Twenty years of nuclear receptors

Conference on Nuclear Receptors: from Chromatin to Disease

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This EMBO Conference on 'Nuclear Receptors: from Chromatin to Disease' took place between 29 September and 1 October 2005, in Gardone Riviera, Italy, and was organized by A. Maggi, J. Schwabe, D. Moore and T. Perlmann.

Keywords: hormonal signalling; nuclear hormone receptors; transcription

substances, such as intracrine regulatory metabolites, signal the status of the organism as a whole. Several synthetic ligands can specifically modulate the communication of a nuclear receptor with its cellular environment. Lipid-soluble hormones and vitamins can pass easily through the lipid bilayer of cell membranes, triggering an intercellular communication network in which nuclear receptors exert genomic and non-genomic actions that are determined by the specific recognition of the ligand. Nuclear receptors are one of the main classes of transcriptional regulators in metazoans—there are 48 in humans—and they first appeared in the earliest stages of animal evolution, long before the divergence of vertebrates and invertebrates. Steroid receptors were first identified biochemically in the 1960s (Jensen, 2004), but nuclear receptors were only recognized as a superfamily of transcription factors 20 years ago (Green *et al*, 1986; Hollenberg *et al*, 1985; Miesfeld *et al*, 1986). The family includes disguised 'orphan receptors', which are awaiting ligand identification, and true orphans, which regulate transcription independently of ligand binding.

Today, the nuclear receptor field has broadened dramatically and covers areas ranging from structural and functional analyses of the molecular mechanisms of transcription regulation to nuclear-receptor-based drug design and (pre)clinical studies aimed at exploiting the potential of these master regulators for therapy, particularly of metabolic diseases and cancer (Chambon, 2004; Evans, 2004). This EMBO Conference on Nuclear Receptors was special because 2005 marked the 100th anniversary of the coining of the term hormone by Ernest Starling (Tata, 2005) and the 20th anniversary of the cloning of the first nuclear receptors. The organizers put together an exciting and dense programme, keeping the participants closely focused on the science despite the tempting opportunities to explore the beautiful surroundings of Lake Garda. The programme was divided into three main themes: one dealt with basic mechanisms, evolution, chromatin and co-regulators, another with differentiation and development, and the third with metabolism and physiology. Adopting this general organization, this report summarizes the main advances reported during the conference.

Basic mechanisms

Evolution. A frequently asked question concerning the evolutionary origin of the nuclear receptor family is whether the ancestral

EMBO reports (2006) 7, 579–584. doi:10.1038/sj.embor.7400711

Introduction

Nuclear receptors are a family of transcription factors the activity of which is modulated by binding to small signalling molecules. These compounds trigger changes in the conformational and dynamic behaviour of the receptors, which in turn regulate the recruitment of co-regulators and chromatin-modifying machineries. Nuclear receptors regulate growth, development and metabolic homeostasis in multicellular organisms in which hormones and non-hormonal

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Submitted 15 February 2006; accepted 19 April 2006; published online 26 May 2006

nuclear receptor was ligand-dependent or whether it evolved independently (Fig 1). J. Thornton (Eugene, OR, USA) presented an argument that the ancestral nuclear receptor was ligand-dependent and that orphan receptors evolved not *de novo* but by modification of the ancestral ligand-binding pocket. Specifically, his group has carried out phylogenetic analyses, which suggest that the structural features that confer ligand independence on orphan receptors could be derived states rather than ancestral. Further evolutionary implications came from work presented by D. Moras (Strasbourg, France). Using structure-based sequence analysis, he has partitioned the superfamily into two classes: homo- and heterodimeric receptors. Two specific molecular pathways constitute the characteristic signature motifs of each class. These motifs connect a loop near the dimeric interface to helix 1 adjacent to the canonical co-factor-binding site (class I) or to another loop near the activation helix 12 (class II) across the ligand-binding domain. They could constitute pathways for the allosteric control of protein binding, such as retinoic acid receptor (RAR) regulation by phosphorylation. Notably, all the receptors in *Caenorhabditis elegans* (>200) belong to the homodimer group, suggesting that heterodimer-forming nuclear receptors, such as retinoid X receptor (RXR) partners, came later in evolution and/or are not necessary for some species. Alternatively, these receptors might have lost the class II signature motif along with its attached function. This evolutionarily puzzling observation requires further investigation. D. Picard's presentation (Geneva, Switzerland) provided a further boost to the discussion of the evolutionary aspects of nuclear receptor research. So far, nuclear receptors have been found exclusively in animals. Picard provided data to suggest that yeast and animals share the same ligand-binding fold. His group found that the heterodimeric transcription factor subunits Oaf1:Pip2 of *Saccharomyces cerevisiae* contain ligand-binding domains and that the activity of this transcription factor depends on the binding of unsaturated fatty acids.

Co-regulators. Another key issue in nuclear receptor signalling is the role of co-factor molecules that mediate transcriptional activation and repression. The number of co-factors implicated in nuclear receptor signalling is in the hundreds, therefore it is a daunting task to assign the contribution of each of these, either alone or in combination with others, to particular receptor-mediated transcriptional events. The complexity of this issue is increased by the fact that these co-factors also participate in other non-receptor-mediated signalling events.

B. O'Malley (Houston, TX, USA) presented data on how membrane signalling might influence co-activator function. His group has shown that activation of kinase cascades, such as that via Pin1, enhances steroid receptor co-activator 3 (SRC-3) co-factor complex formation by phosphoactivating the co-activator. In more general terms, they showed that the phosphorylation of co-factors can lead to their translocation, enhance their transactivation function, alter their preferences for transcription factors and modulate co-factor turnover.

Another well-characterized co-factor complex, thyroid-hormone-receptor-associated protein (TRAP)/Mediator has been implicated in many transcriptional signalling events. X. Zhang and colleagues (R. Roeder's group, New York, NY, USA) examined and quantified complexes containing the MED1/TRAP220 subunit, which targets nuclear receptors. They found that this subunit is present in only 20% of the TRAP/Mediator population, that the subpopulation is enriched

Fig 1 | The evolutionary enigma of nuclear receptors. It remains controversial whether the ancestral receptor was an orphan lacking a ligand (such as Nurr1) or a ligand-sensitive receptor (such as thyroid hormone receptor (TR)). Figure kindly provided by J. Schwabe.

in other newly identified TRAP/Mediator subunits, and that it is selectively used for nuclear-receptor-mediated transcription. K. Yamamoto (San Francisco, CA, USA) tackled the difficult issue of selectivity and the relationship between a physiological programme and a regulatory network. They used glucocorticoid receptor (GR)-mediated transcription and selective GR activators in mammalian cells and regulation of metabolism in *C. elegans*. His group found that the different GR activators regulated only part of the GR regulatory network. Similarly in *C. elegans*, they found that the nuclear hormone receptor 49 (NHR-49) regulated fatty-acid metabolism during normal growth and also controlled a sector of the fasting response. It seems that complex physiological programmes are assembled from sectors of many regulatory networks rather than being dependent on a single master regulator.

Four talks focused on the 'dark' side of nuclear receptors: transcriptional repression rather than activation. M. Lazar (Philadelphia, PA, USA) presented data on the functional analysis of the co-repressor complex containing either the silencing mediator for retinoid and thyroid receptors (SMRT) or the nuclear receptor co-repressor (N-CoR). SMRT and N-CoR are related proteins that mediate transcriptional repression by forming complexes with histone deacetylases (HDACs) to induce local chromatin condensation. Recently, Lazar's group has found that the enzymatic activity of HDAC3 required the deacetylase-activating domain (DAD) of SMRT. Besides this DAD, SMRT also contains two SANT domains, one of which constitutes a histone interaction domain (HID). HID synergizes with the DAD to promote histone deacetylation. Lazar also showed that the constitutive repressor Rev-erbA, a key

transcription factor in regulating circadian rhythm, uses the N-CoR/SMRT–HDAC3 complex to repress transcription of the putative clock gene *Bmal1*. This process is controlled through the stability of the Rev-erbA protein by glycogen synthase kinase 3 β . J. Schwabe (Cambridge, UK) discussed in more detail the nature of the large multi-protein repression complex that is recruited to unliganded nuclear receptors by SMRT and N-CoR. The structural basis of the recruitment of HDAC3 to this complex was discussed along with insights into the recruitment of co-regulators to the orphan nuclear receptor Nurr1. Another form of nuclear-receptor-mediated repression, known as transrepression, was discussed by S. Kato (Tokyo, Japan). In his model gene system, receptors do not bind directly to DNA, but instead interact physically with activators and interfere with transcription. By studying a novel ATP-dependent chromatin remodelling complex (WINAC), which interacts directly with the vitamin D receptor, his group have elucidated how a negative vitamin-D-responsive element (VDRE) is regulated through the recruitment of chromatin remodelling and histone modification activities. E. Treuter (Stockholm, Sweden) is studying the orphan nuclear receptor DAX-1 (dosage-sensitive sex reversal–adrenal hypoplasia congenita critical region on X chromosome, gene 1). This is an unusual member of the family not only because it has no known ligand and acts as a repressor, but also because it has no DNA-binding domain. DAX-1 is involved in the development of reproductive function, in part by acting as a co-repressor for the orphan receptor steroidogenic factor 1 (SF1). By looking for interacting proteins, Treuter's group identified a previously uncharacterized E3 ubiquitin ligase of the RING–IBR–RING family. Their studies suggest that components of the ubiquitin system control the transcriptional repressive activity of the orphan receptor DAX-1.

Chromatin. Co-factor-bound receptors only bind to DNA in the form of chromatin, and epigenetic changes that affect receptor function have been intensively studied. F. Gannon (Heidelberg, Germany) recently described the epigenetic changes taking place during the oestrogen receptor (ER)-mediated transcriptional response. Following on from this elegant characterization of transcription factor cycling, his group has examined different cell lines for common pathways that are regulated by various ER ligands. An amazing complexity of ER signalling was revealed, suggesting the involvement of different complexes in these pathways. Through screening small-molecule libraries for new types of compound that might modulate ER signalling, they identified some molecules that also influence the activities of topoisomerases. This suggests an interplay between topoisomerases and ER-mediated transcription. These findings underscore the importance of chromatin modification in the regulation of transcription. L. Nagy (Debrecen, Hungary) showed that hormonal responsiveness is also controlled at the epigenetic level. Using the retinoid-regulated differentiation of HL60 cells, he provided evidence that transient histone 4 arginine 3 methylation of target genes is a positive epigenetic marker and regulator of retinoid responsiveness in myeloid cells. This mechanism might act as a signal integrator or as an epigenetic transcription memory during hormone-induced differentiation. R. Schüle (Freiburg, Germany) identified the monoaminoxidase lysine-specific demethylase 1 (LSD1) as the first histone demethylase, thereby defining histone demethylation as a new component in gene regulation. The association of LSD1 with the androgen

receptor (AR) in an AR–LSD1 complex results in demethylation on mono- and dimethyl histone 3 at lysine 9 and thus induces androgen-dependent gene expression. Importantly, inhibition of LSD1 either by RNA interference or small chemical inhibitors severely impaired the growth of prostate tumour cells, making LSD1 a potential drug target for fighting prostate cancer.

Identification of new ligands. Around half of the 48 human nuclear receptors are still considered to be orphans as no physiologically relevant ligand has been found. Structural and microanalytical approaches have been used to identify new ligands for some of these receptors. This issue is complicated by the fact that, in several cases, the ligand-binding pocket contains small lipophilic molecules, which might have a structural function. This seems to be the case for hepatocyte nuclear factor 4 α (HNF4 α). This view was challenged by F. Sladek (Riverside, CA, USA), who has purified receptors directly from mammalian cells and developed a new ligand-identification assay by coupling gas chromatography and mass spectrometry (GC/MS). Using this assay, his group could show that linoleic acid preferentially associates with HNF4 α in mammalian cells and might behave as a dissociable ligand. Through structural analyses, I. Krylova (San Francisco, CA, USA) and colleagues reported that phosphoinositide phosphates are potential ligands of SF1 and liver receptor homologue 1 (LRH1). If substantiated, this finding suggests a relationship between the LRH1, SF1 and phosphatidylinositol 3-kinase (PI3K) signalling pathways.

Differentiation and development

Most of the talks in the other two sessions dealt with genetic approaches for identifying the biological roles of nuclear receptors by using total or tissue-specific/inducible knockout strategies. S. Tsai (Houston, TX, USA) identified a developmental role for the orphan receptor chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) in specifying arterial–venous identity by showing that the receptor is expressed in endothelial cells of veins but not arteries. Selective ablation of COUP-TFII in venous endothelial cells led to arterial characteristics, whereas ectopic expression of the receptor in endothelial cells resulted in a fusion of veins and arteries, the result of which had venous characteristics. Another orphan receptor, Nurr77, was also linked to vascular function by C. De Vries (Amsterdam, The Netherlands). This group found that orphan Nurr77 is expressed as an immediate early gene in stretched venous smooth muscle cells and elicits an inhibitory mechanism to limit adverse responses to mechanical strain. Several presentations addressed the role of retinoid receptors (RARs and RXRs) in different tissue compartments using spatiotemporally controlled genetic ablation techniques. P. Chambon (Strasbourg, France) and colleagues engineered a mouse line in which they selectively ablated RXR α and RXR β in adult keratinocytes. The mice developed extensive alopecia and chronic skin inflammation. This condition mimics human atopic dermatitis with regard to clinical, haematological and immunopathological changes, including a T helper 2 (Th2)-type cytokine expression pattern. These findings assign a key role to RXRs in the control of cutaneous inflammation. N. Ghyselinck (Strasbourg, France) has studied the interrelationship between RAR γ :RXR α and peroxisomal-proliferator-activated receptor- β (PPAR β):RXR α heterodimers in the developing epidermis by using a combination of genetic ablation and pharmacological

Fig 2 | Detection of oestrogen-receptor-mediated transcription at the single-cell level. Oestrogen receptor (ER) fused to the green fluorescent protein localizes with the active form of RNA polymerase II at the integrated, oestrogen-regulated prolactin promoter locus. Figure kindly provided by M. Mancini.

tools. His group has found that both heterodimers participate in the generation of lamellar granules, which deliver lipids between the keratinocytes: $RAR\gamma:RXR\alpha$ heterodimers repress whereas $PPAR\beta/\delta:RXR\alpha$ heterodimers activate gene expression. M. Mark (Strasbourg, France) continued this theme by investigating the contribution of RARs to Sertoli cell differentiation and homeostasis. $RAR\alpha$ is required for spermatogenesis whereas cholesterol homeostasis in Sertoli cells depends on $RXR\beta$:liver X receptor- β ($LXR\beta$) heterodimers, in which the $RXR\beta$ AF2 domain is active. Similar to Ghyselinck, Mark also concluded that RXR is most probably activated in these cells by a ligand that is distinct from 9-*cis* retinoic acid. T. Perlmann (Stockholm, Sweden) used the orphan receptor Nurr1 to investigate the transcriptional programme of dopamine neuron differentiation. His group has identified two new transcription factors, *Lmx1a* and *Msx1*, that function upstream of Nurr1. They also found that *Lmx1a* is required and sufficient to induce dopamine neurons, whereas *Msx1* controls the timing of dopaminergic cell differentiation through the activation of other transcriptional pathways. As these cells progressively diminish in Parkinson disease, these pathways are likely to be useful in designing new treatments for it. J.-A. Gustafsson (Stockholm, Sweden) has been systematically analysing the roles of $ER\beta$. He reported that in prostate epithelial cells, $ER\beta$ suppresses proliferation and promotes differentiation. His group has also found that the natural ligand mediating this effect might be 3 β androstenediol. U. Schibler (Geneva, Switzerland), a circadian rhythm aficionado, found that the positive and negative arms of the feedback loop that controls gene

expression in circadian oscillators are coupled by the orphan nuclear receptor Rev-erbA. Using genetic and global gene expression analysis, Schibler showed that Rev-erbA regulates not only the central circuitry but also the output pathways that encode the regulators and enzymes involved in lipid metabolism.

Metabolism and physiology

Another theme of the conference was the role of nuclear receptors in metabolic processes, including energy metabolism, enterohepatic metabolic cycles and mitochondrial processes. Using molecular and genetic tools, A. Kralli (San Diego, CA, USA) has linked the orphan receptor oestrogen-related receptor- α ($ERR\alpha$) to the co-factor PPAR γ co-activator 1 α (PGC-1 α). It seems that these two transcription factors are linked in tissues such as heart, kidney, brown adipose tissue and muscle and regulate mitochondrial biogenesis, fatty-acid oxidation, the tricarboxylic acid cycle and oxidative phosphorylation. $ERR\alpha$ is coactivated by PGC-1 α . A. Chawla (Stanford, CA, USA) reported on the potential crosstalk of cytokine signalling, PPAR γ and PGC-1 β in regulating macrophage metabolism and the activation state. Another co-factor, receptor-interacting protein 140 (RIP140), has important metabolic roles and is being studied by M. Parker (London, UK). He has shown previously that this co-factor has a role in adipose biology and female reproduction. Now, using global gene expression analysis, he has identified some of the processes that are regulated by this transcription factor. During adipogenesis, it blocks genes involved in energy dissipation and mitochondrial uncoupling, whereas in the ovary, its role is to prevent the gene expression of extracellular matrix components and cell-cell interaction. J. Auwerx (Strasbourg, France) has carried out a systematic analysis of the role of members of the p160 co-activator family in mice, including Src3. He showed that as well as having important roles in energy homeostasis, Src3 also participates in lymphoproliferation. Src3-null animals develop malignant B-cell lymphomas on ageing, suggesting an antiproliferative role for this co-factor in this tissue. D. Mangelsdorf (Dallas, TX, USA) pointed out the complex interrelationship between enterohepatic nuclear receptors (that is, $LXR\alpha$, $LXR\beta$ and farnesoid X receptor (FXR)). LXR agonists stimulate pathways leading to bile-acid and fatty-acid synthesis, whereas FXR has the opposite effect. Activation of these pathways can have a beneficial effect on maintaining cholesterol homeostasis, and therefore these findings have implications for treating or preventing cholesterol gallstone disease, obesity and cancer. D. Moore (Houston, TX, USA) extended this theme by showing data that indicate a role for FXR in liver regeneration. FXR and other nuclear receptors maintain liver homeostasis by activating appropriate metabolic pathways and by sending signals to increase liver capacity when it is functionally reduced. R. Evans (San Diego, CA, USA) reported on a comprehensive analysis of PPAR β/δ function using combinations of transgene and knockout strategies to identify the tissue-specific functions of this receptor. PPAR β/δ is able to suppress inflammation by its action in macrophages, and it can regulate fibre type-switching in the muscle and contribute to insulin-sensitizing activity in the liver. This work provides support for considering PPAR β/δ as a target in syndrome X. Also using mouse genetics, D. Metzger (Strasbourg, France) showed that PPAR β/δ and the co-factor transcription intermediary factor 2 (TIF2) are key players in the determination of muscle fibre type-switching and whole-body energy homeostasis, further underscoring the idea that these molecules are relevant targets for the treatment of obesity and type II diabetes.

Fig 3 | The pioneers of the molecular era of nuclear hormone receptor research. Left to right: Bert O'Malley, Pierre Chambon, Keith Yamamoto, Ron Evans and Björn Vennström.

Links to human diseases

Four talks dealt with clinical observations or directly addressed the possibility of generating new therapeutic models. Through a collaboration with several colleagues, H. Gronemeyer (Strasbourg, France) revealed that rexinoids, previously thought to be silent in the context of the RAR–RXR heterodimer, are powerful inducers of the differentiation and apoptosis of acute myeloid leukaemic cells, if the intracellular level of cAMP is increased. Gronemeyer defined the corresponding mechanistic principles as RXR 'desubordination' by protein kinase A agonists and showed that apoptosis is due to the activation of tumour-selective TRAIL signalling. He pointed out that this nuclear receptor–kinase crosstalk might allow the development of anti-leukaemia combination therapies. J. Drouin and colleagues (Montreal, Canada) studied the molecular mechanism of the loss of the negative glucocorticoid feedback loop in Cushing disease. They have identified a chromatin-associated protein that is essential for transrepression between GR and nerve-growth-factor-induced protein B (NGFI-B). Notably, they have shown that this protein is frequently lost in human and dog Cushing microadenomas. B. Vennström (Stockholm, Sweden) studied the effect of a mutation in human thyroid hormone receptor- α (TR α). By generating the corresponding mouse knock-in model, he was able to show that the mutation affects two distinct functions, as mice have both neuromuscular and psychiatric abnormalities. These are reminiscent of the effects of thyroid hormone depletion. K. Chatterjee (Cambridge, UK) identified a range of mutations in the PPAR γ gene in patients with severe insulin resistance. He reported three distinct molecular mechanisms by which these receptors interfere with PPAR γ

signalling: two missense mutants formed dominant-negative receptors through their recruitment of co-repressors; one mutant, which led to the formation of a truncated receptor, acted only in concert with a mutation of an unrelated gene; and other DNA-binding domain and ligand-binding domain mutants acted as dominant-negative receptors by sequestering limiting amounts of co-factors.

New technologies

Several technologies developed recently were reported that open up new possibilities in the field. E. Martinez (Bethesda, MD, USA) spoke about a high-content screen for epigenetic modulators that inhibit DNA methyltransferases and HDACs. His group created mammalian cell lines with a stably integrated green fluorescent protein reporter gene that is repressed constitutively because of its chromatin context. Using this system, they can screen compounds in an automated fashion for reactivation of gene expression. M. Mancini (Houston, TX, USA) introduced methods to interrogate mechanisms of nuclear receptor function at the single-cell level by engineering a HeLa cell line containing an ER-responsive promoter array. Using fluorescently labelled receptors, his group are able to quantify ER dynamics at a physiologically regulated transcription locus, monitor co-factor recruitment, test various ligands and follow chromatin modelling in high-throughput microscopy analyses on a timescale of seconds (Fig 2). A. Maggi (Milan, Italy) also uses imaging, but at the level of the whole animal. Her group has generated transgenic reporter mice (ERE-LUC) to monitor the transcriptional activity of oestrogen receptors. This has allowed them to analyse the contribution of ER α and ER β to oestrogen signalling

during embryonic development and also in adult tissues. Finally, on the genomics front, V. Giguère (Montreal, Canada) presented data on the genome-wide localization of the ER/ERR subfamily of nuclear receptors. Giguère's group has used chromatin immunoprecipitation combined with promoter microarray analysis (ChIP-chip) to identify ER and ERR genomic-binding sites in a breast cancer cell line and in mouse tissues. They showed that the ER α and ERR α bind to distinct sets of genes and regulate separate biological processes. ChIP-chip therefore constitutes a powerful new tool to assign functions to closely related nuclear receptor isoforms.

The meeting ended with a special gala dinner, during which several of the early pioneers in the nuclear receptor field shared some of their memories of the early days of the field. Pierre Chambon (Green *et al*, 1986), Ron Evans (Hollenberg *et al*, 1985), Bert O'Malley (Conneely *et al*, 1986), Keith Yamamoto (Miesfeld *et al*, 1986) and Björn Vennström (Sap *et al*, 1986) were also honoured with the gift of a special handpainted Italian tile prepared for the occasion (Fig 3).

ACKNOWLEDGEMENTS

L. Nagy is an International Scholar of the Howard Hughes Medical Institute and holds a Wellcome Trust Senior Research Fellowship in Biomedical Sciences in Central Europe. Work in the Gronemeyer laboratory is supported by grants from the Association pour la Recherche sur le Cancer (ARC), the Association for International Cancer Research (AICR), the Ligue Nationale Contre le Cancer, and the European Community (EPITRON, CRESCENDO, X-TRA-NET). The Schüle lab is supported by grants from the Deutsche Forschungsgemeinschaft, the Deutsch Krebshilfe and the Hans Messner Stiftung.

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