OF BIRTH** 10 July 1955

**ACADEMIC CAREER**

| | |
|---|---|
| 1978-1982 | Graduate student at the Department of Cell Research, Uppsala University. Research on growth factor binding proteins. |
| 1982-1984 | Postdoctoral fellow at the Department of Cell Research, Uppsala University. Research on vitamin A-binding proteins and on transplantation antigens. |
| 1985-1986 | EMBO long-term fellowship at the Department of Genetics and Development, Columbia University, New York. Research on the mechanisms of genetic recombination in yeast. |
| 1987-1993 | Assistant Member at the Ludwig Institute for Cancer Research, Uppsala, Sweden. Main areas of research interest: negative control of transcription, protein phosphatases, intracellular transport. |
| 1993-1995 | Associate Member at the Ludwig Institute for Cancer Research, Uppsala, Sweden. |
| 1995-1998 | Senior Research Position (särskild forskartjänst) with the Swedish Natural Research Council based at the Department of Medical Immunology and Microbiology, Uppsala University. |
| Since 1998 | Professor of Molecular Genetics at the Department of Plant Biology, Swedish University of Agricultural Sciences. |

**RESEARCH INTERESTS**

Our research is focused on two model organisms: the yeast *Saccharomyces cerevisiae* and the moss *Physcomitrella patens*. Our work with yeast currently involves two projects. One project is funded by NFR and deals with negative control of gene expression. We have previously discovered and characterized the zinc finger protein Mig1, which is the main effector in glucose repression, a global regulatory response in yeast. Our current work within this project involves studies of the Mediator complex which is involved in transmitting regulatory signals from activators and repressors to RNA polymerase II. We are also studying the role of the highly conserved jumonji domain which is found in many eukaryotic transcription factors. The other yeast project, which is funded by the Swedish Cancer Society and the EU, deals with intracellular vesicle transport. In particular, we are studying the function of the yeast syntaxins Sso1 and Sso2. The third project, which is funded by SJFR, involves setting up *Physcomitrella* as a model system in plant research, and using it to study metabolic regulation. The reason for our interest in *Physcomitrella* is that it recently was discovered that the yeast knockout technique works in this organism,

making it the first plant that is amenable to this kind of sophisticated functional genomics studies.

## 5 KEY PUBLICATIONS

Aalto, M. K., Keränen, S. and Ronne, H. (1992). A family of proteins involved in intracellular transport. **Cell** 68, 181-182.

Östling, J., Carlberg, M. and Ronne, H. (1996). Functional domains in the Mig1 repressor. **Mol. Cell. Biol.** 16, 753-761.

Aalto, M. K., Jäntti, J., Östling, J., Keränen, S. and Ronne, H. (1997). Mso1p: a yeast protein that functions in secretion and interacts physically and genetically with Sec1p. **Proc. Natl. Acad. Sci. USA** 94, 7331-7336.

Balciunas, D., Gälman, C., Ronne, H. and Björklund, S. (1999). The Med1 subunit of the yeast mediator complex is involved in both transcriptional activation and repression. **Proc. Natl. Acad. Sci. USA** 96, 376-381.

Balciunas, D. and Ronne, H. (2000). Evidence of domain swapping within the jumonji family of transcription factors. **Trends in Biochem. Sci.** 25, 274-276.

## ACTIVE COLLABORATIONS DURING 2000

* Eurofan II functional genomics program (the Secretion Node).
* Sirkka Keränen and Jussi Jäntti, VTT, Helsinki, Finland.
* Stefan Björklund, Umeå University.
* Stefan Hohmann, Göteborg University.
* Lars Rask, Uppsala University.
* Leif Andersson, SLU, Uppsala.
* Sara von Arnold, SLU, Uppsala.
* Sten Stymne, SLU, Alnarp.

## COMPETETIVE GRANT INCOME 2001 (main applicant)

| | |
|---|---|
| * Swedish Natural Sciences Research Council (NFR) | 604.000 SEK |
| * Swedish Research Council for Forestry and Agriculture (SJFR) | 645.000 SEK |
| * Swedish Cancer Society | 464.000 SEK |
| * European Union | 52.000 SEK |

**Curriculum Vitae - Anthony Wright (590106-1779)**

**APPOINTMENTS**

2000: Senior Researcher (särskild forskartjänst) in the area of functional genome analysis at the Swedish Medical Research Council (MFR). Located at Södertörns University College.

1999: Professor of molecular biology at Södertörns University College.

1998: Professor competence (Biträdande professur) in molecular biology at Södertörns University College.

1998: Lecturer (högskolelektor) in molecular biology at Södertörns University College.

1992: Associate Professorship (docentur) in molecular biology at the Karolinska Institutet.

1992: Assistant Professor (Forskarassistent) in molecular medicine, funded by Karolinska Institutet.

**EDUCATION & POSTDOCTORAL STUDIES**

University of Sheffield, UK: Microbiology and Genetics: B. Sc. (Hons): 1980

University of Sussex, UK: Initiation of DNA replication in fission yeast: D. Phil.: 1984

Imperial College of Science, Technology and Medicine, London, UK: Postdoc working on regulation of carbon source regulation in brewers yeast: 1984-1988

Karolinska Institutet, Huddinge Sweden: Postdoc working on establishment of yeast models for functional studies of human steroid receptors: 1988-1992

**RESEARCH INTERESTS**

My group's research addresses the mechanisms by which DNA-bound transcription factors regulate gene expression in eukaryotic cells. An important aspect has been the use of yeast systems for studying human transcription factors. In recent years three main areas of focus have developed. (i) Identification of auxiliary co-activator/ co-repressor proteins that are recruited by- and mediate the functions of- DNA-bound activator/ repressor proteins. We have found that the most important co-activators/ co-repressors are protein complexes involved in chromatin modification and remodelling. (ii) Determination of the mechanism by which activator proteins interact with target proteins. We have shown that activator proteins interact with targets via an 2-step induced-fit mechanism in which poorly structured activators first make rapid, unstable, ionic interactions with target proteins followed by slower, more stable interactions which are dependent on hydrophobic interactions and protein folding. (iii) Identification of mechanisms by which activator proteins are regulated by protein degradation. We have shown that the c-myc activator is degraded by the 26S proteasome complex via specific target sequences in the c-myc protein.

**PUBLICATIONS (5 SELECTED)**

McEwan IJ, Dahlman-Wright K, Ford J, **Wright APH** (1996) Functional Interaction of the c-myc transactivation domain with the TATA binding protein: Evidence for an induced fit model of transactivation domain folding. *Biochemistry* 35:9584-9593

Almlöf T, Gustafsson J-Å, **Wright APH** (1997) Role of hydrophobic amino acid clusters in the transactivation activity of the human glucocorticoid receptor. *Mol. Cell. Biol.* 17:934-945

Flinn EM, Busch CMC, **Wright APH** (1998) Myc boxes, conserved in Myc family proteins, are signals for protein degradation via the proteasome. *Mol. Cell. Biol.* 18:5961-5969

Wallberg AE, Neely KE, Gustafsson J-Å, Workman JL, **Wright APH**, Grant P (1999) Transcriptional activation from chromatin templates in vitro by the glucocorticoid receptor N-terminal activation domain involves recruitment of coactivator complexes with histone acetyltransferase activity. *Mol. Cell. Biol*. 19:5952-5959

Wallberg AE, Neely KE, Hassan AH, Gustafsson J-Å, Workman JL, **Wright APH** (2000) Recruitment of the SWI/SNF chromatin remodelling complex as a mechanism of gene activation by the glucocorticoid receptor τ1 activation domain. *Mol. Cell. Biol*. 20:2004-13

**NATIONAL AND INTERNATIONAL NETWORK (active contacts during 2000)**

Jerry Workman, Penn State University, USA
Wolfram Hörz, Munich University, Germany
Steen Holmberg, Copenhagen University, Denmark
Martin Vingron & Stefan Haas, DKFZ Heidelberg, Germany
Daniel Marechal, Eurogentec SA, Liege, Belgium
EU Network

- Monique Bolotin, Univeristé Paris Sud, France
- Bernard Dujon, Pasteur Institute, Paris, France
- Jens Nielsen, Technical University, Denmark
- Steve Oliver, Manchester University, UK
- Francine Messenguy & Evelyn Dubois, IRMW Brussels, Belgium
- Enrique Herrero, Lleida University, Spain
- Aventis Pharmaceuticals & Crop Science, France

Jan-Åke Gustafsson, Karolinska Institute
Jan Carlstedt-Duke, Karolinska Institute
Claes Gustafsson, Karolinska Institute
Karl Ekwall, Södertörns University College
Kurt Bernt, Södertörns University College
Torleif Härd, Royal Institute of Technology (KTH)

**COMPETETIVE GRANT INCOME 2001 (main applicant)**

| | |
|---|---|
| Swedish Medical Research Council | 1.003.000 SEK |
| Swedish Natural Science Council | 293.000 SEK |
| Swedish Cancer Society | 250.000 SEK |
| EU Network | 425.000 SEK |