View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Cell, Vol. 90, 391–397, August 8, 1997, Copyright ©1997 by Cell Press

Nuclear Receptors in Sicily: All in the Famiglia

Meeting Review

Thomas Perlmann* and Ronald M. Evans†
*Ludwig Institute for Cancer Research
S171 77 Stockholm
Sweden
†Howard Hughes Medical Institute
The Salk Institute for Biological Studies
La Jolla, California 92037

The EMBO workshop on the structure and function of nuclear receptors (NRs) was held May 2–5, 1997, in the majestic and ancient city of Erice, Sicily. Erice sits high above a coastal mountaintop offering a commanding view of the Mediterranean and the dramatic Sicilian countryside. The fortification walls of the city stem back to more than 700 B.C. and speak of a strategic site that has been invaded dozens of times throughout the tumultuous European history from the Phoenicians, to the Greeks, Romans, Vandals, Ostrogoths, Moors and Normans, to name a few. During the week it was invaded by the NR field in which the intellectual battles were pitched on a projection screen in the Ettore Majorana chapel.

Chromatin-Protecting Armor

NRs function as molecular machines to transduce a hormonal signal into a transcriptional response. As sequence-specific DNA binding proteins, the action of the receptor primarily occurs at the site of the target gene. Quiescent genes could be viewed as being wrapped in a protective chromatin shield to fend off centroviral advances from an errant RNA polymerase. Dramatic effects of hormones on chromatin structure have long been recognized; whether this was causal to the transcriptional response or merely its consequence was unclear. In addition, how such changes in chromatin structure were directed by receptors was simply unknown. One popular model is that mere binding by receptor was sufficient to initiate a change in chromatin by altering nucleosome position. In contrast, the effort to identify the biochemical basis for transcriptional regulation has led in recent years to the consideration that NR cofactors might serve as active mediators of the regulatory effect. From the view of an insider, it has often seemed that those labs studying chromatin remodeling and those studying NR cofactors appeared to be on opposite sides of the arena taking unrelated approaches to an otherwise common quest. However, in the last year, these two fields, like lost allies, have been reintroduced to each other and are enjoying a new-found synergy. M. Beato (University of Marburg) outlined the general pathway by which the MMTV LTR utilizes a chromatin-based structure to control its transcription. In this case, nucleosomes are phased in a fashion that allows glucocorticoid receptor but not other transcription factors to bind to target DNA. Accordingly, hormone treatment leads to a rapid alteration in chromatin structure and at the same time promotes cooperative binding of other transcription factors. Presumably, it is the packaging of DNA into a repressive chromatin structure that restricts the accessibility of the template to the basal transcription machinery. Thus, as suggested by Beato, and supported by experiments reported by Ö. Wrange (Karolinska Institute) using in vitro–reconstituted nucleosomes, the role of the glucocorticoid receptor is to function, at least in part, to counteract chromatin-mediated repression. As discussed by L. Krause (UCSD) and J. Kadonaga (UCSD), the ''ground'' state or default status of an endogenous target gene would be transcriptionally inactive. The role of a transcription factor would be to initially counteract the chromatin effect leading to a derepressed template. This, in turn, would be followed by a ''true activation'' event equivalent to robust transcriptional initiation. Kadonaga described dissection of this putative multistep process by creating chromatin templates to directly assess the action of NRs in an in vitro–reconstituted system. While previous in vitro transcription studies have been described, this is the first with chromatin-based templates. The effects with the estrogen receptor (ER) are clear and dramatic. Fifteen- to fifty-fold inductions were seen, all in a hormone-dependent fashion. The estrogen receptor can be added either before or after chromatin assembly, and the entire process was shown to be dependent on the known activation domains in the ER. The improved regulation appears to be a consequence of chromatin suppressing basal activity of the promoter thus increasing the fold of induction. Interestingly, estrogen antagonists failed to activate although these complexes can be bound to the template. Continuing in this vein, A. Wolffe (NICHD) described the use of replication-dependent chromatin assembly to identify three regulatory steps in the regulation of transcription by the thyroid hormone receptor (TR). These three steps include: (1) the establishment of a repressive chromatin structure; (2) disruption of the chromatin template; and (3) transcriptional activation. As is apparent, this is similar to the events described by Kadonaga and Beato although with a completely different regulatory system. In this approach, cloned templates are microinjected into the Xenopus oocyte nucleus, which are then assembled in a natural chromatin structure. In this way, Wolffe has been able to address one of the more vexing problems in NR transcription by demonstrating that nonliganded RXR:TR heterodimers can not only bind to chromatin but also may facilitate the formation of a repressive chromatin structure. Conclusions that arise out of this important model system include the demonstration that the nonliganded TR:RXR heterodimers bind to nucleosome DNA, make use of chromatin to repress transcription, control nucleosome position, and can influence both nucleosome modification and, ultimately, disruption. One caveat that remains, however, is the accuracy with which the microinjected DNAs reflect true physiologic templates.

HATs off to Histones

These studies on chromatin-based transcriptional control have come to the frontline in part based on the

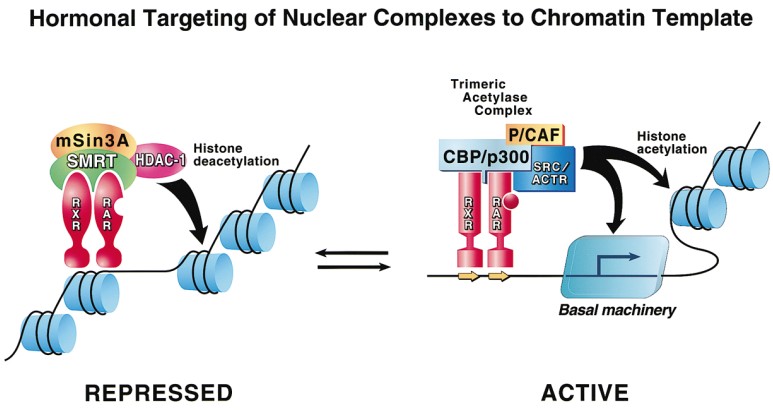

Figure 1. Hormonal Remodeling of Chromatin

Figure illustrates shift in cofactor complexes triggered by hormone binding.

recent discoveries that multiple NR coactivators appear to be directly involved in chromatin remodeling. While many NR-associated proteins have been identified, the CREB binding protein (CBP) and its homolog p300, the CBP associated factors P/CAF, and the steroid receptor cofactor SRC-1 and its homologs (e.g., TIF2) have received considerable attention. R. Evans (HHMI/The Salk Institute) discussed recent studies that suggest the existence of putative coactivator and corepressor complexes. In the first case, a new SRC-1/TIF2 cofactor termed activator of retinoid receptors (ACTR) was described that functions as a novel histone acetyltransferase (HAT). ACTR, in turn, conscripts both CBP and P/CAF and these three proteins form a trimeric activation complex. The link between coactivation and enzymatic activity was established when David Allis first isolated the histone acetyltransferase from tetrahymena (Brownwell et al., 1996), which was shown to be homologous to the yeast coactivator, GCN-5, and subsequently to human P/CAF (Wang et al. 1997). This was strengthened by the discovery that CBP is itself an intrinsic histone acetylase (Ogryzko et al., 1996; Yang et al., 1996). Together, these results suggest that at least one aspect of transcriptional coactivation involves the recruitment by receptors of a multimeric enzymatic complex to the DNA template. Reciprocally, the search for NR corepressors led to the isolation of the silencing mediators, termed SMRT, and N-Cor (Chen and Evans, 1995; Hörlein et al., 1995). These proteins interact with receptors in the absence of hormone and act as transcriptional corepressor for the TR, RAR, and other members of the family. As described by Evans (and recently published in Cell and Nature), SMRT/N-Cor form complexes with the yeast homolog of Sin3 (mSin3A) and RPD-3 (HDAC-1), a histone deacetylase (Alland et al., 1997; Heinzel et al., 1997; Nagy et al., 1997). This work, suggests that transcriptional repression is in part mediated by the action of targeted histone deacetylases recruited to promoters by negative regulatory factors. According to this view, transcriptional regulation by receptors would be controlled by selective recruiting of corepressors or coactivators in response to hormone. This, in turn, would control the balance of enzymatic activity in a target promoter. If the scenario established by this model is correct, then histone tails of nucleosomes may be sites through which a major signal transduction pathway exerts its regulatory effect.

M. Parker (ICRF) described domain mapping studies that led to the identification of a short NR ''signature'' motif sequence present in a variety of coactivators including SRC-1, CBP/p300, TIF-1, TIF-2, and other receptor binding proteins. Peptides, including this 10 amino acid segment, block interactions of SRC-1 with receptors and mutations in the motif block cofactor binding both in vivo and in vitro. However, since this motif is present in thousands of proteins, it alone cannot explain the properties of these cofactors. How coactivators function may not be as simple as their recruitment to the target template. P. Webb (UCSF) described that fusions of coactivators such as SRC-1 with yeast Gal4 DNA-binding domains are insufficient to allow this protein to function at a UAS binding site. However, in the presence of a cotransfected estrogen receptor (ER) ligand binding domain, agonists (but not antagonists) will ''trigger'' Gal4-CBP activation. How such triggering occurs is not clear. Perhaps the HAT activity is potentiated by association of the ER with CBP or the LBD (ligand binding domain) after binding CBP recruits other factors that can cooperate to promote activation.

Is the role of ligand simply to induce a conformation resulting in coactivator recruitment and release of corepressor? Clearly not in the case of steroid receptors whose DNA binding is ligand dependent (Beato et al., 1995). In the absence of ligand, steroid receptors are sequestered in a complex including heat shock protein 90 (hsp90). Ligand is proposed to dissociate the complex and enable receptor dimerization, nuclear translocation, and DNA binding. As most other receptors are apparently not associated with hsp90 and are constitutively localized in the cell nucleus, the common view has been that DNA binding is usually not a ligand-dependent process, a conclusion that is largely supported by in vitro DNA binding experiments. It was therefore somewhat surprising when recent in vivo footprinting indicated that also RAR-RXR heterodimers bound to a specific RARE in the RARβ gene promoter in a ligand-dependent manner (Dey et al., 1994; Chen et al., 1996). In contrast, however, H. Stunnenberg (EMBL, Heidelberg) reported that at least one other receptor, namely the oncogenic variant of TRα (v-erbA), can bind in vivo to a newly identified v-erbA responsive element in the carbonic anhydrase II gene. As mutations in v-erbA prevent ligand binding, this is a clear example of ligand-independent NR DNA binding. Differences may exist between RAR,

RXR, and TR, or alternatively, in vivo footprinting may fail to detect weak protein–DNA interactions in deacetylated and less accessible chromatin. In any event, it is clear that caution is in place before it is concluded that ligands are generally required for NR to bind to DNA.

The End Game

Glucocorticoids are ballistic for T cells functioning as potent immunosuppressives and, when administered to young animals, cause thymic involution by inducing thymocyte apoptosis. They promote cell death, in part, by inhibiting T cell activation by NF-κB signaling and the AP-1 response pathway. In contrast to this inhibition, as reported by B. Thompson (University of Texas, Galveston), they synergize with the protein kinase A pathway although the basis for this synergism is not clear. One clue has come from the study of the expression of the c-*myc* gene. While glucocorticoids and retinoids have previously been shown to lead to rapid downregulation of c-*myc*, Thompson reported strong synergism with forskolin resulting in a near complete loss of c-*myc* mRNA and protein, presumably functioning as another block to T cell stimulation. M. Karin (UCSD) described a potential basis for steroid inhibition of AP-1- and NF-κB-mediated transcription. NF-κB is activated by proinflammatory cytokines, of viral and bacterial infections and, interestingly, can protect cells against apoptosis. Glucocorticoids appear to inhibit NF-κB in part by elevating the expression of IκBα, a specific inhibitor protein reponsible for retaining NF-κB complexes in the cytoplasm. Challenging these results, G. Haegeman (University of Gent, Belgium) suggested that glucocorticoids may act exclusively in the nucleus to inhibit the *trans*-activating capacity of the p65 subunit of NF-κB. Thus, while cross-regulation between these pathways has been well described, the molecular basis for this still remains controversial.

Continuing on the theme of glucorticoid regulation, H. Reichardt (German Cancer Research Center, Heidelberg) described recent studies on the characterization of glucocorticoid receptor–targeted gene mutations in mice. Repression of AP-1 does not appear to require classical GRE DNA binding in AP-1 regulated promoters. This appears to be the case also for the inhibition of NF-κB, as a mutation within the GR DBD dimerization motif (D box) does not prevent repression although it effectively blocks GRE binding (S. Okret, Karolinska Institute). As a consequence, such mutations can distinguish between positive regulation through GREs and negative regulation of AP-1 and NF-κB. Reichardt described a gene replacement approach where a mutation that prevents GRE binding by abolishing dimerization (here referred to as GR^{dim}) was targeted to the *GR* locus in mice. Since GR^{dim} is as effective as wild-type GR in blocking AP-1, the experiment selectively investigates the in vivo contribution of the GRE-dependent functions of GR. Interestingly, in contrast to the previously described early perinatal lethality of $GR^{-/-}$ mice (Cole et al., 1995), the $GR^{dim/dim}$ mice are viable, indicating that the GRE binding function of GR is less essential than had previously been expected. Remarkably, several of the phenotypes previously observed in $GR^{-/-}$ mice were not seen in $GR^{dim/dim}$ animals, including aberrent differentiation of adrenal chromaffin cells, hyperplasia of the adrenal cortex, and abnormal lung development. Although residual GRE binding of GR^{dim} can not be entirely ruled out, these results support the essential role of GR function by mechanisms that may be independent of classical GRE binding and direct transcriptional activation.

Even direct regulation at GRE controlled genes is variably orchestrated by GR as previously demonstrated mainly in K. Yamamoto's lab (UCSF). This group has shown that GR regulation at GREs is dramatically influenced by the DNA binding site itself. Thus, the nature of the GRE influences whether GR will activate or repress gene transcription. The DBD can functionally interact with the N-terminal activation domain to repress its activation function (Lefstin et al., 1994). Yamamoto continued to present structural data on GR DBD complexes with GRE derived by NMR spectroscopy showing that binding to a positive GRE induces a conformational shift in the DNA binding domain primarily involving the D box region. Interestingly, DBDs from mutated versions of GR harboring mutations within the DBD, which previously were shown to relieve the inhibitory function, are in the ''active'' conformation even in the absence of DNA as determined by NMR. The results emphasize the importance of the DNA as a regulatory signal for NRs.

Ten Facts about Fat

Orphan receptors provide a continuing source of new opportunity for discovering new ligands and expanding our understanding of the complex nature of endocrine physiology. Among the most intensively studied over the last several years are the peroxosime proliferator-activated receptors (PPARs), which are believed to control the expression of genes involved in lipid homeostasis. W. Wahli (University of Lausanne) described how the PPARα isoform activates gene transcription in response to a variety of compounds including hypolipidemic drugs, as well as natural fatty acids. In contrast, B. Spiegelman (Dana-Farber Cancer Institute) reviewed evidence suggesting the PPARγ isoform serves as a master factor for adipoctye differentiation. Despite the plethora of PPAR-activators, it is only recently that PPAR ligands have been identified. In a series of recent studies (see Kliewer et al., 1995, and Forman et al., 1995a), the prostaglandin metabolite PGJ2 and the insulin-sensitizing thiozoladinedione (TZD) drugs are described as PPARγ ligands. Why activation of PPARγ in fat leads to insulin sensitization in muscle remains an unresolved but important medical issue. Spiegelman went on to describe that phosphorylation of PPARγ by the MAP-kinase pathway appears to block adipocyte differentiation. Hence, this cross-regulation may represent a very important control mechanism that impacts on adipocyte differentiation as well as insulin sensitivity. The ability of PPARγ to induce terminal adipogenesis raises the possibility that ligand stimulation of this receptor could be beneficial in the treatment of malignancies where the receptor is expressed. Indeed, Spiegelman provided an exciting description of recent experiments demonstrating the expression of PPARγ in liposarcomas, breast

and colon cancer, and the potential of γ activators to dramatically slow cell growth in vitro. Walter Wahli described how a variety of eicosanoids and fatty acids may function as PPARα ligands, which leads to the unusual and important conclusion that some NRs may interact physiologically with a variety of natural ligands. Furthermore, some of these natural ligands consist of common dietary nutrients as opposed to classic hormones. This provides a direct link between nutrition and physiologic homeostasis.

This problem was further addressed by M. Milburn (Glaxo Wellcome, Inc.) who presented one of the three crystallographic talks at the meeting. Milburn specifically described the crystal structure of PPARγ in both the presence and absence of cognate ligands. The ligand used was one of the TZDs, which bind to PPARγ with high affinity. In support of the conclusion that PPARs are designed to interact with a variety of ligands, Milburn reported that the ligand binding pocket of PPARγ is unusually large (1300 Å^3) and only 40% of this space is filled by the TZD ligand in the PPARγ structure. This clearly indicates that TZD binding only represents one out of several possibilities for ligand binding. In contrast, J. Baxter (UCSF) and Dino Moras (IGBMC, France) described the previously published TRα- and RARγ-structures in which ligands are very tightly fitted in their respective ligand binding pockets (Renaud et al., 1995; Wagner et al., 1995). It is therefore noteworthy that RAR can also bind two structurally distinct ligands with high affinity, namely all-*trans* retinoic acid (atRA) and 9-*cis* RA (9cRA). Moras reported new data on the 9cRA-bound RARγ hence allowing comparison of how the two ligands are associated with the RARγ LBD. In contrast to the liganded PPARγ, both atRA and 9cRA are tightly fitted into the ligand binding pocket of RAR with every atom of the ligands in direct contact with one or more proximal residues. Interestingly, although atRA and 9cRA are structurally distinct, when bound, both molecules are distorted by the pocket toward strikingly similar formations. Thus, both receptor and ligand exert reciprocal conformational changes on each other.

The RAR and TR Genetic Battlefield

As a reflection of their important regulatory roles during development, retinoids are also potent regulators of complex differentiation events in vitro. For example, treatment of F9 embryonic carcinoma cells with varying concentrations of RA results in controlled induction of primitive, parietal, or visceral endoderm after exposure to RA. While knock-out experiments are usually performed in whole animals, P. Chambon (IGBMC, Strasbourg) reported on recent gene targeting experiments of RARα, RARγ, and RXRα in F9 cells (Taneja et al., 1996; Chiba et al., 1997). Some of the most striking conclusions can be enumerated. First, despite the functional similarity of RAR isotypes in vitro and in transfected cells, RARα and RARγ mutant cells have distinct effects on F9 cell differentiation. Thus, RARα targeted cells were defective in the formation of parietal endoderm and visceral endoderm differentiation was delayed. On the other hand, RARγ is required for both primitive and parietal but not visceral endoderm. Second, RXRα affects all of the studied processes in F9 cells. This indicates that RARα and RARγ require heterodimerization with RXRα, a conclusion that is further supported from gene targeting experiments in mice (see below). Third, and perhaps most striking, targeting itself can induce functional redundancy. Accordingly, when using synthetic RAR isotype–specific ligands, RARγ was shown to be ineffective in inducing differentiation of F9 cells into parietal endoderm. In contrast, an RARγ-specific ligand was able to induce differentiation in *RARγ*$^{-/-}$ cells demonstrating that RARγ now had acquired an active role in the targeted cells (Taneja et al., 1996). This is an important lesson considering the many phenotypes of knock-out mice with mild or no phenotypes. The possibility of a similar genetic ''adaptation'' in the absence of a particular gene product may have to be considered as a possible explanation for many observations. Finally, rescue experiments have proven very useful in this system and have defined specific roles for distinct functional domains, such as the RARγ N-terminal AF1 domain. Furthermore, a protein kinase A phosphorylation site in RARα was shown to be selectively required for parietal endoderm differentiation while the corresponding site in RARγ is not required. The results emphasize the role of retinoid receptors as integrators of multiple signals and stimuli.

Chambon continued to report on the impressive amount of data that has now been accumulated on phenotypes in retinoid receptor knock-out mice, including compound knock-outs with targeted mutations in more than one retinoid receptor gene. Many of the deficiencies have previously been defined in fetuses from vitamin A–deficient (VAD) dams. While mutations of individual genes have been shown to result in surprisingly mild phenotypes, most VAD phenotypes are observed in various combinations of retinoid receptor knock-outs (Kastner et al., 1995). Furthermore, the results clearly indicate that RAR/RXR heterodimeric complexes are responsible for vitamin A signaling in vivo (Kastner et al., 1997). Specifically, RXRα appears to be the main heterodimerization partner of RARs during embryogenesis. While the function of RXR as an auxiliary partner of many receptors is indicated by the severity of the phenotype observed in *RXR*α$^{-/-}$, *RXR*β$^{-/-}$ double mutant mice, which die around embryonic day 10, it is striking that animals lacking 5 out of 6 RXR alleles, having only a single allele of RXRα, are viable, a quite remarkable demonstration of redundancy (Krezel et al., 1996).

RXR is not only a retinoid receptor which can bind 9-*cis* RA, but can also function as an unliganded auxiliary protein required for high affinity DNA binding of many NRs including RAR (Mangelsdorf et al., 1995). That this is an essential function for RXR is clearly demonstrated by many previous experiments including the strong genetic evidence presented above. Paradoxically, however, the role of RXR as a classical signaling receptor in vivo has remained less clear. The Chambon lab has now addressed this issue in an experiment utilizing a gene replacement strategy in mice. In these animals, RXRα has been manipulated so that a mutation (referred to as RXRα^0) inactivated the ligand-dependent transactivation domain AF2 of RXR while sparing the ability to form heterodimers. These animals are not as severely affected as *RXR*α$^{-/-}$ mice but do display some of the

RXR$\alpha^{-/-}$ phenotypes with variable penetrance. In addition, other abnormalities normally seen in RAR mutant mice were detected, particularly when RXRα^{o} was combined with single targeted RAR isotypes. This led to the conclusion that liganded RXR apparently plays a role in retinoid signaling, not only as a heterodimerization partner, but also as a ligand-activated receptor. Presumably the ligand would be identical to 9cRA or an as-yet-unidentified RXR ligand. Although other explanations for these interesting results can not be entirely ruled out—e.g., that the RXRα^{o} mutation affects the ability of the RAR partner to function optimally, these results are intriguing because they provide the most compelling evidence so far for a role of liganded RXR in vivo.

While it has been presumed that all retinoid receptors have been identified, D. Moore (Massachusetts General Hospital) rattled a provocative sabre at this thesis. The battlefield was defined by the farnesoid X receptor (FXR), which had previously been shown to form a heterodimer with RXR and to respond positively to both juvenile hormone (JH III) and farnesoic acid (Forman et al., 1995b). Moore now provides evidence that a potent synthetic retinoid, tetramethyl-tetrahydro-naphthalenyl-propenyl-benzoic acid (TTNPB) is able to activate the FXR:RXR heterodimer. Since TTNPB is an extremely potent RAR agonist, the thrust of Moore's argument is that perhaps FXR is a new retinoic acid receptor. While direct binding was not established and the activation is approximately 1000-fold less efficient than RAR activation, the results remain provocative as they establish that a common synthetic ligand is able to activate two markedly distinct receptors. Nonetheless, these results raise the issue as to the nature of the endogenous FXR ligand and whether or not FXR responds, as suggested by Moore, to a new vitamin A metabolite and whether its mutation will explain some features of vitamin A deficiency.

In addition to its role in normal physiology, the retinoic acid receptor can be mutated to an oncogenic form as in acute promyelocytic leukemia (APL), which is associated with the t(15;17) translocation by fusion of the *PML* and *RAR* genes. Expression of the resulting *PML/RAR*α fusion gene in transgenic mice results in a disease extremely similar to the human one, demonstrating that formation of the chimeric protein is the molecular basis of this malignancy (Brown et al., 1997). As discussed by H. de The (CNRS), APL is a unique model system in cancer biology as retinoic acid, which binds the PML/RARα fusion, leads to clinical remission by inducing differentiation. Despite the effectiveness of this therapy, patients can become RA-resistant and relapse. The use of Chinese herbal medicine in the treatment of APL led to the recent discovery that arsenic trioxide (As) is a dramatic chemotherapeutic agent. Unlike RA, this new drug appears to target the PML moiety of the fusion protein to promote its degradation, resulting in apoptosis and dramatic clinical remission.

Another of the classical NR ligands is thyroid hormone whose receptors come in two flavors, TRα and TRβ. In addition, several isoforms exist as a result of alternative splicing. In case of TRα, one isoform binds ligand (TRα1) while a second (TRα2) lacks ligand binding ability and has been suggested to downregulate hormone-activated TRα1. Gene targeting that selectively abolished either the TRα1 or TRα2 isoforms has illuminated the in vivo role of each of the isoforms as presented by B. Vennstrom (Karolinska Institute). While the TRα1 mutant mice show decreased heart rate and body temperature, as well as a mild hypothyroidism, TRα2 does not display any dramatic phenotype. Neither TRα1 or TRα2 affects survival of the animals, and the mice are fertile. The phenotype of the TRα1 animals is distinct from previously described TRβ null mutant mice ascribing unique functions for the two *TR* genes (Forrest et al., 1996). Although these data would argue that the TRα2 isoform does not play a significant role in vivo, a new study from J. Samarut (CNRS) provides a somewhat surprising twist to the TR story. Accordingly, a TRα null mutation, which inactivates both TRα1 and TRα2 isoforms, generates a much more severe phenotype than would be expected from looking at the relatively mild phenotypes resulting from mutating either TRα1 or TRα2. TRα null mice are severely growth retarded beginning 1–2 weeks after birth and die between 3–5 weeks. Defects include aberrent ossification and severely reduced thyroid hormone levels. Interestingly, about half of the homozygous animals could be rescued by administration of thyroid hormone. Thus, while TR downregulates hormone levels, as demonstrated previously, these results indicate that TRα plays a key role for thyroid hormone production. Importantly, the results raise new questions regarding the role of the nonliganded TRα2.

Orphan Receptors—Coup d'Etat

Orphan NRs constitute the majority of the members of the NR superfamily. Lacking identified ligands, the functions of these proteins are often rather obscure and the available information is often limited to what can be assayed in transfected cells and in in vitro experiments. Gene targeting of orphan receptors can therefore be unusually rewarding, as the results have the potential of establishing a physiological basis for continuing analysis of these proteins. Two of the earliest orphans to be identified are the two COUPs, TFI and -II (Qiu et al., 1996). These receptors have been shown to efficiently repress activation by several other NRs including RAR, TR, and PPAR. COUP is also one of the most evolutionarily conserved receptors and has a well-characterized counterpart in Drosophila (Sevenup). Homologs have also been cloned from early metazoans including Hydra (V. Laudet, CNRS). Remarkably, Laudet reported a striking conservation also at the functional level as suggested by the observation that the Hydra COUP homolog is expressed in neurons, the major COUP-expressing cell type also in vertebrates.

S. Tsai (Baylor College of Medicine, Texas) presented interesting data showing that COUPs are indeed key regulators during neuronal development. COUP *TFI* gene targeted mice die soon after birth and display deficiencies in the formation of cranial nerves IX and X. These mice also show reduced axonal arborization in the cervical plexus region. Furthermore, the early characterization of the COUP *TFII*$^{-/-}$ mice reveals an early embryonic lethal phenotype involving aberrent vascularization. Interestingly, COUP *TFII* expression in the spinal cord is apparently regulated by another evolutionarily conserved regulatory protein, sonic hedgehog (shh)

(S. Tsai). Data identifying an shh-responsive element in the COUP *TFII* promoter define this gene as one of the first identified target genes for shh signaling in the developing central nervous system, a tissue where shh is a key regulator of cell fate specification.

An important feature of ligands for nucelar receptors is that they are small lipophilic molecules and hence particularly useful as pharmaceuticals. As orphan receptors have yet to find their ligands, their potential as targets for drug development remains to be elucidated. However, the following phenotypes resulting from orphan receptor gene inactivation illustrate some exciting possibilities in disorders as diverse as neurodegenerative disease and atherosclerosis. First, T. Perlmann (Ludwig Institute/Karolinska Institute) reported on a targeted mutation of *Nurr1*, a close relative of *NGFI-B/Nur77* and *Nor1/MINOR*, resulting in early postnatal death and complete agenesis of midbrain dopamine cells (Zetterström et al., 1997). These neurons are the cells that degenerate in patients with Parkinson's disease. Moreover, heterozygous, otherwise healthy mice showed decreased levels of dopamine, indicating that *Nurr1* may play a role also in mature dopamine cells to maintain wild-type levels of the neurotransmitter. In addition to Parkinson's disease, the results have implications for other dopamine-related disorders such as schizophrenia and drug abuse.

Second, nature itself has helped to provide genetic information on yet another orphan receptor, ROR, which recently was shown to be mutated in ''staggerer'' (sg/sg) mice (Hamilton et al., 1996). These animals have been extensively studied because of their deficiencies of cerebellar development. An additional function for ROR has now been defined by the study of sg/sg mice which were found to suffer from increased sucseptibility for atherosclerosis, presumably due to a decreased level of apo A-I and apo A-II plasma levels (B. Staels). Furthermore, transient transfection experiments defined ROR as a direct regulator of the *apo A-I* gene through a specific ROR binding site in the promoter. These results suggest that ROR may be a promising target for drugs modulating lipid metabolism.

Assault on Estrogen

Considering the continual interest in estrogen receptor (ER) function over the years, the word ''renaissance'' would perhaps be an exaggeration when describing the burst of data reported on ER at this meeting. However, an increased interest level is quite evident, not the least stimulated by the recent and quite unexpected identification of a second estrogen binding receptor (ERβ) (Kuiper et al., 1996). This receptor was originally cloned from a rat prostate cDNA library and sites of ERβ expression include the prostate, bone, ovary, bladder, lungs, and brain. Several groups reported new data on this receptor. E. Enmark (Karolinska Institute) reported that the human *ER*β gene has been localized to 14q22–24, a similar position as an early onset Alzheimer's disease locus, an insidious chromosomal location considering the reported protection from Alzheimer's disease by estrogens.

Although the in vivo distribution of ERα and ERβ is partially distinct, the two receptors are colocalized in several tissues. Therefore, as reported in several talks, it is notable that ERα and ERβ form heterodimers that can be activated by hormone (M. Parker, E. Enmark, and V. Giguere, McGill University). Hence, such heterodimers constitute a novel estrogen-dependent mechanism for gene regulation in addition to regulation by ERα and ERβ homodimers. While several groups reported that ERα and ERβ respond in a similar way to a variety of tested compounds, differences do exist. For example, Giguere showed that 4-hydroxy tamoxifene is a partial agonist for ERα but fails to activate ERβ, while another estrogen, Raloxifene, is a more potent antagonist of ERβ than ERα. These and other pharmacological differences between ERα and ERβ may establish a basis for further therapeutic developments of estrogens in the treatment of various disorders including breast cancer and osteoporosis.

Cross-talk between different signaling pathways converging on transcription factors is common, and ER is not an exception in this respect. Thus, ERα can be activated by several nonligand pathways including those triggered by epidermal growth factor and dopamine. Mutations affecting such regulation have been defined including a tyrosine residue preceding helix 12 in the AF2 core (M. Parker and B. Katzenellenbogen, University of Illinois, Urbana). This tyrosine is a target for phoshorylation in vivo. Its regulatory role is illustrated by mutations that transform ERα into a constitutively active receptor also in the absence of estrogens. Continuing on the theme of constitutively active receptors, but diverging from ER to yet another orphan receptor, F. Wiebel (Karolinska Institute) reported on data involving OR-1/UR, a close relative of LXR/RLD-1. Coexpression of GAL4-fused OR-1 and RXR results in constitutively active transcription from a reporter containing GAL4 DNA binding sites, evidently as a result of heterodimerization. Mutations in the RXR helix 12 did not affect this activity while it was completely abolished by the corresponding mutation in OR1. Furthermore, protease protection experiments suggest that the interaction with RXR is responsible for inducing an ''active'' conformation of OR-1. Thus, RXR in effect acts as a ligand for OR1 leading to its activation.

The activity of other types of receptors, expressed in neurons of meeting participants, was eventually silenced at the final hour of an intense meeting. As the weary troops disembarked, Erice was once again returned to its much deserved majesty and tranquility. At the nearby Palermo airport, however, an air traffic controller strike revealed that no individual is truly immune from another's sorrow. However, despite the delays and frustration, the feeling was that much had been accomplished and that the field of nuclear receptors continues to march at a remarkable pace.

References

Alland, L., Muhle, R., Hou, H., Jr., Potes, J., Chin, L., Schreiber-Agus, N., and DePinho, R.A. (1997). Role for N-Cor and histone deacetylase in Sin3-mediated transcriptional repression. Nature *387*, 49–55.

Beato, M., Herrlich, P., and Schütz, G. (1995). Steroid hormone receptors: many actors in search of a plot. Cell *83*, 851–857.

Brown, D., Kogan, S., Lagasse, E., Weissman, I., Alcalay, M., Pelicci, P.G., Atwater, S. and Bishop, J.M. (1997). A PMLRARα transgene initiates murine cute promyelocytic leukemia. Proc. Natl. Acad. Sci. USA *94*, 2551–2556.

Brownell, J.E., Zhou, J., Ranalli, T., Kobayash, R., Edmondson, D.G., Roth, S.Y., Allis, C.D. (1996). Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell *84*, 843–851.

Chen, J.Y., Clifford, J., Zusi, C., Starrett, J., Tortolani, D., Ostrowski, J., Reczek, P.R., Chambon, P., and Gronemeyer, H. (1996). Two distinct actions of retinoid-receptor ligands. Nature *382*, 819–822.

Chen, J.D., and Evans, R.M. (1995). A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature *377*, 454–457.

Chiba, H., Clifford, J., Metzger, D., and Chambon, P. (1997). Distinct retinoid X receptor retinoic acid receptor heterodimers are differentially involved in the control of expression of retinoic target genes in F9 embryonal carcinoma cells. Mol. Cell. Biol. *17*, 3013–3020.

Cole, T.J., Blendy, J.A., Monaghan, A.P., Krieglstein, K., Schmid, W., Aguzzi, A., Fantuzzi, G., Hummler, E., Unsicker, K., and Schutz, G. (1995). Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. Genes Dev. *9*, 1608–1621.

Dey, A., Minucci, S., and Ozato, K. (1994). Ligand-dependent occupancy of the retinoic acid receptor beta 2 promoter in vivo. Mol. Cell. Biol. *14*, 8191–8201.

Forman, B.M., Tontonoz, P., Chen, J., Brun, R.P., Speigelman, B.M., and Evans, R.M. (1995a) 15-deoxy-D12,14-Prostoglandin J2 is a ligand for the adipocyte determination factor PPARγ. Cell *83*, 803–812.

Forman, B.M., Goode, E., Chen, J., Oro, A.E., Bradley, D.J., Perlmann, T., Noonan, D.J., Burka, L.T., McMorris, T., Lamph, W.W., Evans, R.M., and Weinberger, C. (1995b). Identification of a nuclear receptor that is activated by farnesol metabolites. Cell *81*, 687–693.

Forrest, D., Hanebuth, E., Smeyne, R.J., Everds, N., Stewart, C.L., Wehner, J.M., and Curran, T. (1996). Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: evidence for tissue-specific modulation of receptor function. EMBO J. *15*, 3006–3015.

Hamilton, B.A., Frankel, W.N., Kerrebrock, A.W., Hawkins, T.L., Fitz-Hugh, W., Kusumi, K., Russell, L.B., Mueller, K.L., van Berkel, V., Birren, B.W., Kruglyak, L., and Lander, E.S. (1996). Disruption of the nuclear hormone receptor ROR alpha in staggerer mice. Nature *379*, 736–739.

Heinzel, T., Lavinsky, R.M., Mullen, T.-M., Söderström, M., Laherty, C.D., Torchia, J., Yang, W.-M., Brard, G., Ngo, S.D., Davie, J.R., et al. (1997). A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature *387*, 43–48.

Hörlein, A.J., Näär, A.M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Söderström, M., Glass, C.K., and Rosenfeld, M.G. (1995). Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature *377*, 397–404.

Kastner, P., Mark, M., and Chambon, P. (1995). Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? Cell *83*, 859–869.

Kastner, P., Mark, M., Ghyselinck, N., Krezel, W., Dupe, V., Grondona, J.M., and Chambon, P. (1997). Genetic evidence that the retinoic signal is transduced by heterodimeric RXR/RAR functional units during mouse development. Development *124*, 313–326.

Kliewer, S.A., Lenhard, J.M., Willson, T.M., Patel, I., Morris, D.C., and Lehmann, J.M. (1995). A prostoglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. Cell *83*, 813–819.

Krezel, W., Dupe, V., Mark, M., Dierich, A., Kastner, P., and Chambon, P. (1996). RXR-gamma null mice are apparently normal and compound RXR-alpha(+/−)/RXR-beta(−/−)/RXR-gamma(−/−) mutant mice are viable. Proc. Natl. Acad. Sci. USA *93*, 9010–9014.

Kuiper, G., Enmark, E., Pelto-Huikko, M., Nilsson, S., and Gustafsson, J.-Å. (1996). Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc. Natl. Acad. Sci. USA *93*, 5925–5930.

Lefstin, J.A., et al. (1994). Influence of a steroid receptor DNA-binding domain on transcriptional regulatory functions. Genes Dev. *8*, 2842–2856.

Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R.M. (1995). The nuclear receptor superfamily: the second decade. Cell *83*, 835–839.

Nagy, L., Kao, H.-Y., Chakravarti, D., Lin, R., Hassig, C.A., Ayer, D.E., Schreiber, S.L., and Evans, R.M. (1997). Nuclear receptor repression mediated by a complex containing SMRT, Sin3, and histone deacetylase. Cell *89*, 373–380.

Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H., Nakatani, Y. (1996). The transcriptional coactivators p300 and CBP are histone acetyltranferases. (1996). Cell *87*, 953–959.

Qiu, Y., et al. (1996). Chicken ovalbumin upstream promoter-transcription factors and their regulation. J. Ster. Biochem. Mol. Biol. *56*, 81–85.

Renaud, J.-P., Rochel, N., Ruff, M., Vivat, V., Chambon, P., Gronemeyer, H., and Moras, D. (1995). Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid. Nature *378*, 681–689.

Taneja, R., Roy, B., Plassat, J.-L., Zusi, C.F., Ostrowski, J., Reczek, P.R., and Chambon, P. (1996). Cell-type and promoter-context dependent retinoic acid receptor (RAR) redundancies for RARβ2 and Hoxa-1 activation in F9 and P19 cells can be artefactually generated by gene knockouts. Proc. Natl. Acad. Sci. USA *93*, 6197–6202.

Wagner, R.L., Apriletti, J.W., McGrath, M.E., West, B.L., Baxter, J.D., and Fletterick, R.J. (1995). A structural role for hormone in the thyroid hormone receptor. Nature *378*, 690–697.

Wang, L., Mizzen, C., Ying, C., Brownell, J., Candau, R., Barley, N., Allis, C.D., and Berger, S.L. (1997). Histone acetyltransferase activity is conserved between yeast and human GCN5, and is required for complementation of growth and transcriptional activation. Mol. Cell. Biol. *17*, 519–527.

Yang, X.-J., Ogryzko, V.V., Nishikawa, J., Howard, B.H., and Nakatani, Y. (1996). A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature *382*, 319–324.

Zetterström, R.H., Solomin, L., Jansson, L., Hoffer, B.J., Olson, L., and Perlmann, T. (1997). Dopamine neuron agenesis in Nurr1-deficient mice. Science *276*, 248–250.