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EMBO WORKSHOP REPORT

Steroid and nuclear receptors Villefranche-sur-Mer, France, May 25–27, 1999

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Introduction

Ever since the discovery of puff induction by ecdysone, gene regulation by steroid hormones has occupied a position at the frontier of progress in eukaryotic gene expression. Cloning of the hormone receptors revealed that they belong to a large family of nuclear receptors, including those for vitamin D_3, thyroid hormones and retinoids. Because of their inducible nature, these receptors are now among the best characterized transcription factors in eukaryotes. Knowledge of the structure and the molecular mechanism of regulation by nuclear receptors of various cellular processes is progressing very rapidly. All nuclear receptors share a short DNA-binding region encompassing two zinc fingers and a hydrophobic ligand-binding domain, the three-dimensional structures of which have been elucidated. In the last decade, however, the number of nuclear receptors identified has expanded rapidly by the homology cloning of family members, many of which are so-called orphan receptors. These 'receptors in search of a ligand' have attracted considerable interest since they could help to uncover new endocrine regulatory systems. At the end of May, a group of some 200 scientists met in an old fortress at Villefranche-sur-Mer near Nice to discuss novel findings on the structure and function of this large family of transcriptional regulators. The meeting lasted for three full days and covered a vast range of topics, from chromatin and transcription to gene targeting and development, including many aspects of cross-signalling, as well as the identification of new metabolic and endocrine regulatory pathways.

In this report, we will summarize recent findings reported in the different areas with the intention of conveying a feeling for the present state of this rapidly evolving field. Owing to space limitations, we have had to select only a few of the many exciting data presented at the meeting. We apologize to those colleagues whose results are not discussed appropriately, in particular to those who described the advance in understanding the three-dimensional structure of the receptors and their interaction with other factors. This structural work has revealed the basis for agonistic or antagonistic action of receptor ligands, namely the different changes they induce in the C-terminal region of the receptors. Agonistic ligands generate a surface with high affinity for coactivators, whereas antagonistic ligand do not (Darimont *et al*., 1998; Feng *et al*., 1998; Nolte *et al*., 1998; Shiau *et al*., 1998). The difficulties in discussing these issues without detailed graphics preclude the inclusion of this point in the review.

Coactivators

Before entering the description of the presentations, just a warning for readers who are not familiar with the field: please, do not be frightened by the large number of abbreviations and acronyms. They were unavoidable in the first two sections of the report but become progressively less of a nuisance as we progress to the discussion of the physiological aspects of hormone action.

The meeting started with a talk on transcriptional control by Bob Roeder (Rockefeller University, New York), and focused on the function of transcriptional coactivators. Apart from the USA (upstream stimulatory activity) fraction and the derived PCs (positive coactivators, PC2, PC4 and P52), he distinguished two main classes of coactivators: those interacting with sequence-specific transcription factors and those interacting with the general transcriptional machinery on core promoter elements. To this latter class belong the TAFs [TATA box binding protein (TBP) associated factors], which are found in at least two different multiprotein complexes: the TFIID complex and the SAGA complex (Struhl and Moqtaderi, 1998). In the TFIID complex, TAFs are associated with TBP, whereas in the SAGA complex they interact with several other polypeptides involved in chromatin remodelling, including histone acetyltransferases (HATs) such as P/CAF and GCN5. A similar redundancy or promiscuity is found with coactivators, which interact directly with nuclear receptors. Apart from P/CAF, other coactivators including SRC1 and CBP/p300 exhibit HAT activity. Roeder described in detail a large multicomponent TRAP (thyroid hormone receptor-associated proteins) complex, which he isolated via its stable intracellular association with ligand-activated thyroid hormone receptor (Fondell *et al*., 1996; Ito *et al*., 1999). This complex is virtually identical to another coactivator complex that he identified by independent affinity purification methods and functional assays (with VP16 and p53), and named SMCC (Srb/Mediator coactivator complex) (Gu *et al*., 1999), and to another complex, DRIP (vitamin D receptor interacting proteins), described in the subsequent talk by Len Freedman (Memorial Sloan-Kettering Cancer Center, New York) (Rachez *et al*., 1998, 1999). All these complexes share a small subset of components of the Mediator (Kim *et al*., 1994) and Srb complexes (Thompson *et al*., 1993),

Table I. Subunit composition of Mediator-related complexes

yHoloenzyme[a] Thompson, 1993	YMediator Kim, 1994	MMediator Jiang, 1998	HMediator Boyer, 1999	TRAP Fondell, 1996	SMCC Gu, 1999	DRIP Rachez, 1998	NAT Sun, 1998	ARC Naar, 1999	CRSP Ryu, 1999
Srb8 (p166)			N.D.			p250		p250	
Srb9 (p160)			N.D.	p240	p240	p240	p230	p240	
	Nut1 (p130)		N.D.	230	230				
Gal11(p120)	Gal11(p130)	p160a,b	N.D.	p220	p220	p205		p205	200
Rgr1(p123)	Rgr1(p115)	Rgr1(p110)	N.D.	Rgr1(p170)	Rgr1(p170)		Rgr1(p150)		
Sin4 (p111)	Sin4 (p110)		p150[b]	p150	p150	p150		p150	150
			hSur2			Sur2(p130)	Sur2(p140)	Sur2(p130)	Sur2(p130)
								p105[c]	
			N.D.	p100	p100	p100		p100	p100
Srb4 (p78)	Srb4(p98)	p96	N.D.	p97	p97	p97			
		p96	N.D.	p95	p95		p95		
			N.D.	p93	p93	p92	p90	p92	
		p78	N.D.	p80	p80	p77		p77	p77
Med1 (p64)	Med1 (p70)		N.D.			p70-1	p70	p70	p70
			N.D.			p70-2			
Srb10[d] (p63)	Srb10 (p63)	p55	Srb10	Srb10 (p53)	Srb10		Srb10 (p56)		
Med2 (p48)	Med2 (p62)		N.D.				p45		
Pgd1 (p48)	Pgd1 (p48)		N.D.				p37		
Med4 (p32)	Med4 (p38)						p36	p42	
Srb11[e] (p38)	Srb11 (p38)	p34	Srb11	36		36	Srb11 (p31)	p36	p34
Med7 (p26)	Med7 (p31)	Med7 (p36)	hMed7			Med7 (p34)		Med7 (p34)	Med7 (p33)
Med6(p33)	Med6 (p36)	Med6 (p32)	hMed6	Med6(p33)	Med6(p33)	Med6(p33)	Med6(p33)	Med6(p33)	
Srb5 (p34)	Srb5 (p35)								
			N.D.	Srb11 (p32)	Srb11 (p32)		p32	p32	
Med8 (p25)	Med8 (p30)		N.D.						
Rox3 (p25)	Rox3 (p30)		N.D.						
		p28a,b	N.D.				p23		
Srb2 (p23)	Srb2 (p27)		N.D.				p22		
			N.D.	Soh1 (p25)	Soh1 (p25)		p21		
	Med9 (p20)		N.D.						
	Nut2[f] (p19)		N.D.	Nut2 (p18)	Nut2 (p18)		Nut2 (p14)		
Srb7 (p16)	Srb7 (p16)	Srb7 (p21)	N.D.	Srb7 (p20)	Srb7 (p20)		Srb7 (p17)		
	Med11 (p15)		N.D.						
Srb6 (p14)	Srb6 (p15)								

Homologous subunits are indicated by the same shade of grey background.
[a]Only Mediator-related subunits of the yeast RNA polymerase II holoenzyme are listed.
[b]Interacts with AF2 of nuclear receptors.
[c]Interacts with AF1 of nuclear receptors.
[d]Srb10 = Cdk8.
[e]Srb11 = cyclin C.
[f]Nut2 = Med10.

both originally identified in yeast (Table I). Although the yeast Srb complex complements CTD mutations of RNA polymerase II, SMCC works *in vitro* as a coactivator with RNA polymerases lacking the CTD repeat.

The TRAP/DRIP/SMCC complexes are also related to the CRSP (Ryu *et al*., 1999) and the ARC (Naar *et al*., 1999) complexes, described by Bob Tjian as coactivators of other transcription factors such as Sp1 and SREBP, as well as to the histone deacetylase-containing NAT complex (Sun *et al*., 1998), to the mouse mediator (Jiang *et al*., 1998) and to the recently identified human 30 polypeptide mediator complex containing Sur2/DRIP130 (Boyer *et al*., 1999). The general cofactor PC2 (Kretzschmar *et al*., 1994) also contains a subset of TRAP polypeptides and is probably comparable to CRSP. Thus, some of the confusion generated by the multiplicity of coactivator complexes is reduced by the observation that all these complexes share a common core that is very resistant to high salt and urea concentrations (Roeder), but differ with respect to various subsets of other components. An interesting observation is that many of the coactivator complexes do not function as activators or even function as repressors when tested with recombinant general transcription factors (Sun *et al*., 1998; Boyer *et al*., 1999), suggesting that unidentified components present in factors 'purified' from natural sources are required for the coactivator function.

A comparison of some of the identified polypeptides in these various coactivator complexes is presented in Table I. The components interacting with some of the sequence-specific transcription factors have been identified. For instance, TRAP220/DRIP205 interacts in a ligand-dependent manner with TRα, VDR (vitamin D receptor), retinoic acid receptor α (RARα), retinoid X receptor (RXRα), peroxisome proliferator activated receptor α and γ (PPARα, PPARγ) and weakly with estrogen receptor α (ERα) (Yuan *et al*., 1998), whereas Sur-2/DRIP130 interacts with adenovirus E1A (Boyer *et al*., 1999). TRAP80/DRIP77 also interacts with p53 and VP16, explaining how TRAP/SMCC/DRIP complexes can activate both p53- and VP16-mediated transcription. Moreover, two activators (TRα and VP16) can bind simultaneously to a single coactivator complex, thus suggesting a mechanism for activator synergy (Roeder).

As for the physiological role of these coactivator complexes, there is still no convincing experimental evidence. Roeder (Yuan *et al*., 1998) and Freedman have identified a region of TRAP220/DRIP205 that contains two conven-

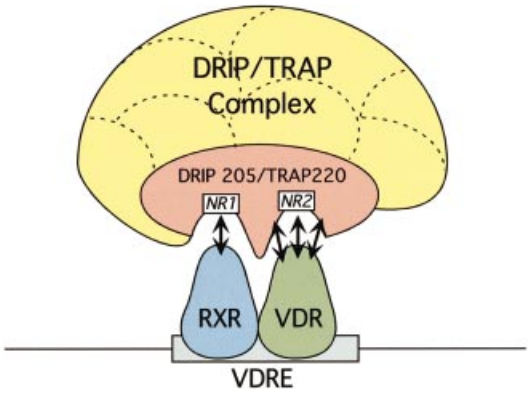

Fig. 1. Interaction of DRIP205/TRAP220 with the two partners of the heterodimer RXR–VDR bound to a vitamin D-responsive element.

tional NR boxes, NR1 and NR2, which share the LxxLL motifs but differ in the flanking amino acid sequence. This region interacts with TRα and VDR in a ligand-dependent manner. A polypeptide containing NR2 interacts more strongly with TRα and VDR than does the region containing NR1, and inhibits TRα and VDR function both *in vitro* (Yuan *et al*., 1998) and in transfection assays. In contrast, a polypeptide containing NR1 has a higher affinity than NR2 for the RXR partner in the heterodimer (Rachez *et al*., 1999). It is therefore conceivable that a single coactivator subunit interacts simultaneously with the two members of the VDR–RXR heterodimer and could mediate their synergism (Figure 1). Along similar lines, Freedman also reported that the N-terminal transactivation function (AF1) of the glucocorticoid receptor (GR) interacts with DRIP150, whereas the C-terminal transactivation function (AF2) interacts with DRIP205, offering a possible explanation for the transcriptional synergism between these two transactivation domains of GR in response to hormone (Hittelman *et al*., 1999). In contrast to a previous report with partially purified DRIP complex, neither a more purified DRIP complex (Rachez *et al*., 1999) nor the TRAP complex (Fondell *et al*., 1999) exhibits HAT activity. While HAT-containing coactivators such as CBP and SRC-1 bind to VDR from nuclear extracts, they do not co-purify with the transcriptionally active DRIP complex but rather sediment in separate fractions on a glycerol gradient, suggesting that these proteins exist as distinct complexes (Rachez *et al*., 1999). Along with observations that the TRAP and SMCC complexes mediate activator functions on DNA templates, these results support an earlier model (Yuan *et al*., 1998; Fondell *et al*., 1999) suggesting that these complexes act in steps of the transactivation process subsequent to chromatin remodelling (Freedman, 1999).

The initial step in promoter activation was the subject of a presentation on the regulation of the mouse mammary tumour virus (MMTV) promoter by glucocorticoids and progestins. Miguel Beato (IMT, University of Marburg, Germany) summarized the evidence demonstrating that the functional synergism between hormone receptors and nuclear factor 1 (NF1), which is essential for hormonal induction *in vivo*, depends on the organization of the promoter into positioned nucleosomes. Using minichromosomes assembled in extracts from *Drosophila* embryos, the synergism can be reproduced *in vitro* and is dependent on pre-incubation of the minichromosomes with purified receptor in the presence of the extract and ATP (Di Croce *et al*., 1999). Intriguingly, the synergism can also be detected with truncated NF1 containing just the DNA-binding domain and lacking any of the known transactivation domains. DNase I footprinting experiments show that the receptors bind synergistically with NF1 or its DNA-binding domain to the minichromosomes, while they compete for the naked MMTV promoter (Di Croce *et al*., 1999). Neither SWI/SNF nor CHRAC appears to play a role in this context, while the receptor recruits nucleosome remodelling factor (NURF) to the MMTV promoter in chromatin. NURF induces an ATP-dependent remodelling of chromatin resulting in an unstable or transient opening of the promoter nucleosome. NF1 plays only a structural role, acting as a wedge to stabilize the open conformation of chromatin, thus facilitating full occupancy of the hormone responsive elements (HREs). It is the full loading with hormone receptors that leads to optimal induction without a direct participation of transactivation functions of NF1. In subsequent steps other coactivators, such as TRAP/DRIP or HAT-containing complexes, may be required for efficient transcription, but there does not seem to be an involvement of histone acetylation in the early steps of chromatin opening and loading with sequence-specific transcription factors.

Another potential coactivator is LEM6 (ligand-enhancing modulator 6), a protein identified by Natasha Kralli and co-workers (Biozentrum, Basel), which interacts with the ligand-binding domain of steroid receptors by means of an LXXLL-containing domain. It appears that LEM6 is the human homologue of the mouse PPARγ coactivator PGC-1 (Puigserver *et al*., 1998). Like PGC-1, LEM6 mRNA is expressed in a subset of tissues, suggesting that such coactivators may contribute to cell-type specificity. PGC-1 has been shown to interact with the DNA-binding and hinge domain of PPARγ, but Kralli reported an agonist-dependent interaction with the ligand-binding domain of steroid receptors. Transcriptional coactivation depends on N-terminal residues, which encompass the LXXLL motif and an activation domain, while dimerization of the protein and subnuclear localization depend on C-terminal residues, which also encompass an RNA-binding motif. The precise role of LEM6/PGC-1 has yet to be elucidated but on the basis of co-transfection experiments it is certainly one of the most potent coactivators described to date. Given its RNA-binding potential, it could be part of the complex containing the recently discovered RNA coactivator SRA (Lanz *et al*., 1999), which also interacts with the steroid receptor coactivator-1 (SRC-1) (Oñate *et al*., 1995).

Many laboratories have been searching for coactivators for AF1, an activation domain found in the N-terminus of steroid hormone receptors. One candidate is p68, an RNA helicase described by Shigeaki Kato (University of Tokyo) that appears to be specific for ERα (Endoh *et al*., 1999). Its *in vitro* interaction with the N-terminal domain of the receptor was potentiated when the domain was phosphorylated by mitogen activated protein kinase (MAPK), implying that it may play a role in cross-coupling between oestrogen and EGF/IGF1 signalling pathways, which have been shown to phosphorylate AF1 and enhance its activity (Kato *et al*., 1995).

Two additional cofactors were described that appear to

promote an interaction between the N- and C-terminal domains of the androgen receptor (AR). Jorma Palvimo (University of Helsinki) reported that a protein, ARIP3, interacts with AR in a ligand-dependent fashion and promotes its ability to stimulate transcription from a subset of promoters, at least at low concentrations. Roland Schule (Clinic for Tumor Biology, Freiburg) reported that Flirt1 (ARC-1), a protein with four copies of a Lim motif and an autonomous activation function, also potentiates the transcriptional activity of AR in a ligand-dependent manner. During embryonic development, Flirt1 expression is initially restricted to the developing heart and smooth muscle cells. In the adult, Flirt1 is expressed exclusively in the heart but its role in mediating androgen action is unclear. Along the same lines, Henk Stunnenberg (University of Nijmegen) reported that RORβ sustains multiple rounds of transcription initiation *in vitro*, in nuclear extracts from a neuronal cell line (Neuro 2A), yet it cannot ensure re-initiation of transcription in HeLa cell nuclear extracts. Together, these data suggest the existence of cell-type-enriched coactivators and initiation factors for specific receptors.

Corepressors

The corepressors N-CoR/SMRT appear to suppress the activity of thyroid hormone and RARs in the absence of ligand while they repress steroid hormone receptors in the presence of antagonists. Cristiana Juge-Aubry (Geneva University Hospital) reported that they are also implicated in the activation of PPARα by MAP-kinases. Insulin treatment results in phosphorylation of serine residues at positions 12 and 21, which increases the transcriptional activity of the receptor. They hypothesized that phosphorylation leads to either enhanced recruitment of coactivators or displacement of corepressors. Evidence was presented, based on cotransfection experiments with chimeric and mutant receptors, that corepressors are likely to bind and silence PPARα, and that phosphorylation might cause its release and lead to transcriptional activation (Juge-Aubry *et al*., 1999).

Aria Baniahmad (University of Giessen) described a novel corepressor, Alien, first identified in *Drosophila*, that is distinct from N-CoR/SMRT and has a different expression profile (Dressel *et al*., 1999). In the absence of hormone, the protein potentiates silencing by TR, but not by RAR, and harbours an autonomous silencing domain. It may also regulate the activity of a number of nuclear receptors in *Drosophila* since it also interacts with the ecdysone receptor and seven-up. Evidence is accumulating to suggest that RIP140, a protein that interacts with activated receptors, also functions as a repressor (Cavailles *et al*., 1995). Sam Okret (Karolinska Insitute, Huddinge) reported that RIP140 antagonizes not only the ability of the GR to stimulate transcription from reporter genes containing either glucocorticoid responsive elements (GREs) or AP1 sites, but also the repressive effect of GR on negative GREs or on NF-κB activation of transcription (Subramaniam *et al*., 1999). When Vincent Cavailles originally cloned the gene, he found that overexpression of RIP140 suppressed the activity of ERα (Cavailles *et al*., 1995). Whether the failure to inhibit oestrogen signalling in female RIP140$^{-/-}$ mice is the cause of their observed infertility (Malcolm Parker, ICRF, London) has yet to be established.

Cross-talk

Cross-coupling between nuclear receptors and growth factors is emerging as a common feature of many physiological responses to hormones but the molecular mechanisms involved are often unclear. Ferdinando Auricchio (University of Naples) described interesting results on the interaction between ERα and the tyrosine kinase protooncogene Src, which leads to a rapid activation of the mitogenic MAPK cascade in breast cancer cells (Migliaccio *et al*., 1996). The same results can be obtained in COS cells transfected with either the wild type or transcriptionally inactive mutants of ERα (Castoria *et al*., 1999). Unexpectedly, progestins also activate the mitogenic cascade in a process that depends on binding to the progesterone receptor PR_B, but that also requires ERα and can be blocked by anti-oestrogens (Migliaccio *et al*., 1998). Immunoprecipitation and pull-down experiments suggest a direct interaction between subpopulations of ERα and PR_B, which pre-exists in a complex in the absence of ligand. Interfering with the activation of the MAPK cascade in various ways completely inhibited the proliferative response of the cells to oestrogens or progestins (Castoria *et al*., 1999). Shigeaki Kato reported that the ability of transforming growth factor β to stimulate transcription by the vitamin D receptor was mediated by Smad3 acting as a coactivator (Yanagisawa *et al*., 1999). However, they were unable to detect a direct interaction between the VDR and Smad3, and instead proposed the existence of a quaternary complex stabilized by a CBP/SRC1 complex forming contacts with Smad3 and VDR, respectively.

Interaction of nuclear receptors with other transcription factors is also a mechanism by which signal transduction is modified. The classical example is the interaction of steroid hormone receptors with members of the AP1 or the NFκB complexes. M.Sjöberg (Karolinska Institute, Stockholm) demonstrated cross-talk between STATs and ERα or ERβ, similarly to the previously reported cross-talk between GR and STAT5. Oestrogens potentiate via their receptors the transcriptional activities of STAT5 on the β-casein promoter. In contrast, STAT5 has no effect on the activity of ER on an ERE-responsive reporter gene. Interestingly, ERβ was more potent in stimulating STAT5 activity than ERα. This is in contrast to the strengths of these receptors on an ERE-controlled reporter gene. Mapping of ER domains important for the cross-talk showed that neither the ligand binding domain (LBD) nor the N-terminal domain was necessary. These data suggest that the well-known oestrogen and prolactin stimulatory effect on mammary gland growth and development may converge on the prolactin-induced STAT5 signalling pathway. In a presentation by A.Aranda (IIB, Madrid), cross-talk involving a novel group of transcription factors was presented. c-ets-1 was shown to cause a ligand-independent activation of VDR. This involved the DNA binding domain (DBD) and N-terminal domain of VDR but not the AF-2 or LBD. A physical interaction between VDR and c-ets was shown in a GST pull-down assay. The interaction between VDR and c-ets-1 may lead to a conformational

change in VDR as an increased resistance to protease digestion was observed. The pituitary-specific transcription factor GHF-1/Pit-1 was also shown to interact with VDR (Tolón *et al*., 1999). These interactions may play an important role in the vitamin D stimulation of the rat prolactin gene.

P.G.Pelicci (European Institute of Oncology, Milan) presented data on the possible correlation between chromatin modification by acetylation and the molecular pathogenesis of acute promyelocytic leukemia (APL). A fusion protein between RARα and PML (or PLZF) interacts with the N-CoR–histone deacetylase (HD) repressor complex (Grignani *et al*., 1998). The presence of these transforming proteins in the multimeric complex inhibits differentiation of haematopoietic precursor cells. A coiled-coil motif located in the N-terminal portion of the RARα–PML is responsible for multimerization as well as for the higher affinity for the N-CoR–HD complex. Dissociation of the corepressor complex from RARα–PML by retinoic acid administration induces differentiation of leukaemic blasts as well as disease remission. One of the potential target genes of retinoid activation is p21, which is upregulated during cell differentiation and contains in its promoter an RAR binding site.

A different aspect of the RAR–PML protein has been elucidated by Anne Dejean (Institut Pasteur, Paris). APL cells have lost PML nuclear bodies, which are recovered after retinoic acid or arsenic trioxide (As_2O_3) treatment. As_2O_3 increases covalent linkage of the ubiquitin-related SUMO1 (small ubiquitin-like modifier) to both PML and RAR–PML. This process seems to be related to protein targeting rather than to protein degradation. Other human diseases, including neurodegenerative disorders and viral infections, present a similar disruption of nuclear bodies. Dejean and co-workers identified the immediate early viral proteins IE1 and ICPO as being responsible for the disruption of nuclear bodies after herpes simplex virus and cytomegalovirus infection, respectively, strengthening the role for SUMO1 modification in the maintenance of the structural integrity of nuclear bodies.

The ability of retinoic acid to induce differention of promelocytes carrying the t(15;17) chromosomal translocation in APL patients and the *in vitro* NB4 cell model is well established. Hinrich Gronemeyer (IGBMC, Strasbourg) reported work done in collaboration with the laboratory of Michel Lanotte (INSERM U496, Paris) demonstrating that the synergism between RXR-selective ligands (‘rexinoids’) and protein kinase A agonists was capable of inducing the maturation of NB4 promyelocytes *in vitro*. The ability of RXR/PKA agonists to stimulate maturation suggests that a distinct RXR-dependent maturation pathway exists that involves cross-coupling with protein kinase A signalling. Since the maturation of retinoic acid-resistant NB4 cells was also promoted by rexinoid/PKA agonists, it is conceivable that patients with retinoic acid-resistant PML would respond to a combination therapy of this type.

Combinatorial regulation

Keith Yamamoto (University of California, San Francisco) presented a conceptual framework for rationalizing how combinatorial regulation involving dozens of polypeptides in several multiprotein complexes can solve the apparent dilemma between the requirement for diversity of possibilities and precision of the individual response. He suggested that diversity is achieved by flexibility in the construction of the multiprotein complexes, while precision results from combinatorial selection of a particular array among the multiple possibilities (Yamamoto *et al*., 1998).

The determinants of diversity and specificity were illustrated by the differential interaction of the ligand-binding domains of TRβ and GR with the coactivator GRIP1. GRIP1 contains three so-called NR boxes encompassing the core motif LXXLL flanked by different amino acid sequences. Whereas TRβ interacts preferentially with NR2, GR prefers NR3 for binding. This reflects different combinations of recognition determinants used by each receptor: each requires the leucine residues of the LXXLL motif, but whereas TRβ specificity is set by the amino acids immediately adjacent to the motif, GR specificity is determined by the motif residues themselves, as well as by an auxiliary site downstream of the NR box region (Darimont *et al*., 1998; Hong *et al*., 1999). GRIP1 also interacts with the integrator CBP (CREB binding protein) via an independent auxiliary site. One can envisage how a single coactivator can choose among various possible partners, depending on the cellular context. A comparison of the interfaces between several transactivators and their respective coactivators (p53–MDM2, VP16–hTAF30, CREB–CBP) reveals in all cases an amphipathic α helix interacting with a hydrophobic groove, suggesting the existence of variations on a common theme.

The effect of the ligand on hormone receptor function is strongly context dependent and offers a separate example of flexibility. Tamoxifen, for instance, is an antagonist on ERα in breast cancer cells but an agonist in endometrial cells, whereas raloxifen is an antagonist in both cell types but an agonist in bone and liver cells. This behaviour has been explained classically by the different relevance in various cells of the ligand-dependent activation function AF2 versus the ligand-independent activation function AF1. Intriguingly, in *Saccharomyces cerevisiae* all ligands are agonistic, suggesting the absence of a factor that couples the activity of AF2 to the other transactivation functions of the receptors. Yamamoto mentioned that there is preliminary genetic evidence for the existence of such coupling factors in mammalian cells. Thus, the positive or negative nature of the response to a ligand is determined by the molecular environment in the target cell.

A similar argument can be developed for the influence of simple or composite HREs on the behaviour of various receptors in the absence or in the presence of ligand (Lefstin and Yamamoto, 1998). The different HREs instruct the receptor and associated factors to adopt alternative conformations, leading to the assembly of different repressing or activating complexes. As a conclusion of these considerations, Yamamoto proposed the existence of a three-dimensional regulatory space in which the response of a gene to a hormone is specified and determined by the values of the three coordinates: cellular context, physiological context and gene (response element) context.

New functions

In the dynamic field of nuclear and steroid hormone receptors, new functions for recently discovered receptors

are continuously being unveiled. It is well established that PPARα plays an important role in energy metabolism (Desvergne and Wahli, 1999). However, the physiological conditions under which PPARα signalling is triggered have remained elusive. In PPARα null mice, a 24 h fast causes a fatty liver, hypoglycaemia, hypoketonaemia, hypothermia and elevated plasma free fatty acid concentrations (Kersten *et al*., 1999). Strikingly, some PPARα target genes are affected by PPARα deletion only in the fed state, whereas others are affected only in the fasted state. The data presented by W.Wahli (University of Lausanne) show that PPARα plays a pivotal role in the management of energy stores during food deprivation. Via modulation of gene expression, PPARα induces hepatic fatty acid oxidation to supply substrates (glucose, ketone bodies) that can be metabolized by other tissues. While insulin levels are low at times of starvation, they are higher after food intake. Insulin binds to a membrane receptor and it has long been realized that signalling through membrane receptors may be able to modulate nuclear and steroid receptor-mediated transcription. In the case of PPARα, insulin can enhance receptor activity via phosphorylation of serines 12 and 21 in the AF1 domain of the receptor (Juge-Aubry *et al*., 1999). Insulin-induced phosphorylation of PPARα goes through the MAPK p42/p44 pathway and may weaken the interaction with some kind of repressor protein, leading to increased transcriptional activation (C.Juge-Aubry, University of Geneva).

A few years ago, it was first proposed that PPARα plays a role in inflammation by being the nuclear receptor for leukotriene B_4 (LTB_4). According to Wahli, some synthetic agonists and antagonists of the membrane receptor for LTB_4 are also potent ligands for PPARα. The data he presented strengthen the concept of a function of LTB_4 as a ligand for PPARα, and point towards a still to be explored cross-talk between the membrane and nuclear receptor for this eicosanoid (Devchand *et al*., 1999; Lin *et al*., 1999). Support for an anti-inflammatory effect of PPARα is provided by a decreased lipopolysaccharide (LPS)-induced inflammatory response by the PPARα ligand fenofibrate in rabbit aorta, and an increased inflammatory response to LPS in aortas of PPARα null mice (Bart Staels, INSERM, Lille). The anti-inflammatory effect of PPARα agonists may be exerted by inhibiting interleukin 6 (IL-6) transcription by interfering with If-κB and AP-1 action. The reverse is also true: p65 and c-Jun, components of If-κB and AP-1, respectively, can repress PPARα-induced transactivation.

FXR (NR1H4) first gained the spotlight when it was proposed to be a nuclear hormone receptor for farnesol metabolites. New data presented by D.Mangelsdorf (UT Texas Southwestern Medical Center) and S.Kliewer (Glaxo-Wellcome) indicate that the true ligands for FXR are not farnesol metabolites or retinoids but bile acids (accordingly, a more appropriate name for FXR is BAR, for bile acid receptor). This work was recently published as a series of three papers from Mangelsdorf's, Kliewer's and Forman's groups arriving at similar conclusions (Makishima *et al*., 1999; Parks *et al*., 1999; Wang *et al*., 1999). We will summarize these important findings briefly, as recent reviews on this topic have already appeared (Gustafsson, 1999; Russell, 1999). The most potent BAR agonist happens to be the bile acid chenodeoxycholic acid.

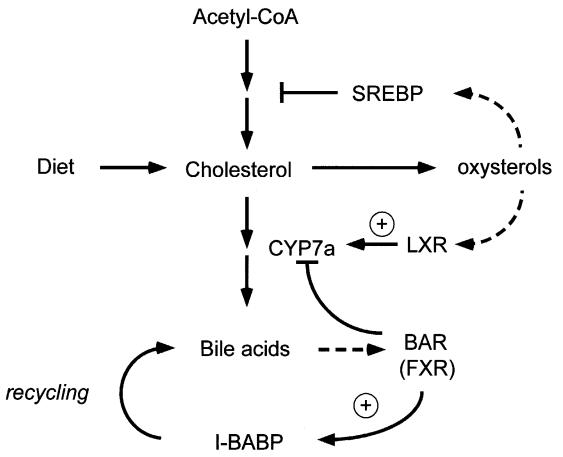

Fig. 2. Role of LXR and BAR in the regulation of bile acid synthesis. Synthesis of bile acids is regulated at the level of the rate-limiting step catalysed by cholesterol 7α hydroxylase. To prevent excessive build-up of free cholesterol in the liver, cholesterol stimulates its own conversion to bile acids by activating the nuclear receptor LXR, which increases transcription of the Cyp7α gene. This so-called feedforward mechanism requires conversion of cholesterol to oxysterols, which are the ligands for LXR. Overproduction of bile acids is prevented by binding to their nuclear receptor BAR (bile acid receptors or FXR), which subsequently downregulates the expression of the Cyp7α gene. In order to stimulate recycling, the bile acids–BAR complex also stimulates recycling of bile acids by inducing transcription of I-BABP (intestinal bile acids binding protein), a protein that is responsible for re-uptake of bile acids in the intestine.

Cholic acid and to a lesser extent secondary bile acids are much weaker ligands for BAR, while conjugated bile acids can be ligands but require the presence of the bile acid transporter I-BAT for uptake into cells. Both negative [cholesterol 7α hydroxylase (Cyp7α)] and positive [intestinal bile acid binding protein (I-BABP)] targets of BAR and chenodeoxycholic acid have been identified. Cyp7α catalyses the rate-limiting step in bile acid synthesis from cholesterol and I-BABP is an ileal transport protein that promotes reuptake of bile acids in the small intestine. An elegant scheme has been proposed in which bile acids in liver feed back on their own synthesis by downregulating Cyp7α expression, whereas bile acids in the gut stimulate expression of I-BABP (Figure 2). This mechanism prevents excessive production of bile acids, and at the same time promotes their recycling from the intestines.

Moreover, Cyp7α is directly regulated by LXRα receptor (Mangelsdorf) due to the presence in its promoter of an LXRα-responsive element. Genes containing this element can be activated by different cholesterol metabolites, of which the oxysterol 24-hydroxycholesterol is the most active in transient transfection experiments. $LXR\alpha^{-/-}$ mice, treated with a high-cholesterol diet, fail to induce Cyp7α transcription, leading to an over-accumulation of cholesterol in the liver. These finding are the basis for understanding the feedforward regulation of bile acid synthesis.

Differentiation

Its now well established that nuclear receptors play an important role in development and tissue differentiation. This knowledge has mainly been acquired through studies in which the effects of inactivation of receptors using

homologous recombination have been analysed. In most cases, activation of nuclear receptors requires their cognate ligands. Thus, temporal and spatial expression of ligands and enzymes necessary for their synthesis is of great importance for receptor activation. Recent studies, particularly in the retinoid field, have suggested that nuclear receptor ligands act not only in a classical endocrine way but also in a paracrine or intracrine manner. T.Perlmann (Ludwig Institute, Stockholm) presented a transgenic approach to analyse temporal and spatial expression of RXR and RAR ligands during development. The best results were obtained with a ligand trap assay using a feedback-inducible RAR-driven expression vector in which both the effector and reporter gene were placed on the same plasmid. In this bicistronic plasmid, the effector was the LBD of the RARα fused to the Gal4 DBD and its expression was controlled by Gal4 binding sites and a minimal promoter. The activation of Gal4–RARαLBD by binding of its cognate ligand *in vivo* not only led to the activation of the fusion protein, but also to binding to the Gal4 sites controlling the expression of a LacZ reporter gene. Using this approach, Perlmann and colleagues could not only identify retinoid synthesis in previously known retinoid-producing locations (spinal cord, limb, neuronal retina, etc.), but also at novel sites. At embryo day (E) 11.5–12.5, retinoid synthesis was identified in the developing forebrain and the lateral eminence ganglion giving rise to the striatum, a brain area involved in motor control and voluntary movements. A fibre-like expression pattern and costaining using specific glial cell markers correlated the retinoid-synthesizing areas to the distribution of radial glial cells. In fact, primary radial glial cells isolated from the lateral eminence ganglion were able to activate the Gal4-LacZ reporter gene in a co-culture assay. This was in line with the ability of exogenously administered retinoic acid to enhance striated neuron differentiation, as judged from the expression of specific differentiation markers. In addition, this is in agreement with data from P.Chambon's group showing that some RAR and RXR knock-out mice exhibit a defect in striatum function (Krezel *et al*., 1998). In summary, ligand trap assays as developed by Perlmann and colleagues may be very useful for the identification of ligands for orphan receptors. In fact, a similar approach was used by C.Thummel (University of Utah, Salt Lake City) aiming at identifying a ligand for the orphan receptor DHR78 involved in *Drosophila* metamorphosis. Several lines of evidence, including DHR78 mutations, suggest that this receptor and its unknown ligand are important during the transition from the mid- to the late-third instar larvae stage. Using a ligand trap assay, C.Thummel and colleagues showed that DHR78 is activated in the early- and mid-, but not in the late-third instar larvae in a subset of neurons. Based on the pleiotropic effect seen following DHR78 mutation, he suggested that these neurons may have a neuroendocrine function regulating a more general *Drosophila* development. The importance of retinoids for proper development was further demonstrated by P.Chambon (IGBMC, Strasbourg), who presented results from mice in which the retinalaldehyde dehydrogenase 2 (RALDH2) gene had been inactivated through homologous recombination. RALDH2 is responsible for the conversion of retinal to retinoic acid (Niederreither *et al*., 1999). Lack of this enzyme resulted in embryonic death at E10.5. Lack of normal development was already seen at age E8.5 with no axial rotation (body turning). Furthermore, RALDH2-deficient mice revealed, for example, disrupted heart, somite and ear development. Expression of the retinoic acid-inducible gene HoxA1 was reduced (Niederreither *et al*., 1999). Most of the phenotype disappeared following maternal administration of retinoic acid during E6.5–10. These results demonstrate that retinoic acid synthesized from maternal retinol is essential for the development of the mammalian embryo. In contrast to most instances of individual RAR or RXR inactivation, no redundancy is seen. This further emphasizes the importance of retinoic acid as a hormonal signal for proper development.

A general problem is that, in many instances, disruption of nuclear receptor genes results in embryonic or early postnatal lethality. Furthermore, it is difficult to evaluate whether the observed phenotype is a consequence of a direct or an indirect effect of the gene disruption. To overcome these problems, several groups are now in the process of developing tissue-specific and inducible gene disruptions. G.Schütz (German Cancer Research Centre, Heidelberg) described the effects of tissue-specific inactivation of GR in the liver and in the brain. Mice lacking GR expression in the hepatocytes were viable but stopped growing after 5 weeks of age. This may be a consequence of reduced gluconeogenesis or growth factor stimulation as expression of PEPCK, TAT and IGF-1 was reduced. Inactivation of GR in the brain, by driving the CRE-recombinase by the nestin promoter, also resulted in viable but smaller animals, which demonstrated phenotypic changes resembling Cushing-like symptoms. The animals revealed increased POMC expression, loss of bone density, impaired emotional learning and reduced anxiety. Further analysis of the $GR^{dim/dim}$ mice (Reichardt *et al*., 1998), i.e. animals where GR cannot dimerize and thus cannot activate GRE-regulated genes, has now revealed that these mice maintain the ability of glucocorticoids to repress TPA- or LPS-induced stimulation of cytokine (IL-2, IL-6, tumour necrosis factor α and interferon α) expression in isolated primary cell cultures. Furthermore, glucocorticoid repression of TPA-induced skin inflammation was unaffected in $GR^{dim/dim}$ mice. This shows that the anti-inflammatory activity *in vivo* of a GR unable to transactivate target genes, but able to transrepress AP-1- and NF-κB-responsive genes, is maintained. Using a tissue-specific and conditional knock-out technique, P.Chambon demonstrated that RXRα plays a crucial role in retinoic acid-induced proliferation of basal keratinocytes. Tissue-specific and conditional inactivation of RXRα in the epidermis was achieved by directing a CRE–ER LBD_{mut} fusion protein to the basal epidermic layer using the keratinocyte (K5) promoter. Using an ER-LBD mutated in the ligand-binding domain, a conditional activation of the recombinase could be obtained with the synthetic anti-oestrogen hydroxytamoxifen, while the natural oestrogens are unable to activate the fusion protein. Administration of hydroxytamoxifen in the latter half of gestation resulted in blistering between the basal layer and the basal membrane, resembling the pathology seen in junctional epidermolysis of man. This type of approach will most likely be very useful for future studies of the role of nuclear receptors.

J.Samurat (Ecole Normale Supèrieure, Lyon) presented

data from a TRα and TRβ double knock-out mouse. Comparing the phenotype of this mouse with the phenotypes of the individual knock-outs revealed that both gene products are involved in the feedback control of the pituitary–thyroid axis and cooperate in the development of the intestine but not of bone. The TRβ effect on the intestine was revealed only in the double knock-out mouse, suggesting that the lack of an intestinal effect in the single TRβ knock-out mice may be due to redundancy. Furthermore, results were presented that identified a new TR signalling pathway and a physiological role for two smaller transcripts generated from a promoter sequence in intron 7 of the TRα gene. These smaller transcripts, TRΔα1 and TRΔα2, are expressed at high levels in the intestine, brain, lung and inner cell mass before gastrulation and generate truncated TRα variants that act as trans-dominant negative regulators of both TRs and RARs (at least *in vitro*).

K.Korach (NIH-NIEHS, Research Triangle Park) described recent developments regarding the ERα$^{-/-}$ mice. The phenotype of these mice has recently been reviewed (Couse and Korach, 1999) and will not be discussed further. J.-Å.Gustafsson (Karolinska Institute, Stockholm) described new developments regarding the physiological role of the novel oestrogen receptor β. Several examples now exist where ERα and ERβ have different, often opposite, effects (yin/yang principle) on gene expression. ERβ is quite widespread in the organism and, by and large, appears to be quantitatively as important or more important than ERα. The recently generated ERβ$^{-/-}$ mice are currently subject to intense studies. As the observed phenotypes display some variability, they will not be discussed in detail. Whereas male and female ERα$^{-/-}$ mice are sterile, only ERβ$^{-/-}$ females show reduced fertility and an ovarian phenotype. Interestingly, the ovarian phenotype in the ERα$^{-/-}$ mice was completely normalized following reduction of the strongly elevated levels of luteinizing hormone (Schomberg *et al.*, 1999), suggesting that the primary cause of their infertility is disturbed function of the hypothalamo-pituitary axis rather than a primary defect in the ovaries.

The orphan nuclear receptor COUP-TF also consists of two isoforms, which harbour completely separate functions. M.-J.Tsai (Baylor College of Medicine, Houston) presented data from mice carrying a disruption of the COUP-TFI gene. COUP-TFI is highly expressed in the central and peripheral nervous system during embryonal development. In the COUP-TFI$^{-/-}$ mice, several defects in the development of the nervous system are observed, including loss of formation of cortical layer IV and impaired axon myelinization. M.-J.Tsai presented data showing that the lack of layer IV formation was due to apoptosis of the neurons normally present. This was a consequence of inadequate trophical activity due to reduced thalamocortical projections into this area, which in turn resulted from apoptosis of the subplate neurons necessary for thalamocortical axon guidance to innervate layer IV neurons. M.-J.Tsai demonstrated that COUP-TFI-deficient mice express a reduced level of the transcription factor Tst-1/Scip/Oct-6, which could partly explain the defective myelinization of axons in the central and peripheral nervous system. COUP-TFII, on the other hand, seems to be more involved in angiogenesis, as presented by S.Tsai (Baylor College of Medicine, Houston). Disruption of the COUP-TFII gene resulted in embryonic lethality at E10, possibly due to an improper heart development and inappropriate blood circulation. Furthermore, these mice exhibited haemorrhages in, for example, the neuronal tube and a defect in angiogenesis (generation and remodelling of the vascular tree) from the age of E9.5. Interestingly, the phenotype of the COUP-TFII knock-out mice very closely resembles the phenotype of angiopoietin-1 or its receptor (Tie2) seen in knock-out mice. Furthermore, angiopoietin-1 expression was reduced in the COUP-TFII knock-out mice, most likely resulting in perturbed angiopoietin-receptor (Tie2) signalling. In summary, the data presented by S.Tsai on the COUP-TFII knock-out mice suggest that COUP-TFII plays an important role in angiogenesis and heart development by contributing to the endothelial–mesenchymal interaction.

Conclusions

At the end of this compact and intense workshop, three main features surface above the plethora of interesting data presented in the many lectures and posters. First, new regulatory pathways on cholesterol and bile acid metabolism have been discovered, starting from the study of the natural ligands for some orphan receptors. In view of the large number of nuclear receptors in search of a ligand, we can expect more of these surprises in the near future. Secondly, the striking complexity of transcriptional regulation by coactivator complexes and chromatin remodelling machines is providing the rational framework to understand the specificity and variation of hormonal effects depending on physiological, cellular and genetic contexts. More complete data on hormonal responses will be generated by the use of DNA microarrays for the analysis of whole-genome expression. The progress expected in the methodologies for structural analysis of multiprotein complexes will furnish the topological information required for a comprehensive description of the multiple receptor networks. All this information will have to be incorporated into complex computer models in order to simulate the cellular response. Thirdly, the elegant experiments on tissue-specific and conditional receptor mutations, as well as the whole-animal ligand trap assay, convey a feeling for the kind of approaches that will help us to refine these models. Eventually, this will lead to a better understanding of hormone physiology and cross-talk with other signalling pathways in molecular terms.

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