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Review

Nuclear receptors, their coactivators and modulation of transcription*

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Nuclear receptors are ligand-dependent transcription factors which can also be activated in the absence of their lipophilic ligands by signaling substances acting on cell membrane receptors. This ligand-independent activation indicates the importance of nuclear receptor phosphorylation for their function. Nuclear receptor-mediated transcription of target genes is further increased by interactions with recruited coactivators forming a novel family of nuclear proteins. CBP/p300, a coactivator of different classes of transcription factors, including the tumor suppressor protein p53, plays a special role acting as a bridging protein between inducible transcription factors and the basal transcription apparatus, and as an integrator of diverse signaling pathways. Coactivators of nuclear receptors and associated proteins forming a multicomponent complex have an intrinsic histone acetylase activity in contrast to nuclear receptor and heterodimer Mad-Max corepressors, which recruit histone deacetylase. Similarly the Rb protein interacts with histone deacetylase to repress transcription of cell cycle regulatory genes. Targeted histone acetylation/deacetylation results in remodeling of chromatin structure and correlates with activation/repression of transcription. Recent data point to the important role of coactivator proteins associated with inducible transcription factors in transcription regulation, and in the integration of multiple signal transduction pathways within the nucleus.

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Abbreviations: CDK, cyclin-dependent kinases; DBD, DNA-binding domain; LBD, ligand-binding domain; SRC-1, steroid receptor coactivator 1; CBP, CREB-binding protein; p/CAF, p300/CBP-associated factor; HAT, histone acetylase (acetyltransferase); HDAC, histone deacetylase; HRE, hormone response element; HSP, heat-shock protein; ODC, ornithine decarboxylase; TBP, TATA box-binding protein.

According to the classic model of lipophilic hormone action the transcriptional control of hormonally responsive genes is mediated by specific high affinity intracellular receptor proteins acting as ligand-dependent transcription factors*. The biochemical methods, in particular the availability of purified hormones and antibodies, and molecular cloning techniques, enabled the discovery of numerous intracellular receptors representing an evolutionary conserved class of proteins. Present in diverse animal species – from worms to insects and mammals – they form a large and quickly growing superfamily of nuclear receptors characterized by homologies in their amino-acid sequences and similarity of the mechanisms of action (Table 1).

Table 1. The nuclear receptor superfamily

- evolutionary old proteins with highly conserved structure
- similarity of structure (functional domains) and mechanism of action
- as ligand-activated transcription factors they act in the nucleus (in contrast to membrane receptors)
- their ligands are small, lipophilic molecules
- very low concentration in the cell
- acting as transcription factors they activate or repress transcription of a target gene (or gene networks) in temporally defined sequence, in cell- or tissue-specific fashion
- functionally diverse, they control and coordinate complex events involved in development, differentiation, metamorphosis, homeostasis

This highly evolved old superfamily of eukaryotic transcription factors is often divided into three large families, depending on the structure of their ligands (the steroid receptor family, nonsteroid – or the thyroid/retinoid/vitamin D – receptor family, and the orphan receptor family of unknown ligand and/or function. This last family comprises numerous subfamilies each having multiple members. The search for the hormonal activators of orphan receptors has led to the identification of several lipophilic compounds as their cognate ligands. It appears, however, that not all of the orphan receptors are likely to be hormone responsive, some of these proteins transactivate target genes in a ligand-independent manner). Another division is based on the nuclear receptor dimerization and DNA-binding properties [1].

Nuclear receptors show a highly conserved modular structure. They share three major structural/functional domains: a variable N-terminal domain containing ligand-independent activation function, AF-1, and two evolutionary conserved parts – a central DNA-binding domain (DBD) and a multifunctional C-terminal domain. Two multicysteine zinc fingers of DBD make contact with the major grove of DNA, while the recognition α-helix is responsible for binding with specific DNA sequences, hormone response elements (HRE) in target genes. The ligand binding domain (LBD) situated with several other subdomains in the C-terminal region has the essential property of hormone recognition and binding. Upon ligand binding it shifts the receptor to a new conformational transcriptionaly active state.

The field of nuclear receptors, showing a remarkable progress, has been the subject of many excellent reviews; a selection of the recent ones, since 1995, will be found as reference numbers [1–10].

*Steroids, in addition to regulating gene transcription, can also evoke non-transcriptional responses interacting with cell surface receptors. Non-genomic action of steroids is not, however, the subject of this review.

CLASSIC *VERSUS* LIGAND-INDEPENDENT MODEL OF NUCLEAR RECEPTOR ACTIVATION

Ligands of nuclear receptors are small lipophilic molecules which, unlike hydrophilic signaling substances namely peptide hormone, growth factors or neurotransmitters, pass freely through the lipid bilayer of cell membranes. Once inside the cell the ligand binds specifically with its cognate receptor inducing a sequence of events:

- release of chaperone proteins (HSP 90, 70, 53, p60 and p23) which keep steroid receptors (but not nonsteroid and orphan receptors) in a „ligand-friendly" conformation
- homo- or heterodimerization
- phosphorylation of specific serine, threonine or tyrosine situated in the N-terminal domain
- direct association of receptor dimer with specific DNA consensus sequences, HRE in the target gene (steroid receptors; nonsteroid and orphan receptors appear to be bound to DNA in the absence of ligand, ligand binding may cause dissociation of corepressor proteins as discussed later)
- induction of transactivation function
- binding of coactivator protein(s) (compare the chapter on coactivators of nuclear receptors).

For years it was generally accepted that binding of a lipophilic ligand to a nuclear receptor is the sole and absolute requirement for its activation resulting in modulation of transcription of a target gene (classic model). However, a few years ago came the first surprising discovery that chicken progesterone receptor can be activated and can induce transcription of its target gene by the neurotransmitter dopamine in the absence of the cognate ligand, progesterone [11]. Now it is well documented that some nuclear receptors (such as estrogen, progesteron, androgen, vitamin D, retinoic acid receptors, but apparently not glucocorticoid and mineralocorticoid receptors) can be alternatively activated in the absence of their lipophilic ligands by a variety of extracellular signals. Data based mainly on transfection experiments but also on experiments in whole animals showed that activation of membrane-receptor-mediated signal transduction pathways by hydrophilic substances including dopamine, epidermal growth factor, gonadotropin releasing hormone, transforming growth factor α, insulin and insulin-like growth factor I induce ligand-independent transcriptional activity of some nuclear receptors [12–18]. Other modulators of kinase/phosphatase pathways, namely cAMP, forskolin and an inhibitor of phosphatases, okadaic acid [13, 16, 17, 19], exert similar alternative activation indicating the importance of phosphorylation of specific amino-acid residues in the receptor molecule for its function. Although the phosphorylation status of steroid receptors correlates with their activity [17, 20], this covalent modification is not the sole event responsible for their activation. Numerous recent reports show that the recruitment of coactivator or corepressor proteins by nuclear receptors is essential for their transcriptional activity as will be discussed later on.

NUCLEAR RECEPTORS AS INTEGRATORS OF DISTINCT SIGNALING PATHWAYS. IMPLICATIONS FOR ANTITUMOR THERAPY

Nuclear receptors activated by lipophilic ligands or, alternatively, by several cell substances acting on surface receptors can be considered the integrators of distinct signal transduction pathways. Thus, the pathways activated by hydrophilic and lipophilic signaling substances *via* the membrane and nuclear receptors, respectively, do not act independently, but are subject to cross modulation. The interaction between different signaling pathways converging on nuclear receptors is shown in Fig. 1.

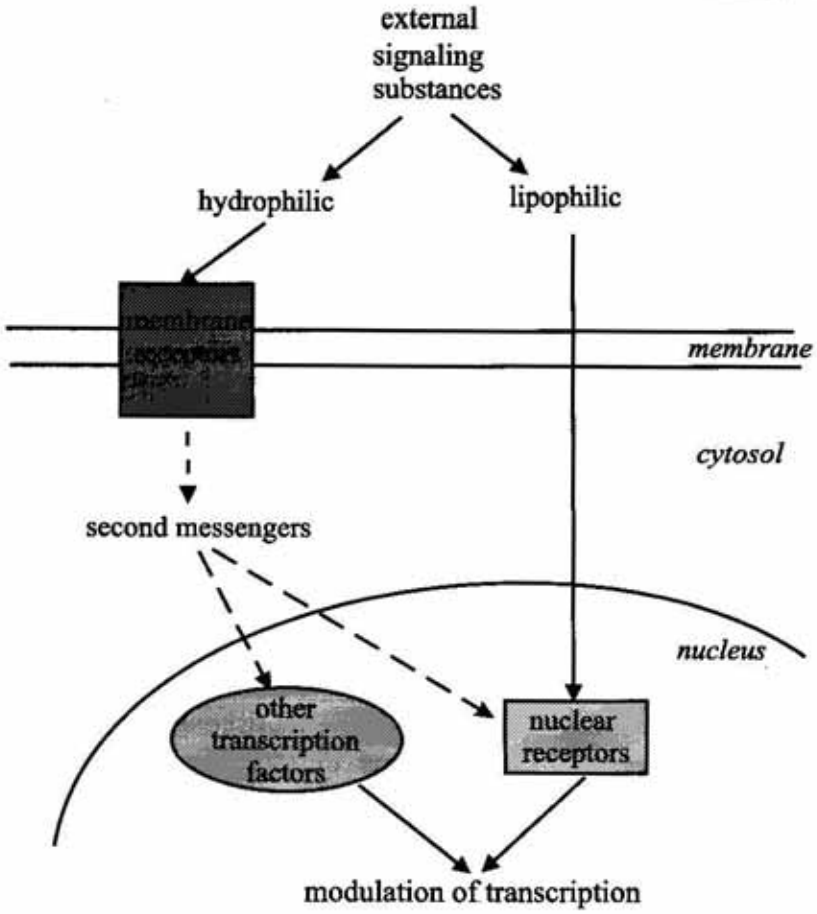

Figure 1. Membrane and nuclear receptors. Interaction between different signaling pathways.

A good example of the interaction (cross-talk) between two signaling pathways triggered at the cell surface and intracellularly is the regulation of ornithine decarboxylase (ODC) expression studied in author's lab [21]. In mouse kidney the expression of ODC, the key enzyme in polyamine biosynthesis, is under control of testosterone. This androgen in the presence of endogenous catecholamines induces spectacular increase of ODC activity (700-fold) and its transcript level (12-fold). However, catecholamine depletion drastically decreases this induction suggesting that both signaling substances – testosterone and catecholamines – interact synergistically to maximally stimulate ODC expression [21].

Steroid hormones and growth factors are two major regulators of breast and prostate normal and cancerous growth [22-24]. The growth of many tumors is initially stimulated by estrogens or androgens, and the use of their antagonists is the most common type of endocrine therapy in the treatment of drug-responsive tumors. The application of antiestrogen and antiandrogen chemotherapy can lead, however, to steroid independence and to antagonist resistance often connected with the expression of mutant forms of estrogen/androgen receptors by resistant tumors [25]. Some of these receptor mutants that fail to bind lipophilic ligand may acquire constitutive transcriptional activity [25], or may still be activated by growth factors [26] and contribute to cancer progression. Thus, understanding the cross-talk between these signaling substances is important for effective antitumor therapy.

Antiandrogens and antiestrogens widely used for the treatment of prostate and breast cancer may act sometimes as agonists leading to tissue-specific stimulation of cellular proliferation. Recent data suggest that the molecular mechanism of agonist activity of antiandrogens depends on the promotion of androgen receptor and its coactivator interaction [27]. Moreover, activation of specific signal

transduction pathways can convert steroid antagonists into agonists by displacing receptor-bound nuclear corepressors [28, 29].

COACTIVATORS OF NUCLEAR RECEPTORS

Contrary to the well known nuclear receptor structure studied by X-ray and NMR spectroscopy their complex molecular mechanism of action is far from being understood. To modulate transcription inducible transcription factors, such as nuclear receptors, have not only to recognize specific DNA motifs, but also to communicate with components of the basal transcription apparatus. Thus, transcriptional regulation may involve a concerted action of associated proteins recruited to nuclear receptors by protein–protein interaction.

crystal structure of nuclear receptor C-terminal domain revealed that a highly conserved subregion termed activation function 2 (AF-2) of ligand-binding domain (LBD) is required for the recruitment of coactivator proteins [31–33]. Upon ligand binding LBD undergoes a conformational change resulting in an extensive shift in position of the AF-2 region located in its last 12-th helix providing a surface with which coactivator proteins can interact.

Nuclear receptor coactivators identified recently with biochemical and molecular biology methods are listed in Table 2. The first coactivator identified in 1995 [34] was SRC-1 (*steroid receptor coactivator 1*) which together with its homologue TIF2 forms the novel, still growing family of coactivators of nuclear receptors. Members of the family interact usually with several nuclear receptors (ARA_{70},

Table 2. Nuclear receptor coactivators

Coactivator	Interaction with a receptor	Remarks
SRC-1/N-CoA1/ p160/ERAP160	PR, ER, GR, RAR, RXR, TR	increases (more than 10×) transactivation induced by nuclear receptors, forms a family of coactivators (sequence motif LXXLL), interacts with CBP/p300 and P/CAF, histone acetylase (HAT) activity (H3, H4) [34–37]
TIF2/GRIP1/N-CoA2	PR, ER, RAR, RXR	30% homology with SRC-1 [38]
CBP/p300 (CREB-binding protein)	PR, ER, TR, RAR, RXR; also: CREB, v-Jun, c-Jun, c-Myb, v-Myb, Sap-1a, c-Fos, STAT, NFκB	MASTER COACTIVATOR, binds nuclear receptors (sequence motif LXXLL) and other classes of transcription factors, interacts with SRC-1 and P/CAF, HAT activity (H2A, H2B, H3, H4) [39–44]
ARA_{70}	AR	specifically increases AR-induced transactivation (prostate and other tissues) [45]
RAC3	RAR, PR	[46]
ACTR	RAR, RXR, TR	binds CBP/p300 and P/CAF, HAT activity (H3, H4) [47]
AIB1	ER	gene amplification and increased expression in breast and ovarian cancer [48]
P/CIP	various nuclear receptors and other transcription factors	forms complex with CBP/p300, homology with SRC-1, sequence motif LXXLL [49]
TRAM-1	TR, ER, RAR, RXR	forms complex with CBP/p300 and P/CAF [50]

The intensive studies of the last few years have led to the identification of a novel family of proteins called nuclear receptor coactivators that interact with nuclear receptors in a ligand-dependent manner and potentiate their transcriptional activity [30, 31]. X-ray

which shows absolute specificity towards androgen receptor, is an exception) enhancing their transactivation. All coactivators contain one or more leucine-rich charged helical sequence motif LXXLL (where X denotes any amino acid) which is the family's signature

[51]. As shown recently these short characteristic motifs responsible for the interaction with the AF-2 region of nuclear receptors are vital in mediating cooperative assembly of coactivator complexes on nuclear receptors [31]. Characteristic for nuclear receptor coactivators and important for their function (as will be discussed later) is their intrinsic activity of histone acetyltransferase (HAT) and recruitment of another HAT protein, p/CAF (which was very recently shown to form a histone acetylase multiprotein complex [52, 53].

CBP/p300 IS A MASTER COACTIVATOR

CBP, a huge protein consisting of 2.4 thousand amino acids was identified in 1993 as CREB-binding protein [39, 54, 55], but very soon it appeared that the role it plays is much more important. Numerous studies revealed that CBP and its close homologue p300, having all properties of a nuclear receptor coactivator (Table 2), interact also with other classes of transcription factors, thus acting as master coactivator and as an integrator of multiple signal transduction pathways [40–44, 56, 57]. Microinjection of anti-CBP antibodies into cell blocks transcriptional activity of several transcription factors causing a functional knock-out [58]. Due to the limited amount of CBP in the cell there is a competition for this coactivator between transcription factors activated by diverse signaling pathways resulting in their antagonism or synergism [56, 58, 59].

Moreover, CBP/p300 interacts with some nuclear receptor coactivators (Table 2) and binds components of the basal transcriptional machinery such as TBP (TATA *box binding protein*) and general transcription factor TFIIB [56]. Thus, CBP/p300 serves as a bridging molecule linking promoter and inducible transcription factors.

Of great interest was the recent finding that CBP/p300 can also interact with the tumor suppressor protein p53 thus becoming its coactivator and potentiating transciptional activity of p53 [60–63]. The formation of an intranuclear complex between p300 and p53 was observed when both proteins were coexpressed in the cell [61]. This colocalization is prevented by virus oncoprotein E1A which interacts with the same CBP binding site as p53. The resulting competition of p53 and E1A for CBP/p300 has dramatic consequences – p53 loses control over cell cycle, and in spite of DNA damage cell proliferation instead of cell cycle arrest is observed [64].

REVERSIBLE HISTONE ACETYLATION MODULATES CHROMATIN STRUCTURE

Active role of chromatin structure in the regulation of transcription has been recently widely recognized. One of the main chromatin remodeling systems is histone acetylation likely to play a key role in gene regulation in eukaryotes [65–68].

Nucleosome, the main unit of chromatin, is a protein–DNA complex that consists of a histone octamer (two copies each of histones H2A, H2B, H3 and H4) wrapped by a superhelix of DNA [69–71]. Each histone, apart from the structured three helix domain, has long unstructured tails projecting out of the nucleosome. N-terminal tails, rich in lysine are positively charged which causes a tight association between DNA and histones, and between nucleosomes. These highly charged core histone tails are target for reversible acetylation mediated by histone acetylases/deacetylases. Acetylation, by reducing net charge, leads to a weakening of histone–DNA contact, and to a loosening of internucleosomal interactions. Thus, acetylation promotes chromatin disruption and facilitates the access for sequence-specific transcription factors and the basal transcription machinery to a target gene. There is a general correlation between the level of histone acetylation and

transcriptional activity – hyperacetylated histones are associated with transcriptionally active chromosomal domains while hypoacetylated histones are detected at silenced regions [66, 67].

It is to be noted, however, that modification of chromatin through acetylation/deacetylation is not the sole determinant of its structure. Among multiple chromatin remodeling systems evolutionary conserved SWI2/SNF2 family that alter nucleosome structure in an ATP-dependent manner is of special importance [68, 72, 73].

HISTONE ACETYLASES/DEACETYLASES ARE INVOLVED IN ACTIVATION/REPRESSION OF TRANSCRIPTION

Highly conserved lysine residues in the N-terminal tails of all four core histones are enzymatically acetylated *in vivo*; their acetylation is regulated by two families of enzymes, the histone acetylases (HAT) and histone deacetylases (HDAC). Recently several transcriptional coactivators, including nuclear receptor coactivators and CBP/p300, have been shown to possess intrinsic HAT activity (Table 2) [37, 47, 74]. Moreover, coactivator proteins interact with the HAT protein, p/CAF, forming a multicomponent coactivator complex [75].

The presence of multiple HATs in the same coactivator complex (which may vary in composition depending on the class of transcription factors [76]) appears to be connected with different substrate specificity of HAT components [31, 76]. Acetylation of individual histones and even of a particular lysine residue on the same histone may exert specific functional effects. Moreover, nonhistone proteins (e.g., p53) may also be substrates for coactivators with HAT activity; acetylation of p53 by p300 dramatically stimulates its DNA-binding activity [77].

In contrast to coactivators known to be histone acetylases, recently characterized multisubunit corepressor complexes have been shown to contain histone deacetylase, which is required for transcriptional repression mediated by nuclear receptors, such as thyroid hormone and retinoid receptors, and by Mad-Max heterodimer [78–80]. Such targeted recruitment of histone deacetylate activity results in localized perturbation of chromatin structure and is a novel mechanism of transcription repression [81].

Recruitment of histone deacetylase/acetylase by nuclear receptors appears to be the basis of the recently proposed three-step model for transcriptional regulation by thyroid hormone receptor [82]. Unliganded receptor binds to its HRE sequence in a target gene and recruits a deacetylase complex thereby repressing transcription. In the presence of thyroid hormone an exchange of corepressor complex for coactivators with multiple HAT activity occurs with concomitant activation of transcription [82].

Several extremely interesting recent reports document that also the retinoblastoma tumor suppressor protein Rb represses transcription by recruiting HDAC [83–86]. pRb regulates progression through the G1 phase of cell cycle by inhibiting transcription of cell cycle regulatory genes controlled by E2F family of transcription factors. Once bound to E2F, pRb recruits HDAC leading to transcription repression reversed by its hyperphosphorylation by G1 cyclin-dependent kinases, CDK4 and CDK6.

Moreover, it appears that also DNA methylation-dependent transcriptional silencing may relay on histone deacetylation. Namely, MeCP2, a protein that selectively binds to methylated DNA, exists as a complex with histone deacetylase and some other proteins [87, 88].

Collectively, these recent data indicate that targeted histone acetylation by recruited HAT coactivators and deacylation by HDAC

corepressors is important for remodeling chromatin structure, and is a mechanism of transcription modulation.

CONCLUDING REMARKS

The role of inducible transcription factors in transcriptional regulation is well established and unquestionable. Transcription factors function by recognizing and binding to specific DNA consensus sequences in target genes. However, from the recent findings it appears that the regulation of transcription requires also the participation of another class of proteins that are recruited to sequence specific transcription factors by protein-protein interactions and potentiate transactivation. These non-DNA binding proteins – coactivators of transcription factors – serve several important roles. Firstly, they can act as bridging proteins between DNA-bound transcription factors and the basal transcription complex, and secondly through histone acetylase intrinsic or recruited activity they alter the architecture of chromatin. Finally, some transcription coactivators, such as CBP/p300, play a critical regulatory role as integrators of diverse signaling pathways with the selective induction of gene expression.

Recently, the class of protein integrators expanded with the surprising discovery that SRC-1, originally identified as coactivator of nuclear receptors, and its homologue p/CIP, may also enhance transactivation mediated by other classes of transcription factors acting as coactivators of NFκB and AP-1 (SRC-1, [89, 90]), and of CREB and STAT-1 (p/CIP [76]).

The cooperation between different coactivator proteins, and competition for limiting amounts of these molecules may explain numerous examples of cross-talk between distinct signal transduction pathways.

REFERENCES

1. Mangelsdorf, D.J. & Evans, R.M. (1995) The RXR heterodimers and orphan receptors. *Cell* **83**, 841-850.

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

3. Beato, M., Herrlich, P. & Schütz, G. (1995) Steroid hormone receptors: Many actors in search of a plot. *Cell* **83**, 851-857.

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

5. Słomczyńska, M. (1995) Receptory hormonów steroidowych – budowa i funkcja. *Post. Biol. Kom.* **22**, 3-21 (in Polish).

6. Weigel, N.L. (1996) Steroid hormone receptors and their regulation by phosphorylation. *Biochem. J.* **319**, 657-667.

7. Czekaj, P. (1996) Interakcje receptorów hormonu tarczycy i pochodnych witamin A i D z DNA. *Post. Biol. Kom.* **23**, 261-278 (in Polish).

8. Sokół-Misiak, W. (1997) Receptory jądrowe; w *Receptory struktura charakterystyka funkcja* (Nowak, J.Z. & Zawilska, J.B., red.) pp. 335-358, PWN, Warszawa (in Polish).

9. Perlmann, T. & Evans, R.M. (1997) Nuclear receptors in Sicily: All in the famiglia. *Cell* **90**, 391-397.

10. Kumar, M.V. & Tindall, D.J. (1998) Transcriptional regulation of the steroid receptor genes. *Prog. Nucleic Acid Res. Mol. Biol.* **59**, 289-306.

11. Denner, L.A., Weigel, N.L., Maxwell, B.L., Schrader, W.T. & O'Malley, B.W. (1990)

Regulation of progesterone receptor-mediated transcription by phosphorylation. *Science* **250**, 1740–1743.

12. Power, R.F., Mani, S.K., Codina, J., Conneely, O.M. & O'Malley, B.W. (1991) Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science* **254**, 1636–1639.

13. Aronica, S.M. & Katzenellenbogen, B.S. (1993) Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-1. *Mol. Endocrinol.* **7**, 743–752.

14. Ignar-Trowbridge, D.M., Teng, C.T., Ross, K.A., Parker, M.G., Korach, K.S. & McLachlan, J.A. (1993) Peptide growth factors elicit estrogen receptor-dependent transcriptional activation of an estrogen-responsive element. *Mol. Endocrinol.* **7**, 992–998.

15. Mani, S.K., Allen, J.M.C., Clark, J.H., Blaustein, J.D. & O'Malley, B.W. (1994) Convergent pathways for steroid hormone- and neurotransmitter-induced rat sexual behavior. *Science* **265**, 1246–1249.

16. O'Malley, B.W., Schrader, W.T., Mani, S., Smith, C., Weigel, N.L., Conneely, O.M. & Clark, J.H. (1995) An alternative ligand-independent pathway for activation of steroid receptors. *Rec. Prog. Horm. Res.* **50**, 333–347.

17. Bunone, G., Briand, P.-A., Miksicek, R.J. & Picard, D. (1996) Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO J.* **15**, 2174–2183.

18. Ankrapp, D.P., Bennett, J.M. & Haslam, S.Z. (1998) Role of epidermal growth factor in the acquisition of ovarian steroid hormone responsiveness in the normal mouse mammary gland. *J. Cell. Physiol.* **174**, 251–260.

19. Nazareth, L.V. & Weigel, N.L. (1996) Activation of the human androgen receptor through a protein kinase A signaling pathway. *J. Biol. Chem.* **271**, 19900–19907.

20. Blok, L.J., de Ruiter, P.E. & Brinkmann, A.O. (1998) Forskolin-induced dephosphorylation of the androgen receptor impairs ligand binding. *Biochemistry* **37**, 3850–3857.

21. Manteuffel-Cymborowska, M., Pęska, M., Chmurzyńska, W. & Grzelakowska-Sztabert, B. (1997) Catecholamines are required for androgen-induced ODC expression but not for hypertrophy of mouse kidney. *Biochim. Biophys. Acta* **1356**, 292–298.

22. de Cupis, A. & Favoni, R.E. (1997) Oestrogen/growth factor cross-talk in breast carcinoma: A specific target for novel antioestrogens. *Trends Pharmacol. Sci.* **18**, 245–251.

23. Lu, S., Tsai, S.Y. & Tsai, M.-J. (1997) Regulation of androgen-dependent prostatic cancer cell growth: androgen regulation of *CDK2, CDK4,* and CKI *p16* genes. *Cancer Res.* **57**, 4511–4516.

24. Knudsen, K.E., Arden, K.C. & Cavenee, W.K. (1998) Multiple G_1 regulatory elements control the androgen-dependent proliferation of prostatic carcinoma cells. *J. Biol. Chem.* **273**, 20213–20222.

25. Zhang, Q.-X., Borg, A., Wolf, D.M., Oesterreich, S. & Fuqua, S.A.W. (1997) An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. *Cancer Res.* **57**, 1244–1249.

26. Trowbridge, J.M., Rogatsky, I. & Garabedian, M.J. (1997) Regulation of estrogen receptor transcriptional enhancement by the cyclin A/Cdk2 complex. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10132–10137.

27. Miyamoto, H., Yeh, S., Wilding, G. & Chang, C. (1998) Promotion of agonist activity of antiandrogens by the androgen receptor coactivator, ARA70, in human prostate cancer DU145 cells. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 7379–7384.

28. Lavinsky, R.M., Jepsen, K., Heinzel, T., Torchia, J., Mullen, T.-M., Schiff, R., Del-Rio, A.L., Ricote, M., Ngo, S., Gemsch, J., Hilsenbeck, S.G., Osborne, C.K., Glass, C.K., Rosenfeld, M.G. & Rose, D.W. (1998) Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 2920–2925.

29. Wagner, B.L., Norris, J.D., Knotts, T.A., Weigel, N.L. & McDonnell, D.P. (1998) The nuclear corepressor NCoR and SMRT are key regulators of both ligand- and 8-bromo-cyclic AMP-dependent transcriptional activity of the human progesterone receptor. *Mol. Cell. Biol.* **18**, 1369–1378.

30. Glass, C.K., Rose, D.W. & Rosenfeld, M.G. (1997) Nuclear receptor coactivators. *Curr. Opin. Cell Biol.* **9**, 222–232.

31. Torchia, J., Glass, C. & Rosenfeld, M.G. (1998) Co-activators and co-repressors in the integration of transcriptional responses. *Curr. Opin. Cell Biol.* **10**, 373–383.

32. Moras, D. & Gronemeyer, H. (1998) The nuclear receptor ligand-binding domain: Structure and function. *Curr. Opin. Cell Biol.* **10**, 384–391.

33. Nolte, R.T., Wisely, G.B., Westin, S., Cobb, J.E., Lambert, M.H., Kurokawa, R., Rosenfeld, M.G., Willson, T.M., Glass, C.K. & Milburn, M.V. (1998) Ligand binding and coactivator assembly of the peroxisome proliferation-activated receptor-γ. *Nature* **395**, 137–143.

34. Onate, S.A., Tsai, S.Y., Tsai, M.-J. & O'Malley, B.W. (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270**, 1354–1357.

35. Smith, C.L., Onate, S.A., Tsai, M.-J. & O'Malley, B.W. (1996) CREB binding protein acts synergistically with steroid receptor coactivator-1 to enhance steroid receptor-dependent transcription. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 8884–8888.

36. Yao, T.-P., Ku, G., Zhou, N., Scully, R. & Livingston, D.M. (1996) The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10626–10631.

37. Spencer, T.E., Jenster, G., Burcin, M.M., Allis, C.D., Zhou, J., Mizzen, C.A., McKenna, N.J., Onate, S.A., Tsai, S.Y., Tsai, M.-J. & O'Malley, B.W. (1997) Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature* **389**, 194–198.

38. Voegel, J.J., Heine, M.J.S., Zechel, C., Chambon, P. & Gronemeyer, H. (1996) TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO J.* **15**, 3667–3675.

39. Chrivia, J.C., Kwok, R.P.S., Lamb, N., Hagiwara, M., Montminy, M.R. & Goodman, R.H. (1993) Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature* **365**, 855–859.

40. Nordheim, A. (1994) CREB takes CBP to tango. *Nature* **370**, 177–178.

41. Chakravarti, D., LaMorte, V.J., Nelson, M.C., Nakajima, T., Schulman, I.G., Juguilon, H., Montminy, M. & Evans, R.M. (1996) Role of CBP/P300 in nuclear receptor signalling. *Nature* **383**, 99–103.

42. Janknecht, R. & Hunter, T. (1996) Transcriptional control: versatile molecular glue. *Curr. Biol.* **6**, 951–954.

43. Shikama, N., Lyon, J. & La Thangue, N.B. (1997) The p300/CBP family: Integrating signals with transcription factors and chromatin. *Trends Cell Biol.* **7**, 230–236.

44. Giles, R.H., Peters, D.J.M. & Breuning, M.H. (1998) Conjuction dysfunction: CBP/p300 in human disease. *Trends Genet.* **14**, 178–183.

45. Yeh, S. & Chang, C. (1996) Cloning and characterization of a specific coactivator, ARA_{70}, for the androgen receptor in human prostate cells. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5517–5521.

46. Li, H., Gomes, P.J. & Chen, J.D. (1997) RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 8479–8484.

47. Chen, H., Lin, R.J., Schiltz, R.L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M.L., Nakatani, Y. & Evans, R.M. (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90**, 569–580.

48. Anzick, S.L., Kononen, J., Walker, R.L., Azorsa, D.O., Tanner, M.M., Guan, X.-Y., Sauter, G., Kallioniemi, O.-P., Trent, J.M. & Meltzer, P.S. (1997) AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* **277**, 965–968.

49. Torchia, J., Rose, D.W., Inostroza, J., Kamei, Y., Westin, S., Glass, C.K. & Rosenfeld, M.G. (1997) The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* **387**, 677– 684.

50. Takeshita, A., Cardona, G.R., Koibuchi, N., Suen, C.-S. & Chin, W.W. (1997) TRAM-1, a novel 160-kDa thyroid hormone receptor activator molecule, exhibits distinct properties from steroid receptor coactivator-1. *J. Biol. Chem.* **272**, 27629–27634.

51. Heery, D.M., Kalkhoven, E., Hoare, S. & Parker, M.G. (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* **387**, 733–736.

52. Struhl, K. & Moqtaderi, Z. (1998) The TAFs in the HAT. *Cell* **94**, 1–4.

53. Ogryzko, V.V., Kotani, T., Zhang, X., Schiltz, R.L., Howard, T., Yang, X.-J., Howard, B.H., Qin, J. & Nakatani, Y. (1998) Histone-like TAFs within the PCAF histone acetylase complex. *Cell* **94**, 35–44.

54. Kwok, R.P.S., Lundblad, J.R., Chrivia, J.C., Richards, J.P., Bächinger, H.P., Brennan, R.G., Roberts, S.G.E., Green, M.R. & Goodman, R.H. (1994) Nuclear protein CBP is a coactivator for the transcription factor CREB. *Nature* **370**, 223–226.

55. Arias, J., Alberts, A.S., Brindle, P., Claret, F.X., Smeal, T., Karin, M., Feramisco, J. & Montminy, M. (1994) Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. *Nature* **370**, 226–229.

56. Janknecht, R. & Hunter, T. (1993) A growing coactivator network. *Nature* **383**, 22–23.

57. Montminy, M. (1997) Something new to hang your HAT on. *Nature* **387**, 654–655.

58. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.-C., Heyman, R.A., Rose, D.W., Glass, C.K. & Rosenfeld, M.G. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* **85**, 403–414.

59. Hanstein, B., Eckner, R., DiRenzo, J., Halachmi, S., Liu, H., Searcy, B., Kurokawa, R. & Brown, M. (1996) p300 is a component of an estrogen receptor coactivator complex. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 11540–11545.

60. Avantaggiati, M.L., Ogryzko, V., Gardner, K., Giordano, A., Levine, A.S. & Kelly, K. (1997) Recruitment of p300/CBP in p53-dependent signal pathways. *Cell* **89**, 1175–1184.

61. Lill, N.L., Grossman, S.R., Ginsberg, D., DeCaprio, J. & Livingston, D.M. (1997) Binding and modulation of p53 by p300/CBP coactivators. *Nature* **387**, 823–827.

62. Scolnick, D.M., Chebab, N.H., Stavridi, E.S., Lien, M.C., Caruso, L., Moran, E., Berger, S.L. & Halazonetis, T.D. (1997) CREB-binding protein and p300/CBP-associated factor are transcriptional coactivators of the p53 tumor suppressor protein. *Cancer Res.* **57**, 3693–3696.

63. Gu, W., Shi, X.-L. & Roeder, R.G. (1997) Synergistic activation of transcription by CBP and p53. *Nature* **387**, 819–823.

64. Somasundaram, K. & El-Deiry, W.S. (1997) Inhibition of p53-mediated transactivation

and cell cycle arrest by E1A through its p300/CBP-interacting region. *Oncogene* **14**, 1047–1057.

65. Strenglanz, R. (1996) Histone acetylation: A gateway to transcriptional activation. *Trends Biochem. Sci* **21**, 357–358.

66. Wolffe, A.P. & Pruss, D. (1996) Targeting chromatin disruption: Transcription regulators that acetylate histones. *Cell* **84**, 817–819.

67. Pazin, M.J. & Kadonaga, J.T. (1997) What's up and down with histone deacetylation and transcription? *Cell* **89**, 325–328.

68. Gregory, P.D. & Hörz, W. (1998) Life with nucleosomes: Chromatin remodelling in gene regulation. *Curr. Opin. Cell Biol.* **10**, 339–345.

69. Kornberg, R. (1974) Chromatin structure; a repeating unit of histones and DNA. *Science* **184**, 868–871.

70. Richmond, T.J., Finch, J.T., Rushton, B., Rhodes, D. & Klug, A. (1984) Structure of the nucleosome core particle at 7 Å resolution. *Nature* **311**, 532–537.

71. Luger, K., Mäder, A.W., Richmond, R.K., Sargent, D.F. & Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251–260.

72. Tsukiyama, T. & Wu, C. (1997) Chromatin remodeling and transcription. *Curr. Opin. Genet. Dev.* **7**, 182–191.

73. Workman, J.L. & Kingston, R.E. (1998) Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu. Rev. Biochem.* **67**, 545–579.

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

75. Jenster, G., Spencer, T.E., Burcin, M.M., Tsai, S.Y., Tsai, M.-J. & O'Malley, B.W. (1997) Steroid receptor induction of gene transcription: A two-step model. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7879–7884.

76. Korzus, E., Torchia, J., Rose, D.W., Xu, L., Kurokawa, R., McInerney, E.M., Mullen, T.-M., Glass, C.K. & Rosenfeld, M.G. (1998) Transcription factor-specific requirements for coactivators and their acetyltransferase functions. *Science* **279**, 703–707.

77. Gu, W. & Roeder, R.G. (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* **90**, 595–606.

78. 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., Seto, E., Eisenman, R.N., Rose, D.W., Glass, C.K. & Rosenfeld, M.G. (1997) A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature* **387**, 43–48.

79. Nagy, L., Kao, H.-Y., Chakravarti, D., Lin, R.J., Hassig, C.A., Ayer, D.E., Schreiber, S.L. & Evans, R.M. (1997) Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell* **89**, 373–380.

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

81. Kadosh, D. & Struhl, K. (1998) Targeted recruitment of the Sin3-Rpd3 histone deacetylase complex generates a highly localized domain of repressed chromatin *in vivo*. *Mol. Cell. Biol.* **18**, 5121–5127.

82. Wolffe, A.P. (1997) Sinful repression. *Nature* **387**, 16–17.

83. DePinho, R.A. (1998) The cancer – chromatin connection. *Nature* **391**, 534–536.

84. Brehm, A., Miska, E.A., McCance, D.J., Reid, J.L., Bannister, A.J. & Kouzarides, T. (1998) Retinoblastoma protein recruits histone deacetylase to repress transcription. *Nature* **391**, 597–601.

85. Magnaghi-Jaulin, L., Groisman, R., Naguibneva, I., Robin, P., Lorain, S., Le Villain, J.P., Troalen, F., Trouche, D. & Harel-Bellan, A. (1998) Retinoblastoma protein represses transcription by recruiting a histone deacetylase. *Nature* **391**, 601–605.

86. Luo, R.X., Postigo, A.A. & Dean, D.C. (1998) Rb interacts with histone deacetylase to repress transcription. *Cell* **92**, 463–473.

87. Bestor, T.H. (1998) Methylation meets acetylation. *Nature* **393**, 311–312.

88. Eden, S., Hashimshony, T., Keshet, I., Cedar, H. & Thorne, A.W. (1998) DNA methylation models histone acetylation. *Nature* **394**, 842.

89. Na, S.-Y., Lee, S.-K., Han, S.-J., Choi, H.-S., Im, S.-Y. & Lee, J.W. (1998) Steroid receptor coactivator-1 interacts with the p50 subunit and coactivates nuclear factor κB-mediated transactivations. *J. Biol. Chem.* **273**, 10831–10834.

90. Lee, S.-K., Kim, H.-J., Na, S.-Y., Kim, T.S., Choi, H.-S., Im, S.-Y. & Lee, J.W. (1998) Steroid receptor coactivator-1 coactivates activating protein-1-mediated transactivations through interaction with the c-Jun and c-Fos subunits. *J. Biol. Chem.* **273**, 16651–16654.