THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

# EPIGENETIC REGULATION OF HORMONAL RESPONSE

by

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# INTRODUCTION

## 1. Nuclear receptors and their epigenetic environment

Nuclear receptors are ligand activated transcription factors. They act as sensors of lipid soluble molecules, hormones or metabolites. Our main research interest focuses on a subclass of nuclear receptors that forms heterodimers with RXR, the 9-cis-retinoic acid receptor, with major implications in development and in diseases such as atherosclerosis and diabetes. A common attribute of this subclass of nuclear receptors is that they are DNA-bound sensors of intra or extra cellular lipid soluble molecules. Nuclear receptors translate the lipid soluble small molecular environment of the cell into genomic actions. By this nuclear receptors can be viewed as molecular nano-switches that change the status of the chromatin and open genomic scripts depending on the lipid environment of the cell.

The HL-60 cell line is a well-characterized M2 myeloid leukemia cell line that can be induced to undergo differentiation along different pathways, myeloid versus granulocytic, in response to a variety of physiological and pharmacological stimuli. The process of myeloid differentiation itself involves two distinct and sequential steps. The first is an identifiable intermediate state termed the precommitment or primed state and the second, a series of late events that lead to the onset of lineage specific terminal differentiation. Altered nuclear structure and feature retention of phenotype, a form of cellular memory that can last for several cell cycles characterize primed cells. Very little is known about the molecular characteristics of the precommitment or primed state and in particular about how these molecular changes impact the regulation of gene expression. Some of the molecular mechanisms that drive differentiation after retinoid induction are coded by the chromatin context.

The structure of the chromatin plays a fundamental role in regulating gene expression by controlling the access of transcription factors to the regulatory regions of genes. Two classes of enzymes are known to play a role in regulating chromatin structure, the ATP-dependent chromatin remodeling enzymes and the chromatin modifying enzymes. These latter ones are responsible for the post-translational modification of histone tails. According to the "histone code" hypothesis the covalent modifications of the histone tails maintain and modulate the patterns of gene expression. Modified histone tails have been reported to form binding sites of specific classes of proteins; bromodomain–containing proteins and chromodomain–containing proteins. It is thought that these histone-binding proteins form a functional link between the covalently modified histone tails and the effectors of transcription initiation. Chromatin immunoprecipitation (ChIP) enables us to study in vivo DNA-protein interactions at the chromatin level. By using antibodies raised against particular proteins specific DNA fragment libraries can be generated and studied.

In order to investigate the molecular mechanisms that may be associated with the priming of HL-60 cells and the establishment of a pre-committed state, we have characterized the covalent modification of the tails of histones specifically associated with the promoter of tissue transglutaminase, a gene that undergoes marked up-regulation during retinoid-induced myeloid cell differentiation. We have carried out a detailed analysis of the covalent modifications of histones bound to different regions of the transglutaminase promoter during three distinct states of differentiation: the naive state that occurs prior to the initiation of differentiation, the primed or precommited state that is induced by brief exposure of the naive cells to Vitamin D or DMSO and the differentiated state that occurs following the addition of a retinoid to the primed cells.

The analysis of histone tail modifications during both priming and transcriptional activation revealed distinct mechanisms that mark both of these

processes. Priming itself appears to be linked to major changes in histone tail methylation. In particular, methylation of K4 on histone H3 (H3K4) associated with the core promoter is rapidly decreased after the initiation of priming. Priming increases the methylation of arginine on histone H4 (H4R3) specifically on histones associated with a prominent enhancer element in the transglutaminase promoter. Arginine methylation of histones seems particularly important for the induction of the primed state since its elimination with a pharmacologic inhibitor of methyltransferases, resulted in the loss of priming for the induction of tissue transglutaminase gene. Therefore we concluded that H4R3 and H3K4 are modulators of hormonal responsiveness.

## 2. Genome wide location analysis studies

In the second part of my project I have extended my experimental approach from a closed (measurements of a single promoter by QPCR) to an open one (genome wide receptor-binding site survey by cloning and sequencing). This method was successfully used to map transcription factor binding sites in the genome (Weinmann et al, MCB. 2001 Oct.). With this method we mapped genomic loci marked by H4R3 methylation.

Genomic location analysis, a method that involves the combination of chromatin immunoprecipitation (ChIP) with high throughput DNA detection methods, such as microarrays enables us to correlate the epigenetic context of specific genes contributes with their transcriptional activity in a whole genome context (Weinmann et al, Genes Dev. 2002 Jan.). We employed a microarray that contains 12 000 CpG enriched loci of the human genome and analyzed the histone acetylation (H4 acetylation) status of the putative regulatory regions spotted on this microarray. We have also developed a web based bioinformatic interface to link the global gene expression data obtained on the Affymetrix platform with genomic location analyses data of the 12k CpG platform.

## MATERIALS AND METHODS

### Cells

HL-60/CDM-1, Monomac6, HT29 and 293T cells were cultured using standard cell culture conditions.

### Ligand treatments, materials

HL60/CDM1 cells were treated with 9-cis Retinoic Acid in a concentration of 1μM dissolved in ethanol: DMSO. Monomac6, HT29, 239T cells were treated with Rosiglitazone in a concentration of 1μM dissolved in ethanol: DMSO. Priming of cells was done with 1.25% DMSO or 10nM Vitamin D for 16 hours. Blocking of methyltransferases was achieved by treatment with 10μM of Adenosine dialdehyde for the same period. If not specified, all materials were purchased from Sigma. Antibodies for flow cytometric analysis if not otherwise mentioned were purchased from DAKO A/S as well as the appropriate control antibodies.

### Plasmids

Mammalian expression vectors for PRMT1 and the catalytic mutant PRMT1 were received from Uta Maria Bauer (University of Marburg). Mammalian expression vectors for PAD4 and the PAD4 C645S mutant were received from Yanming Wang (Allis Laboratory, Rockefeller). All other plasmids used were described previously by members of our laboratory.

### Transfection

HL-60 cells were transfected with AMAXA Electroporator System. 293T cells were transfected with Poliethylene-imine reagent.

**Extraction of total RNA**

Total RNA was extracted with Trisol Reagent (Invitrogen) according to the manufacturer‘s instructions.

**Real Time QPCR**

Real time quantitative PCR (QPCR) was carried out on an ABI7900 Real Time Sequence Detection System. All PCR reactions were done in triplicate with the appropiate control reactions. The comparative Ct method was used to quantify various PCR products. For the promoter assays the reactions were carried out similarly without the reverse transcription step.

**Chromatin Immunoprecipitation**

Chromatin immunoprecipitation was carried out as described by Kuo and Allis with modifications. Immunoprecipitation was carried out with specific antibodies purchased from Upstate Biotech and Abcam against modified histones as it follows: Upstate: #06–866 Anti Acetyl H4, #07–213 Anti dimethyl H4 Arg3, #07–030 Anti dimethyl H3 Lys 4, #07 212 Anti dimethyl H3 Lys9, and from Abcam: #ab5823 H4 methyl R3 antibody, #413–200 Pan dimethyl arginine. Complexes were collected with blocked protein A Agarose beads (Upstate #16–157). All chromatin results were verified from independent chromatin preps.

**Ligation and Plasmid Preparation**

Protruding ends of the isolated DNA fragments were filled by treatment with Klenow enzyme (Fermentas). Ligation was performed with T4 DNA Ligase (Fermentas). We used Zero Blunt® PCR cloning vector (Invitrogen). The ligation product was transformed in competent E.coli (DH5α). Plasmids were isolated with Miniprep Wizard (Promega) according to the manufacturers

recommendations. The size of the insert was verified by double digestion and PCR. Results were visualized by standard agarose gel electrophoresis.

**Sequencing**

Sequencing of the insert was performed with standard M13+ sequencing primer according to the manufacturers recommendations of the ABI Big Dye Terminator 2.1 kit.

**In Silico Analysis**

The sequences were first analyzed with NCBI Blast. Sequences that past the quality control were further analyzed with BLAT program on the UCSC Human Genome Browser. Conserved regions, known genes, conserved transcription factor binding sites and the position of the analyzed sequence towards these genomic elements was monitored. For annotation we used PANTHER Analysis. The PANTHER (Protein ANalysis THrough Evolutionary Relationships) database allows complex annotation of proteins and genes.

**Cell cycle analysis**

Cell cycle analysis was performed as described in Current Protocols in Cell Biology (1999) edited by Juan S. Bonifacio (et al.) Chapter 8.4.1.

**Intracellular staining and flow cytometry**

Cells were fixed and permeabilised with a buffer containing 0.5% saponine. Immunostaining was carried out with a mouse monoclonal antibody, which specifically recognizes the TGM2 protein and an isotype control antibody.

**Microarray analysis**

For the Affymetrix global gene expression analysis total RNA was isolated by using the RNeasy kit (Qiagen). cRNA was generated from total RNA by using

the SuperScript kit (Invitrogen) and the High Yield RNA transcription labeling kit (Enzo Diagnostics). Fragmented cRNA was hybridized to Affymetrix (Santa Clara, CA) arrays (HG_U133_plus2.0 chips) according to Affymetrix standard protocols. Preliminary data analysis was performed by the Microarray Core Facility at EMBL, Heidelberg.

For genomic location analysis studies primer elongation reaction was performed with the chromatin immunoprecipitation product using Sequenase enzyme, and subsequently PCR amplification was carried out (DeRisi Lab Protocol, UC San Francisco). Labeling of the amplified fragment was performed with the protocol suggested by the developer of the CpG array (UHN, Toronto, Canada). Data analysis was performed using GeneSpring 7.0 (Agilent).

**Quantification of DNaseI sensitivity**

Quantification of DNaseI sensitivity was performed with QPCR analysis. Briefly, nuclei were isolated, treated with different concentrations of DnaseI. Extracted DNA was treated with EcoR1, purified and measured with QPCR for the specific promoter regions.

## RESULTS AND DISCUSSION

### 1. Retinoid regulation of tissue transglutaminase gene expression in naive and primed myeloid leukemia cells.

The expression of tissue transglutaminase type 2 (TGM2) is very tightly regulated in myeloid leukemia cells. Priming of the cells by pretreatment with differentiating agents such as vitamin D or the polar–planar solvent dimethyl-sulfoxid (DMSO) increases retinoid-induced TGM2 expression. This experimental system has allowed us to study the effects of priming on the expression of a specific gene the induction of which is increased by the process of pre-commitment. We will refer to the un-primed and uncommitted cells as "naive" and those that advanced to precommitment as "primed". Retinoid induction of TGM2 in naive cells is strikingly enhanced (approx. 100 fold) upon DMSO priming, while the kinetics of the induction was very similar whether or not the cells had been primed.

A key question is whether increased induction of transglutaminase expression in the primed cells is a result of a higher mRNA expression level in each individual cell or is due to an increase in the fraction of the cells responding to the inducer (retinoid). We addressed the issue of the induction of the enzyme by using a coupled immunohistochemical/flow cytometric analysis to evaluate the levels of TGM2 in individual cells prior to and following retinoid treatment. Using these techniques we found that the basal level of TGM2 was undetectable in both untreated HL-60 cells and HL-60 cells treated with DMSO alone. In naive cells 9-cis retinoic acid induced detectable levels of TGM2 in 19.7% of the cells. When DMSO primed cells were treated with 9-cis retinoic acid 63.7% had detectable levels of TGM2

In order to investigate possible explanations for the increased frequency of response by primed cells we tested two obvious hypotheses. The first is that

the effect of priming is to increase the level of expression of retinoid receptors in individual cells. There was also no change in the levels of RAR or RXR receptors. The second is that differentiation is linked to cell cycle arrest. Our results confirmed the previously reported findings, DMSO priming had only a minor effect on the cell cycle distribution of cells.

**2. Alterations in chromatin modifications in naive and primed cells upon retinoid treatment**

To find out whether priming is producing changes at the chromatin level on the regulatory regions of this gene, we performed a DNaseI hypersensitivity analysis of the promoter of TGM2. As a negative control we used the RARβ gene which is not inducible in this cell line by retinoid treatment and priming. On the promoter of RARβ, DMSO priming did not induced DNaseI hypersensitivity. On the other hand, the core promoter of the TGM2 gene became more sensitive to DNaseI solely by DMSO priming suggesting that priming induces changes at the chromatin level of TGM2.

**3. Epigenetic map of the promoter of TGM2**

The next step in our studies was to characterize the effects of DMSO priming on the post-translational modifications of histones associated with regions of the tissue transglutaminase gene promoter. We have used chromatin immunoprecipitation in combination with QPCR (real-time quantitative PCR with TaqMan® probes) to obtain accurate quantitation of the level of post-translational modification of specific histone tails in chromatin isolated from naive and DMSO-primed HL-60 cells. We designed five promoter-specific probe sets spanning the 1800 bp fragment from the HR1 enhancer to the core promoter.

We first examined the effect of retinoid treatment on H4 acetylation in naive and DMSO-primed cells. In naive cells, retinoid treatment produced little change in the level of acetylation of H4 histones associated with the HR1 region of the transglutaminase promoter. In primed cells, on the other hand, retinoid treatment for 2 hours resulted in increased levels of H4 acetylation. Next we carried out a more comprehensive analysis of the effects of DMSO-priming and retinoid-treatment of primed cells on histone modifications (acetylation of H3, H4 and methylation of H3K4, H3K9 and H4R3). In the case of H4 acetylation, retinoid treatment of primed cells resulted in a significant and uniform increase in the level of acetylation of this histone H4 in all five regions of the promoter. This increase in acetylation started within 2 hours of the addition of the retinoid and reached a plateau in 6 to 8 hours.

In the case of H3K4, DMSO priming resulted in a marked decrease in the level of methylation of histones on the core promoter region. While DMSO priming decreased methylation of the H3 histone side chains (K4), it increased the level of methylation on the H4 histone (R3). These results demonstrate that the priming of HL-60 cells results in a coordinated set of histone modifications that are likely to be linked to alterations in chromatin structure. Among the most prominent of these effects are the suppression of H3K4 methylation at the core promoter and the increased methylation of the R3 residue of H4 on histones associated with the distal regulatory regions of the promoter.

**4. Role of H4R3 methylation and H3K4 demethylation**

To test if there is a functional link between the changes in H4R3 methylation induced by priming and altered gene expression, we used adenosine dialdehyde (ADOX), an inhibitor of methyltransferases to suppress methylation. Co-treatment of HL-60 cells with ADOX and DMSO eliminated H4R3 methylation and also reduced arginine methylation in general on the enhancer element

studied, as measured by ChIP QPCR with an anti-Pan-methylated arginine antibody. The decrease in H3K4 methylation induced by DMSO priming was not blocked with ADOX. Inhibition of methylation with ADOX also blocked retinoid-induced acetylation of H4 histones. In parallel with the inhibition H4R3 methylation and H4 acetylation, there was marked but not complete inhibition in the retinoic acid induced expression of tissue transglutaminase. Collectively, these data suggest that the inhibition of methyltransferases by ADOX leads to the inhibition of H4 arginine3 methylation and a concomitant decrease in retinoid-induced transglutaminase promoter activation.

To address the issue of a potential general effect on transcription by DMSO and ADOX on primed gene expression, we used a general expression profiling approach. Out of the 75 retinoid-induced genes the expression of which was selectively increased in DMSO-primed cells, the induction of 62 was blocked by co-adminsitration of DMSO plus ADOX.

**5. Modulation of transcription by altering the epigenetic context**

In order to gain a more mechanistic insight into this process and also to take advantage of recent developments in the field we have evaluated the role of the enzymes proposed to be responsible for H4R3 methylation. These are PRMT1, a methyltransferase and PAD4 a peptidylarginine deiminase recently identified as the enzyme responsible for methyl arginine's conversion into citrulline and thereby removing the methyl group. We examined the expression level of PRMT1 and PAD4 during priming and subsequent retinoid response in HL-60 cells. We found that priming itself induces PAD4 and further induced it during retinoid treament.

If one assumes that H4R3 methylation is the cause of the priming effect a few predictions can be put forward and tested.

A. One is that according to the previouslly published in vitro data (Wang et al, Science. 2001 Aug.), preacetylation of chromatin interferes with H4R3 methylation therefore the priming is likely to be attenuated. We tested this by using Trichostatin A (TSA) a histone deacetylase inhibitor. The DMSO priming effect was completely abolished if cells were pretreated with TSA suggesting that acetylation of histones interfering with H4R3 methylation eliminates the priming effect.

B. Another prediction is that activation of PAD4, the enzyme converting methyl arginine to citrulline also attenuates the priming effect (Wang et al, Science. 2004 Oct.). The presence of A23187, a calcium ionophore agent that activated PAD4, reduced retinoid responsiveness and priming effect. TSA pretreatment and also PAD4 activation prevented/eliminated H4R3 methylation, respectively as our chromatin immunoprecipitation results showed. Moreover, comparison of H4 acetylation and H4R3 methylation revealed that TSA treatment enhanced acetylation, whilst prevented H4 R3 methylation.

C. The third prediction we tested was that increased level of PRMT1, the methylase responsible for H4R3 methylation would lead to increased priming. Transfection of PRMT1 does not induce gene expression, on the other hand it can further induce the priming effect.

D. Finally, we have evaluated the combined effect of transfected wild type or mutant PRMT1 and PAD4 on the retinoid regulated expression of TGM2 and also that of a retinoid inducible reporter gene. Our data suggest that PRMT1's enzymatic activity is required for its co-activator activity. PAD4 does not act as a co-repressor, but its enzymatically inactive mutant synergizes with PRMT1 in enhancing transcription.

**6. Cloning genomic loci marked by H4R3 methylation**

Methylation of histones by PRMT1 makes them a better substrate for histone acetyltransferases and by this provides a mechanistic link between gene expression regulation and arginine methylation. We decided to map genomic loci of arginine methylation in a differentiation primed myeloid leukemia cell line using an unbiased technique. The „ChIP to clone" strategy was used previously to identify binding sites of transcription factors. The localization of H4R3 methylated loci was achieved by using chromatin immunoprecipitation with an antibody raised against methylated H4 arginine. This antibody should bind specifically to genomic loci marked by PRMT1.

Generated DNA fragments were cloned in a low background vector, transformed and the individual colonies isolated. Plasmids from individual colonies were purified, inserts identified by restriction enzyme analysis and by standard PCR with vector specific M13 primers and agarose gel electrophoresis. Further analysis was carried out after sequencing of the isolated plasmids.

**7. Sequencing and analyses of the clones and their genomic loci**

By sequencing 111 of these cloned sequences we found that 57 fragments contained fragments longer than 150 bp, corresponding to mono-, di- or trinucleosomes. We decided to analyse in detail the genomic localization of these. First we performed a BLAST analysis with the identified sequences. 48 of them were of human origin, 7 of bacterial origin and two of them of salmon origin. The bacterial origin was probablly due to contamination with bacterial genomic DNA fragments and the salmon DNA from the salmon sperm DNA used as a blocking agent for the protein A agarose beads.

From the 48 human sequences 38 of them provided us with a perfect match, that means the whole length of our query sequence could be alligned with more than 98% identity to a human sequence The 38 human sequences contained 26 unique sequences, 12 repeted unique sequnces and 10 repetitive

sequences. From the 10 repetitive sequences we found 6 unique repetitive sequences and 4 that were repetition of a single clone from the 6 previously mentioned. We decided to further analyse the genomic localisation of the 26 unique sequence hits with the UCSC genome browser. From the 26 analysed sequences we found 14 nearby known genes. 12 of them had no genes within 10kb. 6 of these had regions with conserved TF binding sites within 5 kb.

The PANTHER (Protein ANalysis THrough Evolutionary Relationships) database allows complex annotation of proteins and genes. This annotation allowed us to align the identified genes along biological processes. Signaling, cell differentiation, immune response and development are frequently associated with the identified genes.

Next we decided to analyse the conserved transcription factor binding sites (TFBS) in the 5kb region of the 26 identified immunoprecipitated fragments regardless wheter there were located in the proximity of genes or not. We found 39 conserved TFBS, with 12 of them beeing present more than one time. 6 TFBS were present at least three times and two of these were published to have conections with the family of protein arginine methyltransferases, namely MEF2 with PRMT4 (or CARM1) and FOXO as being an inducer of BTG1 the activator of PRMT1.

## 8. Genomic location analysis studies

In order to broaden the study of epigenetic changes from individual genes to a high troughput platform and to move our research to a genomic scale we decided to implement chromatin immunoprecipitation methods combined with microarray analysis (ChIP on Chip).

Using this approach, we can integrate the gene expression patterns with molecular determinants of chromatin. The microarray we use are from UHN (Toronto, CA) and contains 12 000 human CpG rich regions. By now our results show that ChIP on Chip is suitable for mapping regulatory sequences of PPARγ target genes. For this analysis we used two independent methods. First we located PPARγ binding sites by using PPARγ specific antibodies. We found occupancy of approximately 1% of the spots represented on the CpG array and identified novel genes with lower level of induction by PPARγ specific ligands as potential target genes.

In an independent set of experiments we analyzed the changes in H4 acetylation of the chromatin regions represented on the CpG microarray after treatment with PPAR specific ligands. By this approach the hit rate for spots that showed an increase in H4 acetylation after PPARγ ligand treatment was 0.6%.

In order to correlate ChIP on Chip results with global gene expression data generated on the Affymerix platform, we developed a web based bioinformatic interface in collaboration with the Hungarian Bioinformatics Institute. Location of the CPG annotation window is:
http://genomics.dote.hu/jetspeed/air/cpg.jsp

## SUMMARY

We propose a new model for the epigenetic regulation of retinoid response and differentiation competence in myeloid leukemia cells. In our studies, we were able to dissect some of the epigenetic changes taking place on a retinoid regulated gene tissue transglutaminase type 2. We found that H4R3 methylation, a modification identified previously but without a well defined biological role, is a hallmark of the primed cell state and precedes gene activation. This modification represents a transcriptional silent (unproductive), but primed state that marks key histones and makes them better substrates for receptor bound acetyltransferases (HAT-s). We propose that this mechanism accounts for the increased susceptibility of the cell to respond to a terminal differentiating agents, such as a retinoid, with increased gene expression and an increased potential for phenotypic differentiation. Preacetylated histones are refractory to this mechanism. This model is consistent with the proposal that histone tail modifications function as the physical mediators of cellular memory. By providing docking sites for transcription factors and marking histones for subsequent covalent modifications, these methylation reactions serve as silent switches of gene expression. Our findings suggest an active and physiological role for arginine 3 methylation on H4 tails in retinoid response and provide a model amenable to further investigation and potentially to pharmacological exploitation.

In order to further characterize the role of arginine methylation in signal integration and developmental processes we carried out mapping of the genomic loci marked by PRMT1 via histone H4 arginine 3 methylation. We used chromatin immunoprecipitation and cloning to isolate genomic loci marked by H4R3 methylation. After sequencing and in silico analysis we found that all of the genomic hits identified were intronic or at 5‘ end of specific genes. The

locations identified were enriched in conserved transcription factor binding sites of POU2F1, MEF-2 and FOXL1 factors. A great number of the genes in the proximity of the identified genomic loci were involving signaling pathways and developmental processes. Furhter analysis of the identified loci needs to be done in order to decipher the role of arginine methylation in regulation signaling pathways and gene expression.

**This thesis is built on the following publications:**

Balint BL, Szanto A, Madi A, Bauer UM, Gabor P, Benko S, Puskas LG, Davies PJ, Nagy L.
Arginine methylation provides epigenetic transcription memory for retinoid-induced differentiation in myeloid cells.
Mol Cell Biol. 2005 Jul;25(13):5648-63.
IF: 8.142

Balint BL, Gabor P, Nagy L,
Genome-wide localization of histone 4 arginine 3 methylation
in a differentiation primed myeloid leukemia cell line
Immunobiology 2005 Aug 210 (2005) 141–152
IF: 1.773

**Other publications:**

Szanto A, Benko S, Szatmari I, Balint LB, Furtos I, Rühl R, Molnar C, Csiba L, Garuti R, Calandra S, Larsson H, Diczfalusy U, Nagy L: Transcriptional regulation of human CYP27 integrates retinoid, PPAR and LXR signaling in macrophages. Mol. Cell. Biol. (2004) 24: 8154-8166
IF: 8.142

Balint BL, Nagy L,
Selective modulators of PPAR activity as new therapeutic tools in metabolic diseases
Current Drug Targets (accepted for publication on 28.04.2005.)
IF: 3.77

Lóránt Székvölgyi, Bálint L Bálint, László Imre, Katalin Goda, Miklós Szabó and Gábor Szabó,
ChIP-on-beads: a robust flow-cytometric method for the evaluation of chromatin immunoprecipitation results (Submitted for publication)

Balint LB, Nagy L: Genome wide location analysis of nuclear receptors
Abstract published in: The FEBS Journal vol 271 Suppl 1 July 2005
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**Posters:**
First author of posters on the following meetings:

* IMP Spring Conference, Vienna, "Epigenetic Programming of the Genome" 2002 May: Epigenetic regulation of Tissue Transglutaminase Type 2,

* Meeting of the Hungarian Biochemical Society/ Keszthely, Hungary 2002 may: Egy retinoid indukálta gén epigenetikus szabályozása

* EMBO Conference on Nuclear Receptors, Villefranche sur Mer 2003 June: Epigenetic Regulation of Hormone Response

* EMBO Practical Course Naples: Deciphering Chromosomes by Chromatin Immunoprecipitation 2003 september: In vivo Genomic Localization of Nuclear receptors

* Semmelweis Symposium: New Trends in Medical Genomics, Budapest, 2003 October: Epigenetic Regulation of Hormonal Response

*6-th Transcription Meeting, Heidelberg, EMBL 2004 September: Analysis of epigenetic determinants of hormonal in a Whole Genome approach

And: Arginine Methylation provides Epigenetic Transcription Memoryfor Differentiating Myeloid Cells

* EMBO-HHMI Joint Meeting Budapest 2004 December: Genome-wide Location Analysis of Nuclear Receptors

* Keystone Symposia PPAR/LXR Meeting, Vancouver, Canada, 2005 April: Genome-wide Location Analysis of Nuclear Receptors

* 30-th FEBS Congress, Budapest, Hungary, 2005 July: Genome-wide Location Analysis of Nuclear Receptors

* EMBO Symposium on Nuclear Receptors, Lake Garda, Italy, 2005 September: Lipid Sensors that Modify the Genome

Co-author:

*Meeting of the Hungarian Society for Cell Biology/ Eger, Hungary 2005 april ( Co-author of poster with Lorant Szekvolgyi: DNS-fehérje interakciók az MLL-gén töréspont klaszter régiójában)

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*What is the chief end of man? Man's chief end is to glorify God, and to enjoy him forever.*

*Question 1, Westminster Shorter Catechism*

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