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# Histone demethylation and androgen-dependent transcription

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Covalent modifications of histones play important roles in the regulation of chromatin dynamics and gene activity. Until recently, it was believed that methyl groups could not be removed from histones; as such, the discovery of the first demethylases opens a novel era in understanding how chromatin dynamic is regulated and shows that active demethylation is linked to both transcriptional repression and activation. During androgen-dependent gene activation, specific demethylases are involved in the control of gene expression. These new findings represent a milestone in elucidating the function of demethylases in gene expression. Furthermore, they show that active demethylation of repressive histone marks is a hallmark in the control of specific gene expression.

## Addresses

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## Introduction

In eukaryotic cells, genomic DNA is complexed with histones and non-histone proteins to form chromatin. Chromatin plays an important role in the regulation of gene expression. The basic unit of chromatin, the nucleosome, consists of 146 base pairs of DNA wrapped around an octamer of the four core histones, H2A, H2B, H3 and H4. The N-terminal tails of histones are positioned peripheral to the nucleosomal core and are subject to various covalent modifications, such as acetylation, phosphorylation, ubiquitination and methylation. Such modifications act sequentially or in combination to specify the so-called ‘histone code’ [1]. Depending on the distinct codes, genes will be either expressed or silenced [1]. The role of methylated histone lysines or arginines within histone tails has been linked to either transcriptional activation or repression [2,3]. Lysine residues in histone tails can be monomethylated, dimethylated

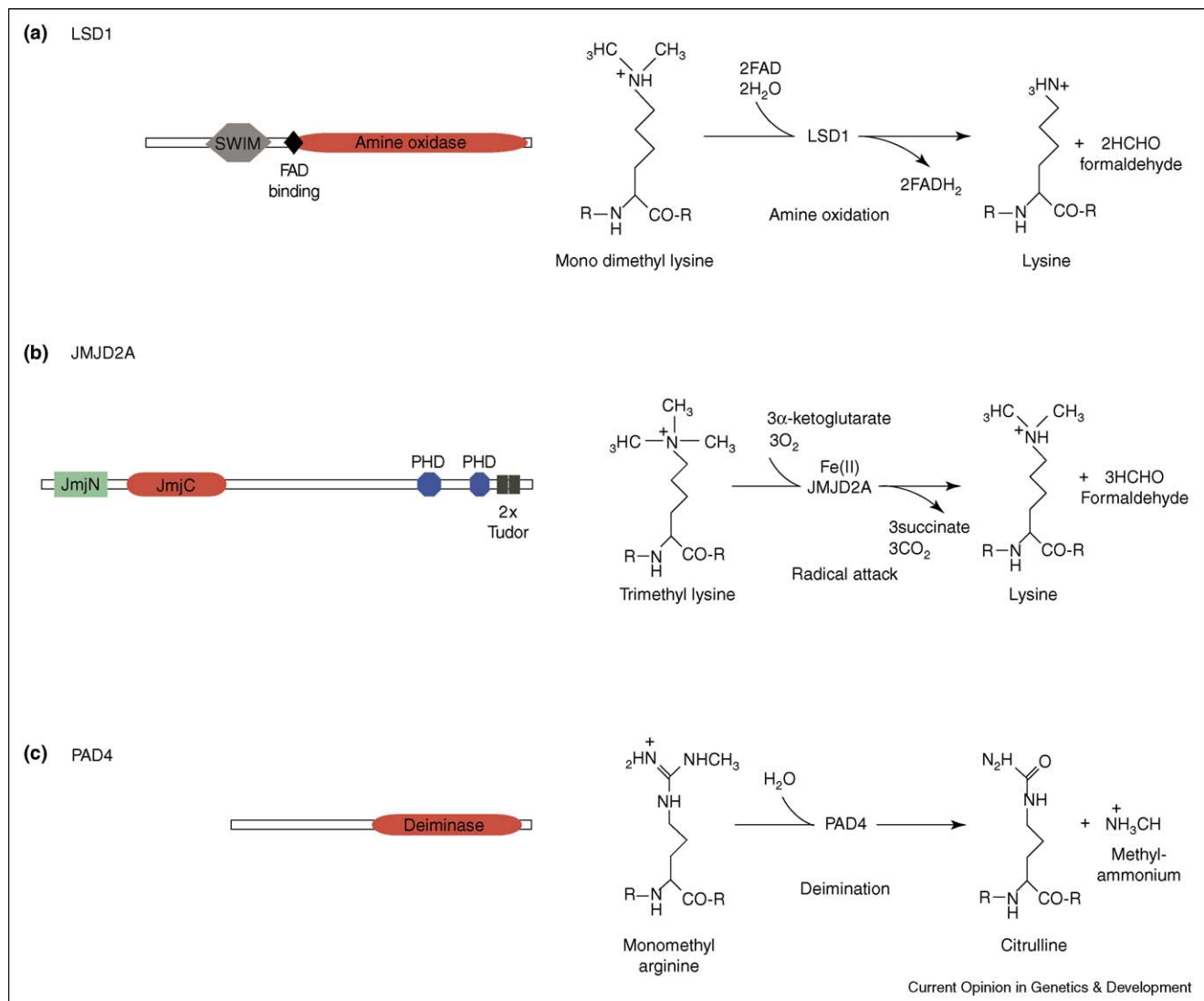
or trimethylated. The differentially methylated lysine residues serve as docking sites for various effector proteins and chromatin modifiers, which results in diverse physiological responses such as transcriptional repression and activation. Whereas evidence has demonstrated that acetylation and phosphorylation are reversible, methylation was considered as a stable and irreversible epigenetic mark that committed the chromatin to a specific transcriptional state [4,5]. In this review, we briefly refresh the knowledge concerning the recent findings in the field of demethylation and discuss the specificity of histone demethylation during androgen-dependent transcription.

## The growing family of demethylases

The first bona fide histone lysine demethylase to be identified was lysine-specific demethylase 1 (LSD1) [6••]. LSD1 is a nuclear amine oxidase homolog containing a C-terminal amine oxidase domain and a centrally located SWIRM (Swi3p, Rsc8p and Moira) domain. Interestingly, SWIRM domains are found in many chromatin-associated proteins and might function as chromosomal DNA binding motifs [7,8]. The oxidation reaction catalyzed by the amine oxidase domain of LSD1 is FAD-dependent and generates an unmodified lysine and a formaldehyde byproduct at the end of its catalytic cycle (Figure 1a) [6••]. LSD1 is highly specific for monomethylated and dimethylated histone H3 at lysine 4 (H3K4), whereas demethylation of trimethylated lysine is prevented by the absence of protonated nitrogen, which is required for the oxidation reaction (Figure 1a) [6••]. Consistent with its role in removal of the active methylation mark on H3K4, LSD1 is found in co-repressor complexes and promotes repression of gene expression [9–12]. Importantly, LSD1 is also associated with activated gene expression. During androgen receptor-mediated gene expression, LSD1 forms chromatin-associated complexes with the ligand-activated androgen receptor and is responsible for demethylation of the H3K9me1 and H3K9me2 [13••].

Very recently, the novel JmjC domain-containing histone demethylase 1 (JHDM1) protein was purified [14••]. The JmjC domain is conserved from bacteria to eukaryotes and belongs to the cupin superfamily of metalloenzymes [15]. JmjC domain proteins are predicted to be hydroxylases and are chemically compatible with demethylation of methylated substrates. JHDM1 specifically demethylates H3K39me2. This reaction occurs in the presence of Fe<sup>2+</sup> and  $\alpha$ -ketoglutarate and generates formaldehyde and succinate [14••]. Overexpression of JHDM1 reduces the level of H3K36me2 *in vivo*. In addition, the same group identified JHDM2A, another

Figure 1



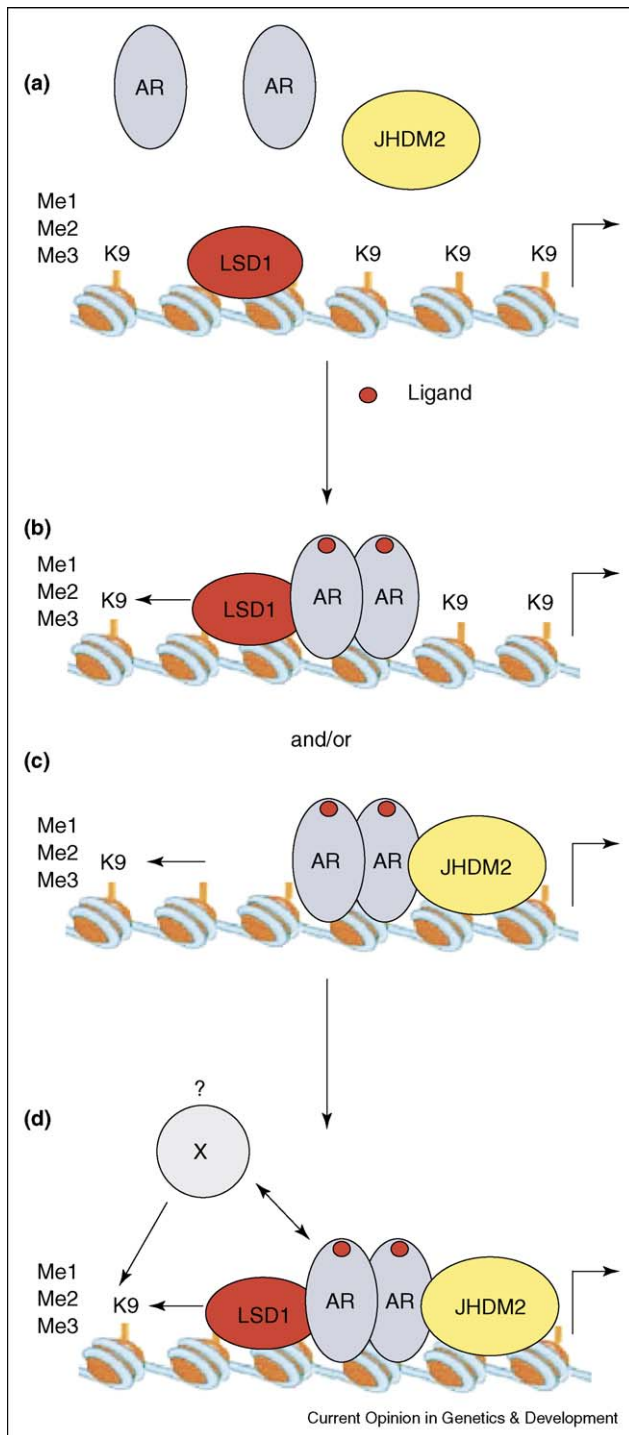
Schematic representation of three different members of the major enzyme-families that are involved in histone demethylation, and their different mechanisms of histone demethylation. **(a)** Removal of methyl group(s) from lysines is an oxidative process catalysed by flavin-dependent amine oxidases of the LSD1 family. The substrate is oxidised by FAD to generate an imin intermediate, which is subsequently hydrolysed. This mechanism requires a protonated nitrogen, and therefore precludes trimethylated lysines as a substrate [6\*\*]. **(b)** Histone demethylation catalysed by a novel group of JmjC domain-containing proteins. These metalloenzymes use the same oxidative demethylation mechanism used by members of the AlkB family of DNA demethylases, which require Fe<sup>2+</sup> and α-ketoglutarate as co-factors. No chemical restriction exists for JmjC domain-mediated demethylation [14\*\*,17\*\*]. **(c)** Protein arginine deiminases (PADs) mediate deimination of histone arginine methylation and produce citrulline and methylamine as reaction products [18\*,19\*\*].

JmjC domain-containing protein, which demethylates H3K9me<sub>2</sub> during transcriptional activation by the androgen receptor [16\*\*]. Additionally, members of the JMJD2 family have been shown to convert H3K9me<sub>3</sub> and H3K36me<sub>3</sub> to the dimethyl but not to the monomethyl or unmethylated versions both *in vivo* and *in vitro* (Figure 1b) [17\*\*].

Another category of enzymes, including peptidylarginine deiminase 4 (PAD4), regulate histone arginine

methylation by converting methylarginine to citrulline and releasing methylamine (Figure 1c) [18\*,19\*\*]. PAD4 targets multiple sites in histones H3 and H4, including H3R17 and H4R3, which are methylated by the co-activators CARM1 (co-activator-associated arginine methyltransferase 1) and PRMT1 (protein arginine methyltransferase 1), respectively [18\*,19\*\*], and PAD4 activity has been linked with the transcriptional regulation of estrogen-responsive genes in MCF-7 cells [18\*,19\*\*].

Figure 2



Demethylases regulate the transcriptional activity of the androgen receptor. LSD1 specifically associates with chromatin on the promoter regions of androgen receptor (AR) target genes in either the absence or the presence of ligand (a). Chromatin association is independent of the presence of the androgen receptor. Once the ligand-activated androgen receptor translocates to the nucleus and binds to the ARE, LSD1 and AR form a transcriptionally active multi-protein complex that demethylates H3K9me1 and H3K9me2 but fail to demethylate H3K9me3 (b) [13\*\*]. In addition, the ligand-activated AR recruits a

### Specificity of histone demethylation during androgen-dependent transcription

The androgen receptor belongs to the steroid hormone receptor family of ligand-activated transcription factors, members of which regulate diverse biological functions including cell growth and differentiation, development, homeostasis and various organ functions in the adult [20,21]. The androgen receptor shares a common modular structure with other nuclear receptors and is composed of several domains that mediate DNA binding, dimerization, ligand binding and transcriptional activity [20]. Upon hormone binding, the cytoplasmic androgen receptor dissociates from chaperones and translocates to the nucleus where it binds to androgen response elements (AREs) of target genes and regulates gene expression [22,23]. During this step, LSD1 forms a chromatin-associated complex with the ligand-activated androgen receptor and is responsible for demethylation of the H3K9me1 and H3K9me2 at androgen receptor target genes such as *prostate specific antigen (PSA)* or *kallikrein2* (Figures 2a and 2b) [13\*\*]. This step is crucial in the regulation of androgen receptor-dependent gene expression, because knockdown of *LSD1* expression is able to block androgen receptor-mediated gene expression. Interestingly, LSD1 specifically binds to the chromatinized *PSA* promoter in either the absence or the presence of ligand (Figure 2). Given that LSD1 is present on the *PSA* promoter in the absence of the androgen receptor, it is possible that LSD1 is part of a co-repressor complex and regulates K4 demethylation to silence androgen receptor target genes. However, decreases in H3K4 methylation were observed [13\*\*]. Therefore, it would be interesting to analyse the nature of the LSD1-containing co-repressor complex present on the *PSA* promoter in the absence of liganded androgen receptors. Is it the same co-repressor complex as described by Shi and co-workers [6\*\*]? Are there other important androgen receptor co-activators associated with this complex, but in an inactive form?

Recently Yamane *et al.* [16\*\*] identified JHDM2A, a second demethylase that is involved in androgen receptor-dependent gene expression. The mechanism of action of JHDM2A differs from that of LSD1. JHDM2A, which demethylates H3K9me2, does not associate with chromatin on androgen receptor-responsive genes in the absence of the receptor. As shown by Yamane *et al.*, JHDM2A interacts with the androgen receptor in a ligand-dependent manner and is recruited by the androgen receptor onto chromatin in response to hormone treatment during activation of the androgen receptor target genes *PSA* and *NKX3.1* (Figure 2c) [16\*\*]. In the regulation

second demethylase, JHDM2, which in concert with LSD1 regulates demethylation of H3K9me2, but never H3K9me1 or H3K9me3 (c) [16\*\*]. Given that ligand-dependent activation of AR target genes is associated with demethylation of H3K9me3, there must be additional demethylases that remove H3K9 trimethyl marks (d).

of androgen receptor-dependent gene activation, this second demethylase is as necessary as LSD1, because knockdown of *JHDM2A* blocks expression of androgen receptor target genes [16\*\*]. We now know of two different mechanisms involving demethylases in the transcriptional regulation of the androgen receptor (Figure 2). Both LSD1 and *JHDM2A* participate in the ligand-dependent demethylation of H3K9me2 at the *PSA* enhancer. Naturally, it would be interesting to elucidate the chronology of action of these two demethylases. Do they act sequentially in two different chromatin-recruited co-activator complexes or do they act in concert as part of the same co-activation complex? Further studies will answer these questions.

Importantly, neither LSD1 nor *JHDM2A* are able to completely remove trimethyl marks at H3K9, although complete demethylation of H3K9me3 occurs during androgen receptor-dependent gene expression on androgen receptor target genes. Thus, one hypothesis put forward is that yet another class of demethylases that removes H3K9me3 is involved in androgen receptor-dependent gene regulation (Figure 2d). Considering the pool of JmJ proteins that remain to be analysed, there is a fair likelihood of identifying members that correspond to the required specificity.

### The LSD1 demethylase is a prognostic marker in prostate cancer

We have demonstrated that LSD1 co-localises with the androgen receptor in normal human prostate and in prostate tumours (Kahl *et al.*, unpublished). Prostate cancer biology varies from locally confined tumours with a low risk of relapse to tumours with a high risk of progression even after radical prostatectomy. Currently, there are no reliable biomarkers to predict clinical outcome. It now appears that *LSD1* expression in prostate tumours correlates significantly with relapse of the tumour during anticancer treatment. LSD1 protein levels are significantly increased in high-risk tumours, making LSD1 a novel biomarker predictive of prostate cancer with aggressive biology. This suggests that LSD1 is involved in the constitutive activation of androgen receptor-mediated growth signals, thereby linking for the first time the level of expression of histone demethylase to tumour progression.

### Conclusion

Androgen receptor-mediated gene expression is a prototypic system to study demethylation in correlation with transcriptional gene activation. By now, two independent mechanisms that act either independently or in concert during androgen receptor-mediated gene expression have been described. However, this is just the beginning, and in the near future interplay between demethylases and additional transcription factors will be described. A huge bundle of open questions remains to be answered in the field of demethylation. Of those, a very exciting issue to

address is the *in vivo* function of demethylases in knockout mice models. Furthermore, it will be interesting to identify the signalling pathways that are involved in the regulation of demethylation and to reveal whether methylated non-histone proteins such as transcription factors are bona fide targets of demethylase.

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### References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Strahl BD, Allis CD: **The language of covalent histone modifications.** *Nature* 2000, **403**:41-45.
  2. Kouzarides T: **Histone methylation in transcriptional control.** *Curr Opin Genet Dev* 2002, **12**:198-209.
  3. Zhang Y, Reinberg D: **Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails.** *Genes Dev* 2001, **15**:2343-2360.
  4. Jenuwein T, Allis CD: **Translating the histone code.** *Science* 2001, **293**:1074-1080.
  5. Rice JC, Allis CD: **Histone methylation versus histone acetylation: new insights into epigenetic regulation.** *Curr Opin Cell Biol* 2001, **13**:263-273.
  6. Shi Y, Lan F, Matson C, Mulligan P, Whetstone JR, Cole PA, Casero RA, Shi Y: **Histone demethylation mediated by the nuclear amine oxidase homolog LSD1.** *Cell* 2004, **119**:941-953. The authors describe LSD1, the first histone demethylase.
  7. Aravind L, Iyer LM: **The SWIRM domain: a conserved module found in chromosomal proteins points to novel chromatin-modifying activities.** *Genome Biol* 2002, **3**: RESEARCH0039.
  8. Qian C, Zhang Q, Li S, Zeng L, Walsh MJ, Zhou MM: **Structure and chromosomal DNA binding of the SWIRM domain.** *Nat Struct Mol Biol* 2005, **12**:1078-1085.
  9. Shi Y, Sawada J, Sui G, Affar el B, Whetstone JR, Lan F, Ogawa H, Luke MP, Nakatani Y, Shi Y: **Coordinated histone modifications mediated by a CtBP co-repressor complex.** *Nature* 2003, **422**:735-738.
  10. Humphrey GW, Wang Y, Russanova VR, Hirai T, Qin J, Nakatani Y, Howard BH: **Stable histone deacetylase complexes distinguished by the presence of SANT domain proteins CoREST/kiaa0071 and Mta-L1.** *J Biol Chem* 2001, **276**:6817-6824.
  11. Hakimi MA, Bochar DA, Chenoweth J, Lane WS, Mandel G, Shiekhhattar R: **A core-BRAF35 complex containing histone deacetylase mediates repression of neuronal-specific genes.** *Proc Natl Acad Sci USA* 2002, **99**:7420-7425.
  12. Hakimi MA, Dong Y, Lane WS, Speicher DW, Shiekhhattar R: **A candidate X-linked mental retardation gene is a component of a new family of histone deacetylase-containing complexes.** *J Biol Chem* 2003, **278**:7234-7239.
  13. Metzger E, Wissmann M, Yin N, Muller JM, Schneider R, Peters AH, Gunther T, Buettner R, Schule R: **LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription.** *Nature* 2005, **437**:436-439. The authors show that LSD1 demethylates the repressive histone marks H3K9me1 and H3K9me2, thereby promoting androgen receptor target gene expression.

14. Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME,  
 ●● Borchers CH, Tempst P, Zhang Y: **Histone demethylation by a family of JmjC domain-containing proteins.** *Nature* 2006, **439**:811-816.

The authors show for the first time that JHDM1, a member of the JmjC family, is an H3K36me2 demethylase.

15. Clissold PM, Ponting CP: **JmjC: Iloenzyme-like domains in jumonji, hairless and phospholipase A2 $\beta$ .** *Trends Biochem Sci* 2001, **26**:7-9.

16. Yamane K, Toumazou C, Tsukada YI, Erdjument-Bromage H,  
 ●● Tempst P, Wong J, Zhang Y: **JHDM2A, a JmjC-Containing H3K9 demethylase, facilitates transcription activation by androgen receptor.** *Cell* 2006, **125**:483-495.

The authors show that JHDM2A is a H3K9me2 demethylase that regulates the transcriptional activity of the androgen receptor.

17. Whetstone JR, Nottke A, Lan F, Huarte M, Smolnikov S,  
 ●● Chen Z, Spooner E, Li E, Zhang G, Colaiacovo M *et al.*: **Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases.** *Cell* 2006, **125**:467-481.

The authors provide evidence that JMJD2 proteins are histone trimethylases.

18. Wang Y, Wysocka J, Sayegh J, Lee YH, Perlin JR, Leonelli L,  
 ●● Sonbuchner LS, McDonald CH, Cook RG, Dou Y *et al.*:

- Human PAD4 regulates histone arginine methylation levels via demethylation.** *Science* 2004, **306**:279-283.

The authors demonstrate that human PAD4 regulates histone arginine methylation by converting methyl-arginine to citrulline and releasing methylamine.

19. Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H,  
 ●● Hagiwara T, Yamada M, Schneider R, Gregory PD, Tempst P, Bannister AJ *et al.*: **Histone deimination antagonizes arginine methylation.** *Cell* 2004, **118**:545-553.

The authors show that deimination by PAD4 is a novel mechanism for antagonizing the transcriptional induction mediated by arginine methylation.

20. Mangelsdorf DJ, Evans RM: **The RXR heterodimers and orphan receptors.** *Cell* 1995, **83**:841-850.

21. Peterziel H, Mink S, Schonert A, Becker M, Klocker H, Cato AC: **Rapid signalling by androgen receptor in prostate cancer cells.** *Oncogene* 1999, **18**:6322-6329.

22. Glass CK, Rosenfeld MG: **The coregulator exchange in transcriptional functions of nuclear receptors.** *Genes Dev* 2000, **14**:121-141.

23. McKenna NJ, O'Malley BW: **Combinatorial control of gene expression by nuclear receptors and coregulators.** *Cell* 2002, **108**:465-474.