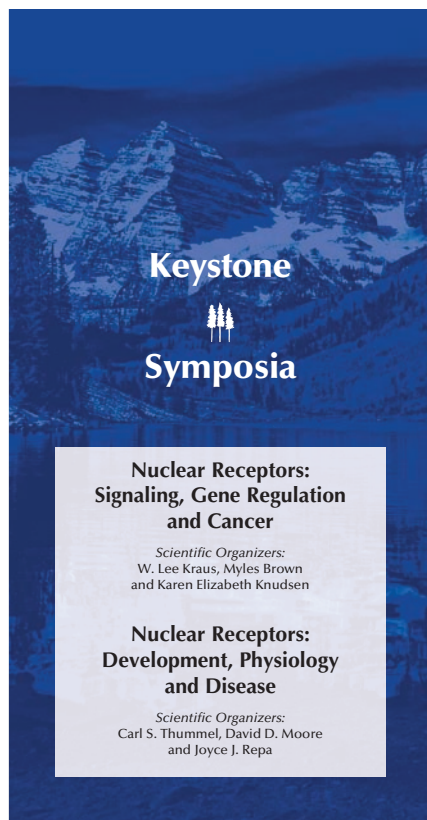


Expanding horizons for nuclear receptors

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The nuclear receptor joint Keystone meetings, formerly organized as 'Steroid Sisters' and 'Orphan Brothers', met this year under the banners, 'Signalling, Gene Regulation and Cancer' and 'Development, Physiology and Disease'. The change reflected both the excellent progress that has been made, as well as new directions that are now central to the field. The meeting covered all aspects of regulation by nuclear receptors and provided a good orientation for investigators new to the area, as well as a timely update for those with long tenure in the field.

In recent years, rapid advances have been made in genome-wide studies of receptor action. The main efforts underway are directed towards the identification of both cell-type-specific and disease-specific chromatin landscapes. Chromatin accessibility has emerged as a crucial prerequisite necessary for nuclear receptor binding. The global analysis of regions hypersensitive to DNase I (John *et al*, 2008) shows a surprising requirement of constitutively open chromatin for glucocorticoid receptor binding when other factors, including activator protein 1 (AP1) initiate chromatin opening (G. Hager, National Cancer Institute). Susanne Mandrup's group (Rasmus Siersbaek and Ronni Nielsen, U. Southern Denmark, Odense) presented findings from studies using the 3T3-1 adipocyte differentiation model, which show clearly that nuclear receptor binding correlates with the presence of local regions of accessible chromatin. Creating and maintaining these regions is fundamental to the adipocyte developmental programme. C/EBP β is necessary for the binding of several transcription factors induced early in the differentiation process and might function as a pioneering factor for PPAR γ (Mandrup). Similarly, FOXA1 has been implicated in chromatin interaction at about 50% of oestrogen receptor- α (ER α) binding sites (Jason Carroll, Cancer Research UK), as well as at a significant number of androgen receptor (AR) sites (Myles Brown, Dana Farber Cancer Institute). PU.1 binding to regulatory elements is also defined by cell-type-specific motifs with enrichment for AP1 and C/EBP β in macrophages, and E2A, EBF, nuclear factor- κ B and OCT in B cells, in which C/EBP β motifs are in fact under-represented (Christopher Glass,



University of California, San Diego). Glass and colleagues further proposed a model for sequential events that establish macrophage and signal-dependent gene regulation, with PU.1 binding as the first step, followed by collaborative loading of other factors. In an extensive study reported by Ralf Kittler (U. Chicago), 24 nuclear receptors, as well as 14 transcription factors and co-regulators, were expressed in tagged BACs in MCF-7 cells, and their binding was analysed by chromatin immunoprecipitation

(ChIP)-chip. Kittler and colleagues observed considerable binding redundancy of nuclear receptors and cooperating factors at regions referred to as 'chromatin hubs', which are associated with active chromatin regions identified by FAIRE, polymerase II (Pol II) occupancy, H2A.Z and histone modifications H3K4me1 and H3R17me2—findings that parallel the glucocorticoid receptor studies (John *et al*, 2008). The genomics of ER signalling in breast cancer was discussed by Benita Katzenellenbogen (U. Illinois), who reported the novel observation that extracellular-signal-regulated kinase ERK2 is activated rapidly by E2, interacts with ER α , and co-localizes with 65% overlap at enhancer regions in an ER α -dependent manner, leading to cell proliferation. The concept of stimulus-specific binding by ERs was advanced by Brown. He showed that growth factors stimulate ER occupancy at a distinct set of sites compared with those stimulated by oestrogen, and that these sites regulate genes that characterize breast tumours that overexpress ErbB2.

Brown and colleagues also pointed to H3K4me2 as the most sensitive enhancer marker for AR binding in LNCaP cells. Enhancers distinguished by AR and FoxA1 binding are characterized by two well-positioned nucleosomes, which undergo remodelling after hormone treatment with a transition from one to two peaks at AR regulatory sites. They further suggested that the hormone-induced changes of H3K4me2 levels reflected the changes in nucleosome occupancy, rather than erasure of the mark. This feature was incorporated in a nucleosome stabilization–destabilization scoring model for the prediction of transcription factor binding sites.

Lee Kraus (Cornell U.) presented the results of the exciting global run-on sequencing (GRO-seq) method for analysing transcriptional activation, in which only engaged polymerase activity is monitored. In oestrogen-stimulated MCF-7 cells, a reasonably altered view of the transcriptional landscape is obtained compared with that observed by steady-state messenger RNA analysis. Surprisingly, E2 treatment leads to transcriptional activity for as much as 26% of the genome, with only 50% of transcripts assigned to annotated genes and noncoding RNAs. The remaining fraction represents antisense and diverse transcripts, as well as transcripts from intergenic and enhancer regions. The presence of Pol II was detected at most enhancers, with transcripts identified at only 18%—the function of this phenomenon remains to be established. Additionally, 322 of 750 microRNA genes are upregulated by oestrogen, with their expression coordinated with the activity of their potential target genes. Application of the GRO-seq method shows a broad response that prepares cells for additional signalling, further translational burden and increased metabolism.

The analysis of long-range chromatin interactions—an emerging methodology that is crucial to understand gene regulatory networks—was described by Edwin Cheung (Genome Institute, Singapore). ER-ChIP, 3C and paired end tag (PET) sequencing were combined in the novel ChIA-PET methodology to characterize “all-to-all” chromatin interactions (Fullwood *et al.*, 2009). Hormone treatment (E2) of MCF-7 cells induces almost 1,500 intra-chromosomal interactions, whereas inter-chromosomal contacts were relatively rare (~15). The functional significance of these was confirmed by the finding that most ‘anchor’ genes are hormone-activated, whereas most ‘loop’ genes stay silent. The *RET* gene was presented as an example of complex regulation by ER-mediated long-range interactions. Rafael Metivier (U. de Rennes I) also discussed long-range interactions between enhancers in the oestrogen response TFF cluster (21q22.3) and the potential role of CTCF/cohesin in this process.

Keith Yamamoto (U. California, San Francisco) presented an update on the important concept that the precise sequence of a GRE will regulate glucocorticoid receptor protein conformation, in turn leading to selective interactions with co-regulators (Meijsing *et al.*, 2009). This work

was extended using NMR-based analysis and molecular dynamic simulations to show both the DNA sequence impact on glucocorticoid receptor structure (particularly the conformation of the ‘lever arm’), and to delineate the differences between GR α and GR γ isoforms.

Great interest was stimulated by the zebrafish-based screening of new ligands for human receptors developed by Henry Krause (U. Toronto) and colleagues. These investigators validated their system by using PPAR γ as a trap, obtaining quantifiable signals in a cell- and tissue-autonomous manner. They have shown that the system can be used for small molecule screens to identify potential novel drugs (Tiefenbach *et al.*, 2010).

The subject of post-translation modifications and the role of co-regulators also brought new insights into the action of nuclear receptors. GlcNAcylation of the nuclear multisubunit MLL5 complex—which manifests histone methyltransferase activity—seems to be crucial for epigenetic control by retinoic acid receptor alpha (RAR α) (Shigeaki Kato, U. Tokyo). Joshua Stender (Glass lab, U. California, San Diego) reported the development of a *Drosophila* high-throughput screen to search for novel proteins involved in nuclear-receptor-mediated repression of proinflammatory genes. This approach led to the discovery of a new mechanism of H4K20me3 demethylation that occurs at promoters of proinflammatory genes after LPS treatment. The reaction is performed by a specific demethylase, PH2F, which opposes the activity of the methyltransferase Smyd5. Kato showed that when the PH2F/ARID5B complex is isolated from Hep2G cells as a part of a fasting response, PH2F is phosphorylated by protein kinase A. The complex then binds to HNF4-RE and demethylates H3K9me₂, thus activating PEPCK expression.

Roland Schule (U. Freiburg) and colleagues have shown previously that PKC-related kinase 1 (PRK1) phosphorylates H3T11, which makes histone H3 a better substrate for JMJD2C—the first histone tridemethylase (H3K9) identified. In this scenario, PRK1 functions as a gatekeeper of AR-regulated gene expression and is considered a promising therapeutic target, as blocking PRK1 activity can suppress AR-induced tumour proliferation. The Schule lab has now identified a novel epigenetic mark (phosphorylated H3T6), as well as an enzyme (PKC β 1) responsible for its deposition. This modification blocks demethylation of H3K4

at the promoters of AR-activated genes (Metzger *et al.*, 2010). Reduced expression of AR-activated genes is observed on PKC β 1 knockdown with RNA interference, and suppressed proliferation of androgen-stimulated tumour cells and tumour growth is observed in xenograft models.

Histone deacetylases (HDACs) have long been regarded to function as co-repressors. However, Catharine Smith (U. Arizona) showed that glucocorticoid receptor requires HDAC activity for both activation and repression. In fact, almost 50% of dexamethasone-activated genes are affected by the HDAC inhibitor VPA. A study presented by Mitchell Lazar (U. Pennsylvania) was focused specifically on HDAC3. Liver-derived ChIP-seq data for HDAC3 shows a binding profile that resembles general nuclear receptor binding, with most sites localized in the distal intergenic region. Furthermore, a motif analysis of HDAC3 binding sites revealed enrichment for the nuclear receptor half site (AGCTCA). Thus, HDAC3 function in liver is linked strongly to enhancer elements and the action of nuclear receptors. HDAC3 was further discovered to be a crucial regulator of hepatic lipid metabolism in a circadian manner. Nearly 50% of HDAC3 binding sites overlap the Rev-Erba profile, and the two complexes interact in a circadian rhythm to promote histone deacetylation and repress transcription.

Nuclear receptors interact in complex ways with the circadian cycle. Urs Albrecht (U. Fribourg), showed that the Per2 LXXLL motif is responsible for dark-phase-specific interactions with PPAR α in liver nuclear extracts, and for regulation of the Bmal1 promoter. We learned from Vincent Giguere (McGill U.) that ERR α is also recruited to circadian gene promoters (Bmal and Clock). ERR α -null mice displayed the altered expression of clock genes, the altered circadian expression of metabolic genes normally regulated by ERR α , and altered blood glucose levels and locomotive activity. A remarkable study from Ueli Schibler's lab (U. Geneva) described circadian expression in real time by using a 250 copy tandem array of the DBP locus driving a luciferase reporter. YFP-tagged Bmal binding to the array was recorded in high resolution (3 min), revealing dynamic waves of Bmal-template interactions. These oscillatory waves were accompanied by parallel cycles of array compaction and decondensation. Residence time of the Bmal regulatory protein on binding sites was also examined

by using FRAP analysis and was found to be very brief, on the timescale of seconds. These findings recall the brief site occupancy reported originally for glucocorticoid receptor, and further emphasize the generality of the 'hit-and-run' exchange mechanism for transcription factors.

Nuclear receptors can also function through non-genomic mechanisms. Coactivators can directly activate enzymes, as described by Bert O'Malley (Baylor College of Medicine) and colleagues for SRC3. An SRC3 splice isoform promotes EGF-mediated cancer cell migration in a FAK-dependent manner. Also, a dominant negative mutant of thyroid hormone receptor (TR β PV) activates the PI3K pathway through interaction with p85a (Sheue-Yann Cheng, National Cancer Institute)—a step that was shown to be regulated negatively by NCoR through competition with the PV mutant for binding to p85a. Treatment with PI3K inhibitors leads to significant reduction of tumour growth and metastasis and increased apoptosis and survival rate in β PV mice.

Several other examples with potential therapeutic relevance involving the modulation of nuclear receptor and co-regulator activity were discussed. Inez Rogatsky (Cornell U.) described a novel facet of the immunosuppressive properties of glucocorticoids. Type I interferon (IFN) is a unique cytokine, controlled by glucocorticoid receptor at the levels of both production and signalling; GRIP1 plays an important role in this regulation. This might explain the effectiveness of glucocorticoids in systemic lupus, a devastating autoimmune disease in which type I IFN and massive over-expression of IFN target genes have been causally linked to pathogenesis.

In the intestine, previous results suggest that local glucocorticoid production and regulation of the anti-inflammatory response is driven by LRH1. A new potential treatment with LRH1 agonist ligands (DLPC and DUPC) activates LRH1 in the gut and ameliorates chemically induced (TNBS and DSS) inflammatory bowel disease in mouse models (David Moore, Baylor College of Medicine). Ronald Evans (Salk Institute) showed that suppressing RXR/TR owing to the PTU/MM1 treatment leads to the upregulation of the *Klf2* gene (essential for lung development) and further rescues type I pneumocyte immaturity. Finally, LXR was identified as a crucial regulator

in the skin and a potential target for atopic dermatitis and other dermal indications (Catherine Thompson, Pfizer). LXR regulates the expression of genes crucial for keratinocyte differentiation, and treatment with LXR ligands can reverse some effects of skin ageing both *in vitro* and *in vivo*. Importantly, Thompson's study involved the development of a new plasma-degradable compound to avoid systemic side-effects.

Among the metabolic pathways regulated by nuclear receptors, the activation of mitochondria through AMPK/PGC1 α was broadly discussed. Increased neovascularization activation of the oxidative network, vascular mapping and type I muscle endurance is achieved in a transgenic mouse model when ERR γ is overexpressed in type II fast muscles (Evans). Anastasia Kralli (Scripps Research Institute, La Jolla) showed that both ERR γ and ERR β can activate pathways similar to ERR α , but with important distinctions. Expression of ERR α correlates with an adverse clinical outcome for breast cancer, while reintroduction of ERR γ to the human MDA MB231 breast cancer cell line reduces tumour growth in mice and promotes the mesenchymal–epithelial transition (MET). Reintroduction of the coactivator PGC1 α , which activates endogenous ERR α does not affect tumour growth or promote MET, suggesting distinct ERR α and ERR γ roles in breast cancer. As discussed by Johan Auwerx (Ecole Polytechnique, Lausanne), the PGC1 α pathway can be modulated by NAD $^{+}$ sensors. Mitochondrial activation was enhanced through PGC1 α deacetylation by SIRT1, activated beforehand by AMPK, NAD $^{+}$ and the recently discovered interaction with PARP1. This work shows a direct link between cellular redox status and signalling/transcription regulation. Giguere described two novel regulators for ERR-dependent transcription pathways, Prox1 and mir-378. Prox1 functions as a negative ERR α regulator and mir-378 targets the PGC1 β –ERR γ –GABPA pathway, which correlates with breast cancer progression.

The nuclear receptor–FGF pathway was discussed by David Mangelsdorf (U. Texas Southwestern Medical Center) and Moore. Moore presented a parental nutrition-associated liver disease (PNALD) neonatal piglet model that lacks the normal production and circulation of bile acids (BA); the BA–FGF19–FXR pathway is disrupted in these animals, but reintroduction of BAs (specifically chenodeoxycholic acid

reverses deleterious effects. According to Mangelsdorf, FGF15 activation by BA and FXR leads to effects similar to insulin action, although through a different pathway. A mechanism of PPAR γ phosphorylation by CDK5 was described by Bruce Spiegelman (Harvard Medical School). In obesity, both inflammatory cytokines and lipids lead to the activation of CDK5, which in turn phosphorylates PPAR γ -S273. This affects a precise subset of PPAR γ -regulated genes (including CD36, adiponectin, adipin and leptin). Treatment with PPAR γ ligands (including MRL24) blocks the phosphorylation event by inducing a conformational change in PPAR γ that makes it less prone to phosphorylation.

The role of nuclear receptors in maintaining pluripotency was covered in a presentation by Austin J. Cooney (Baylor College of Medicine). Cooney's work shows that canonical Wnt signalling acting through β -catenin promotes pluripotency gene expression (Oct4) in an Lrh-1 dependent manner. Differentiation of embryonic stem cells by application of retinoic acid leads to loss of Lrh-1 and activation of the Oct4 repressor GCNF, which in turn initiates the silencing of the gene. These findings suggest a potential mechanism for the regulation of pluripotency by small molecules.

The nuclear receptor field continues to expand and develop new opportunities. Increasing attention is being devoted to the vast and complex roles of nuclear receptors in human disease, while fundamental mechanisms of receptor action remain the subject of intensive investigation. With the explosion of information and detail now available from genome-wide studies, as well as real time studies in live cells, we are clearly at the beginning of a new era in receptor biology.

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Published online 16 July 2010

EMBO reports (2010) **11**, 569–571.
 doi:10.1038/embor.2010.111