

## **Protein arginine methylation in estrogen signaling and estrogen-related cancers.**

Catherine Teyssier, Muriel Le Romancer, Stéphanie Sentis, Stéphan Jalaguier,  
Laura Corbo, Vincent Cavallès

► **To cite this version:**

Catherine Teyssier, Muriel Le Romancer, Stéphanie Sentis, Stéphan Jalaguier, Laura Corbo, et al.. Protein arginine methylation in estrogen signaling and estrogen-related cancers.. Trends in Endocrinology and Metabolism = Trends in Endocrinology & Metabolism , Elsevier, 2010, 21 (3), pp.181-9. 10.1016/j.tem.2009.11.002 . inserm-00452350

**HAL Id: inserm-00452350**

**<https://www.hal.inserm.fr/inserm-00452350>**

Submitted on 2 Feb 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **REVIEW ARTICLE**

2

3

4

5

6 **Protein arginine methylation in estrogen signaling and estrogen-related cancers**

7

8

9

10

11 Catherine Teyssier<sup>1</sup>, Muriel Le Romancer<sup>2</sup>, Stéphanie Sentis<sup>2</sup>, Stéphan Jalaguier<sup>3</sup>, Laura  
12 Corbo<sup>2</sup> and Vincent Cavailles<sup>3</sup>

13

14

15

16 <sup>1</sup>*INSERM, U554, Montpellier, F-34090, France ; CNRS, UMR5048, Centre de Biochimie*

17 *Structurale, Universités Montpellier 1 & 2, Montpellier, F-34090, France*

18 <sup>2</sup>*Université de Lyon 1, ISPB and IFR62, Lyon F-69003, France ; Equipe labellisée “La*

19 *Ligue,” ; U590 INSERM, Centre Léon Bérard, 28 rue Laennec, Lyon, F-69003, France*

20 <sup>3</sup>*IRCM, Institut de Recherche en Cancérologie de Montpellier, Montpellier, F-34298, France;*

21 *INSERM, U896, Montpellier, F-34298, France ; Université Montpellier1, Montpellier, F-*

22 *34298, France ; CRLC Val d’Aurelle Paul Lamarque, Montpellier, F-34298, France*

23

24 *Corresponding author: Dr Vincent Cavailles (v.cavailles@valdorel.fnclcc.fr)*

26 **Abstract:**

27

28 Estrogen signaling pathways regulate cellular processes such as proliferation and  
29 differentiation, and if deregulated, are involved in several human pathologies. Post-  
30 translational modifications (PTMs) play important roles in estrogen signaling pathways. This  
31 review focuses on recent findings pertinent to arginine methylation of non-histone proteins  
32 and their implications in estrogen signaling. We describe protein arginine methyltransferases  
33 and demethylases, the role of methylarginine proteins in estrogen action and cross-talk with  
34 other PTMs such as phosphorylation and lysine methylation. The relationships between  
35 various PTMs form a specific code which might play an important role in hormone signaling.  
36 In addition, deregulations of arginine methylation or of enzymes responsible for these  
37 modifications could be key events in estrogen-dependent cancers such as breast cancer.

38

39 Arginine methylation was first described as a post-translational modification (PTM) of  
40 histones in the late 1960s. However, only recently the responsible enzymes (Box 1) and wide  
41 variety of substrates of this modification have been identified. While the addition of each  
42 methyl group does not modify the charge of the residue, it does increase its bulkiness and  
43 hydrophobicity. Interactions of a methylated protein with its binding partners can therefore be  
44 affected by this modification and impact the physiological functions of the substrate protein.  
45 Methylarginine substrates include transcription factors, nucleic acid-binding factors, signal  
46 transducers, splicing factors and histones. Because of the large number of substrates, protein  
47 arginine methyltransferases (PRMTs) and arginine methylation regulate various cellular  
48 processes such as cell differentiation, DNA repair, RNA processing, signal transduction,  
49 cellular localization, and apoptosis [1-3]. In this review, we describe the recent studies  
50 implicating arginine methylation in estrogen transcriptional regulation and likewise, in  
51 estrogen-related diseases.

52

53

#### 54 **Role of PRMTs and methylarginine proteins in estrogen signaling**

##### 55 **Estrogen action – Genomic and non-genomic effects**

56 Nuclear estrogen receptors act mainly as ligand-activated transcription factors [4]. The  
57 binding of estrogens such as  $17\beta$ -estradiol (E2) induces a protein conformation change in the  
58 receptor that allows recruitment of coactivator complexes with chromatin-remodeling or  
59 histone-modifying activities [5] (Box 2). Steroid-regulated promoters recruit the ligand-bound  
60 receptors and regulatory proteins in an ordered, cyclical manner with multiple rounds of  
61 coactivator assembly and disassembly [6]. Histone acetylases and some methyltransferases  
62 lead to a more open chromatin and increase gene transcription. P300, an acetyltransferase, and  
63 PRMT1 and CARM1, two well-characterized PRMTs, cooperate synergistically to regulate

64 hormone target genes [7]. The activated estrogen receptor can also bind corepressors, such as  
65 RIP140 [8], which recruit enzymes with histone deacetylase activity (HDAC) to repress  
66 transcription. Anti-estrogen compounds like tamoxifen prevent steroid action by inducing a  
67 conformation in the ligand binding pocket of the receptor that fails to bind coactivators and  
68 allows the recruitment of corepressor proteins (such as NCoR and SMRT) together with  
69 HDACs [9].

70 In addition to transcriptional regulation, ER $\alpha$  also mediates events through its association with  
71 signaling molecules outside the nuclei and independent of its direct influence on the genome  
72 [10]. For example, estradiol triggers cell proliferation and cell survival through activation of  
73 MAPK kinases and Akt pathways [11]. These non-genomic actions of estrogens occur rapidly  
74 and independently of protein synthesis. At the molecular level, ER $\alpha$  palmitoylation anchors a  
75 pool of ER $\alpha$  at the plasma membrane [12] where it interacts with Src, PI3K and other scaffold  
76 proteins as MNAR (modulator of non-genomic activity of ER). This complex can therefore  
77 activate the downstream pathways [13]. Recently, novel non-genomic action of estrogens in  
78 breast cancer cells has been described, involving the association of membrane ER $\alpha$  with  
79 HDAC6. This association induces tubulin deacetylation, potentially contributing to estrogen-  
80 induced cell migration [14].

81

## 82 **Methylarginine proteins involved in estrogen action**

83 Arginine methylation affects estrogen-mediated transcription by modifying both histone and  
84 non-histone proteins. Since histone methylation has been widely described in a various  
85 number of reviews [15-17], this section focuses on methylation of non-histone proteins and  
86 their role in estrogen action (Table 1 and Figure 1).

### 87 ***Estrogen receptor***

88 Because PRMT1 [18] and CARM1 (PRMT4) [19] are ER $\alpha$  coregulators (Box 2), ER $\alpha$  could  
89 be a target for arginine methylation. Concordant with this, a recent study described ER $\alpha$  as a  
90 methylarginine substrate [20]. In this study, Le Romancer *et al.* used *in vitro* methylation  
91 assays and showed that PRMT1, but not CARM1, methylated ER $\alpha$  within the DNA binding  
92 domain. Mutation of arginine 260 into alanine (R260A) specifically abolished the  
93 modification by PRMT1. An antibody specific to methylated R260 confirmed ER $\alpha$   
94 methylation in living cells. Perhaps more interestingly, estradiol treatments of MCF7 cells  
95 drastically increased ER $\alpha$  methylation within 5 minutes of treatment. A decrease of the  
96 methylated form was observed within less than one hour, suggesting enzymatic removal of  
97 the methyl group. Indeed, this disappearance was not due to ER $\alpha$  degradation by the  
98 proteasome. Moreover, immunohistochemical experiments performed on human breast  
99 tumors showed that the methylated form of ER $\alpha$  was exclusively localized in the cytoplasm  
100 of breast epithelial cells. Since rapid effects have been described for non-genomic estrogen  
101 actions, the role of methylated ER $\alpha$  in those pathways was investigated. Interestingly,  
102 methylated ER $\alpha$  was essential for E2-induced assembly of ER $\alpha$  with Src, the p85 subunit of  
103 PI3K and the focal adhesion kinase (FAK), a Src substrate involved in the migration process.  
104 E2 activation of Akt was not observed if ER $\alpha$  R260 was mutated to an alanine. Collectively,  
105 these results show that ER $\alpha$  methylation is a prerequisite for its association with certain  
106 molecules involved in growth factor signaling. Since formation of the ER $\alpha$ /Src/PI3K/FAK  
107 complex activates Akt and corresponding downstream pathways, ER $\alpha$  methylation is likely  
108 involved in regulating cell proliferation and cell survival.

### 109 ***Transcriptional coregulators***

110 Transcriptional coregulators play critical roles in controlling ER-mediated transcription. They  
111 function through protein-protein interactions, by facilitating or inhibiting recruitment of other  
112 coregulators or specific components of the transcription machinery (Box 2). Methylation of

113 these cofactors may influence complex formation, enzymatic activity, subcellular localization,  
114 and stability, leading to a subtle regulation of ER-mediated transcription (Figure 1).

115 **SRC-3:** p/CIP/AIB1/SRC-3 is a member of the p160 coactivator family involved in CARM1  
116 and PRMT1 recruitment to ER target genes [7]. SRC-3 (steroid receptor coactivator-3)  
117 protein levels are amplified in breast cancer and associated with poor prognosis [21, 22]. In  
118 fact, studies classified SRC-3 as an authentic oncogene [23, 24]. Two independent groups  
119 found that CARM1 modifies SRC-3 [25, 26], methylating it at the C-terminal region which  
120 contains the p300 and CARM1-binding sites. SRC-3 methylation, which is induced by  
121 estradiol, leads to the dissociation of CBP and CARM1 from SRC-3. Moreover, SRC-3  
122 arginine methylation reduces its stability and causes an increase in its turnover. These results  
123 show that arginine methylation is implicated in the regulation of coregulator stability in  
124 response to estradiol. In addition to this role, arginine methylation also regulates the balance  
125 between coactivator complex assembly and disassembly. Studies suggest that repetitive  
126 association and dissociation of steroid receptors and coactivators from their target promoters  
127 may be required to maintain an activated state of transcription [6]. Altogether, these results  
128 highlight coactivator methylation as an important regulatory mechanism in hormonal  
129 signaling.

130 **CBP/p300:** Recently, Lee *et al.* reported that p300 is methylated by CARM1 at R2142 which  
131 is located within the C-terminal GRIP1 binding domain. Interestingly, methylation of R2142  
132 inhibits the interaction between p300 and GRIP1 whereas PADI 4 removes this methylation  
133 mark, thereby enhancing the p300–GRIP1 interaction. These methylation and demethylation  
134 events alter the conformation and activity of the coactivator complex and regulate estrogen  
135 receptor-mediated transcription [27]. This provides another example of arginine methylation  
136 regulating coactivator complex assembly, conformation and function.

137 **PGC-1 $\alpha$** : PGC-1 $\alpha$  (peroxisome proliferator-activated receptor gamma coactivator 1 alpha)  
138 serves as a coactivator for several nuclear receptors, including ER $\alpha$ , as well as other  
139 transcription factors, such as nuclear respiratory factor 1 (NRF-1). Expression of PGC-1 $\alpha$  is  
140 induced by a variety of physiological stimuli that regulate metabolic activity, such as  
141 exposure to cold, exercise, and fasting. PGC-1 $\alpha$  regulates metabolic processes by affecting  
142 genes involved in mitochondrial biogenesis, respiration and gluconeogenesis. Teyssier *et al.*  
143 showed that the C-terminal region of PGC-1 $\alpha$  is methylated by PRMT1 at one or more sites  
144 within a glutamate and arginine rich region. PGC-1 $\alpha$  coactivator activity and the ability of  
145 PGC-1 $\alpha$  to induce expression of target genes are both compromised by mutation of modified  
146 arginine residues of PGC-1 $\alpha$  or as a result of reduced PRMT1 levels by siRNA. Because  
147 inhibition of PRMT1 leads to inhibition of the expression of some PGC-1 $\alpha$  target genes  
148 involved in mitochondrial biogenesis, it is tempting to speculate a role for PRMT1 in this  
149 regulatory pathway [28]. However, the binding partners of methylated PGC-1 $\alpha$  remain to be  
150 determined.

151 **RIP140**: RIP140 (Receptor Interacting Protein of 140 kDa) is a well-known ER hormone-  
152 dependent binding corepressor [8]. *In vitro* and *in vivo* arginine methylation of RIP140 by  
153 PRMT1 has been described in 3T3-L1 adipocytes [29], and liquid chromatography-tandem  
154 mass spectroscopy identified that arginine methylation occurs on R240, R650, and R948 as a  
155 mono-methyl mark, suppressing RIP140 repressive activity by two mechanisms. First,  
156 methylation of R240, located in the HDAC3 interaction domain of RIP140, impairs its  
157 interaction with HDAC3, reducing its repressive function. Second, arginine methylation of the  
158 three sites increases RIP140's interaction with CRM1, a component of the export machinery;  
159 this leads to RIP140 export to the cytoplasm, thereby reducing its nuclear repressive function.  
160 So far, RIP140 arginine methylation links to adipocyte differentiation in a physiological  
161 context. RIP140 expression enhances fat accumulation in differentiated adipocytes cells by



162 inhibiting lipolysis enzyme expression, whereas a constitutive hypermethylated mutant  
163 (where arginine is replaced by phenylalanine) failed to exert an effect on fat accumulation  
164 because of reduced repressor activity [29]. Because RIP140 repressive activity is inhibited by  
165 arginine methylation and because RIP140 is a key factor in estrogen signaling, it will be of  
166 interest to verify whether RIP140 arginine methylation plays a role in estrogen genomic and  
167 non-genomic pathways.

168

169

## 170 **Arginine methylation cross-talks with other modifications**

171

### 172 **A code for protein PTMs**

173 This concept has been initially proposed for histone tails which are heavily modified by  
174 methylation, in addition to other modifications like acetylation, phosphorylation, and  
175 ubiquitination. These epigenetic marks (defined as the “histone code”) extend the genetic  
176 message beyond DNA sequences. They can be recognized or “read” by non-histone proteins  
177 containing for instance bromo- or chromodomains and participate in chromatin remodelling  
178 and transcriptional regulation [30, 31]. An important point deals with cross-talks that exist  
179 between the different PTMs targeting histone tails, i.e. the modification of one residue  
180 influencing the modification of neighbouring amino acids. Finally, recent publications  
181 extended these observations to non-histone proteins, with nuclear receptor and coregulator  
182 PTM coding emerging as a major level of regulation [32].

183

### 184 **Lysine methylation of RIP140**

185 RIP140 has been described as a substrate for several PTMs, including phosphorylation,  
186 acetylation and arginine methylation [33]. PTMs affect its subcellular distribution, protein-

187 protein interaction, and biological activity in adipocyte differentiation. Huq *et al.* found  
188 recently that endogenous RIP140 is also modified by lysine methylation in differentiated 3T3-  
189 L1 cells [34]. Using mass spectrometry, they found three lysine residues (K591, K653, and  
190 K757) as potential methylation sites. The loss of lysine methylation by mutation of the target  
191 sites enhances arginine methylation, suggesting a communication between lysine and arginine  
192 methylation. This study unraveled a potential code of modifications between lysine and  
193 arginine methylation, which regulates the functionality of a non-histone protein.

194

### 195 **Phosphorylation regulates arginine methylation**

196 The proximity of SRC-3 methylation and phosphorylation sites suggests potential cross- talk  
197 between methylation and phosphorylation of SRC-3. Indeed, Naeem *et al.* showed that  
198 phosphorylation of SRC-3 decreased its methylation by approximately fivefold, indicating  
199 that prior phosphorylation antagonizes methylation at least *in vitro* [26]. By contrast, RIP140  
200 phosphorylation by PKC $\epsilon$  triggers its arginine methylation by inducing subsequent  
201 recruitment of the chaperone 14-3-3 necessary for PRMT1 recruitment and therefore RIP140  
202 methylation [35]. The combination of these PTMs stimulates RIP140 nuclear export and  
203 decreases its repressive activity. Altogether, these studies show that arginine methylation of a  
204 non-histone protein can be influenced by phosphorylation, enhancing the arguments in favor  
205 of a code of modifications extended to coregulators.

206

### 207 **Regulation of PRMT activity: effects on estrogen signaling**

#### 208 **Protein-protein interactions**

209 Recent studies demonstrate that the methyltransferase activity of PRMTs can be modulated by  
210 protein–protein interactions. PRMT1 was initially identified as an interactor of the  
211 antiproliferative proteins BTG1 (B-cell translocation gene 1) and TIS21/BTG2, stimulating its

212 activity towards selected substrates [36, 37]. PRMT1 activity is also modulated in a substrate-  
213 dependent manner by the BTG protein partner, hCAF1 (CCR4 associated factor 1) [38],  
214 which specifically inhibits Sam68 and histone H4 Arg3 methylation [39]. Notably histone H4,  
215 when methylated by PRMT1 at Arg3, becomes a better substrate for p300, whereas  
216 acetylation of H4 by p300 inhibits its methylation by PRMT1 [40, 41]. This cross-talk has  
217 been described to contribute to the complex "histone code" in hormone signaling. Because  
218 both hCAF1 [42] and PRMT1 [18] have been described as transcriptional regulators of the  
219 nuclear receptor response, these results suggest a putative mechanism for hCAF1 in estrogen-  
220 stimulated transcription through participation in PTM coding.

221 CARM1 activity is also regulated by protein-protein interactions. Indeed, CARM1 is a  
222 component of a nucleosomal methylation activator complex (NUMAC) and interacts with  
223 BRG1 (brahma-related gene 1), among others [43]. Once CARM1 interacts with BRG1, it can  
224 then methylate histones. Moreover, CARM1 and BRG1 are both recruited to ER-target genes  
225 and cooperatively activate ER-dependent transcription [43]. Therefore, modulation of PRMT1  
226 and CARM1 activities by protein-protein interaction can be considered an important  
227 component of regulation in estrogen signaling pathway.

228

### 229 **Phosphorylation of CARM1**

230 The methyltransferase activity of CARM1 is negatively regulated by phosphorylation. Two  
231 groups describe phosphorylation of CARM1 at two different serine residues (S229, S217)  
232 during mitosis [44, 45]. Both phosphorylations abolish CARM1's ability to bind the methyl  
233 donor adenosyl-methionine and subsequently inhibit CARM1 methyltransferase activity. In  
234 both cases, CARM1 transactivation of estrogen receptor-dependent transcription is reduced.  
235 Moreover, phosphorylation at S217 promotes CARM1 cytoplasmic localization, which occurs  
236 mainly during mitosis, suggesting that the CARM1 methyltransferase activity is turned off

237 during mitosis when gene transcription is silent and turned on in G1 phase when gene  
238 transcription becomes active. Deregulation of this precise switch of CARM1 activity may  
239 affect progression of the cell cycle in breast cancer cells. Indeed, it was shown that CARM1  
240 is involved in estrogen-induced cell cycle progression of MCF-7 breast cancer cells [46].

241

242

### 243 **Arginine methylation and estrogen-dependent cancers**

244 *Prmt* knockout mice have been developed, providing interesting findings on the relevance of  
245 arginine methylation *in vivo* [1]. For instance, *Prmt1* and *Carm1* gene disruptions result in an  
246 embryonically lethal phenotype and neonatal death respectively, confirming a fundamental  
247 role for these enzymes in cellular metabolism [47, 48]. However, until now, these genetically  
248 modified animals have not provided information regarding roles for PRMT in estrogen-  
249 dependent physiological processes. By contrast, recent studies using microarray and  
250 quantitative PCR-based approaches described the aberrant expression of arginine methylation  
251 enzymes in estrogen-dependent cancers (Table 2).

252

### 253 **PRMT expression in estrogen-related cancers**

254 Several recent papers analyzed PRMT expression in estrogen-dependent cancers. *Prmt1* and  
255 *Fbxo11* expression is up-regulated in high tumor grade breast carcinomas [49, 50] and *Prmt2*,  
256 5 and 10 expression is down-regulated in breast carcinomas [51, 52]. In ovarian  
257 adenocarcinomas, *Prmt1*, 2 and 5 expression is down-regulated compared to normal tissues  
258 [53, 54].

259 *Prmt1* isoforms exist as a result of alternative mRNA splicing, and amino acid sequence  
260 comparison indicates that they are all enzymatically active, but with different N-terminal  
261 hydrophobic regions. Goulet *et al.* found that the expression profile of *Prmt1* splicing variants

262 is altered in breast cancer [55]. Furthermore, this study showed that increased *Prmt1*  
263 expression was detected in human breast tumor samples compared with adjacent normal  
264 breast tissue, confirming the studies mentioned previously. Strikingly, increased arginine-  
265 methylated protein levels were also observed in breast cancer cell lines. Therefore, an altered  
266 *Prmt1* isoform expression profile correlates with a differential pattern of arginine methylation  
267 in breast cancer cell lines, suggesting that misregulation of arginine methylation could  
268 contribute to the propagation of breast cancer.

269

#### 270 **Increased PADI4 expression in breast tumors**

271 Immunohistochemistry detected significant PADI4 expression in various malignancies  
272 including breast carcinomas, endometrial carcinomas and uterine adenocarcinomas, with no  
273 detectable PADI4 expression in benign and healthy tissues [56]. Quantitative PCR and  
274 western blot analyses also showed higher PADI4 mRNA and protein levels in malignant  
275 tissues compared to benign and non-tumor tissues [57]. Interestingly, in MCF7 breast cancer  
276 cells, *PADI4* mRNA expression gradually increased with time after estradiol stimulation  
277 through both classical and non-classical ER-mediated pathways [58]. Altogether, these results  
278 suggest increased PADI4 expression in breast cancer tissues, probably in response to  
279 estradiol.

280

#### 281 **CARM1/E2F1 breast cancer growth induced by estrogens**

282 ER $\alpha$  controls the expression of cell cycle genes which in turn mediate breast cancer  
283 proliferation. Frietze *et al.* showed that CARM1 is essential for estrogen-induced cell cycle  
284 progression in MCF-7 breast cancer cells. Upon silencing of CARM1 by siRNA, the E2-  
285 mediated stimulation of MCF-7 cell cycle progression was strongly reduced. This silencing  
286 resulted in decreased expression of E2F1 and E2F1-target genes (cyclin E1, cyclin A,

287 cdc25A), providing a direct link between CARM1 and cell cycle regulation and identifying  
288 CARM1 as a potential new target in the treatment of estrogen-dependent breast cancer [46].  
289 Aberrant expression of CARM1 has also been linked to human breast cancer [59], with  
290 elevated CARM1 levels found in aggressive breast tumors that also express high levels of the  
291 oncogenic coactivator AIB1 (amplified in breast cancer 1). Compiled high levels of CARM1  
292 and AIB1 could work in synergy to enhance target gene expression and thereby cell  
293 proliferation.

294

### 295 **ER $\alpha$ methylation in breast cancer**

296 In MCF-7 cells, E2-induced ER $\alpha$  methylation is transitory suggesting the involvement of a  
297 not yet identified arginine demethylase whose expression or activity could be deregulated in  
298 breast cancer [20]. The evaluation of methylated ER $\alpha$  with a specific antibody showed that  
299 ER $\alpha$  is hypermethylated in 50% of human breast tumors [20]. Because ER $\alpha$  methylation is  
300 necessary for estrogen-induced cellular kinase pathways, this deregulation in breast cancer  
301 could lead to sustained activation of those kinases which in turn would activate estrogen  
302 signaling. This bidirectional crosstalk appears to play a critical role in breast cancer  
303 development by maintaining the activation of signaling pathways and survival of breast  
304 cancer cells even in the presence of tamoxifen [60]. It is then tempting to speculate that the  
305 deregulation of ER $\alpha$  methylation may be involved in breast tumorigenesis and resistance to  
306 hormonal therapy. Further analyses are needed to consider methylated ER $\alpha$  a new prognostic  
307 marker.

308

### 309 **Concluding remarks**

310 Arginine methylation impacts various levels of regulation in estrogen signaling, and thereby  
311 appears to be a key regulatory event in genomic and non-genomic estrogen actions. In

312 genomic events, arginine methylation of transcriptional coregulators influences coregulators  
313 complex formation, activity, subcellular localization, and stability. Methylation of histones  
314 participates in chromatin remodeling in concert with other PTMs according to the histone  
315 code. In non-genomic events, estrogen receptor methylation is necessary for the formation of  
316 a transduction signaling complex and may participate in downstream events. Although, recent  
317 findings strongly enhanced our knowledge of the role of arginine methylation in estrogen  
318 signaling, a complete understanding will only be realized through answering fundamental  
319 questions (Box 3). For instance, since expression of other PRMTs beside PRMT1 and  
320 CARM1 is deregulated in estrogen-dependent cancers (Table 2), could other PRMTs be also  
321 involved in genomic and non-genomic estrogen regulatory mechanisms? Are other substrates  
322 implicated in estrogen signaling?

323 Deregulation of arginine methylation, methyltransferases and demethylases are described in  
324 estrogen-related cancers, strengthening the notion of a connection between arginine  
325 methylation patterns and cancer progression. Moreover, hypermethylation of ER $\alpha$  in human  
326 breast cancers may indicate that methylarginine proteins represent novel interesting  
327 prognostic biomarkers. Moreover, inhibiting methyltransferase activity, in particular that of  
328 PRMT1 and CARM1, by selective molecules appears as a potential therapeutic tool.  
329 Recently, using an approach based on a protein virtual screen, Spannhoff *et al.* identified  
330 specific PRMT1 inhibitors. These compounds operate as a brake on steroid hormone actions,  
331 suggesting their potential for future drug development in cancer therapy [61]. There is little  
332 doubt that detailed insights into the function and regulation of arginine methylation will  
333 unravel the pathogenesis of various diseases, in particular hormone-dependent cancers, and  
334 eventually contribute to the discovery of novel biological markers or therapeutic targets.

335

336 **Box 1. Arginine methylation enzymes**

337 The enzymes responsible for arginine methylation are called protein arginine  
338 methyltransferases (PRMTs). So far, eleven members have been identified in the PRMT  
339 family [62, 63]. PRMT-encoding genes are well-conserved through evolution [63], sharing  
340 common structural and functional domains (Figure I). Although circumstantial evidence over  
341 the past 40 years depicted arginine methylation as an irreversible PTM, some data suggest  
342 that this modification could be reversed. For instance, histone H4 is transiently and cyclically  
343 methylated on arginine 3 [64] and estrogen receptor  $\alpha$  methylation also appears transient [20].  
344 While enzymes capable of removing or preventing such methylation have been identified,  
345 their roles are still controversial. Peptidylarginine deiminase 4 (PADI4) has been described to  
346 convert monomethylated arginine to citrulline by deimination [27, 65, 66]. However, a full  
347 reversion would then need an enzyme to convert citrullines to arginine residues. More  
348 importantly, PADI enzymes do not deiminate methylated arginine residues *in vitro* [67, 68],  
349 and it seems rather that histone citrullination simply interferes with methylation of arginine  
350 residues. The first histone demethylase removing asymmetrical dimethylation at arginine 2 of  
351 histone H3 and symmetrical dimethylation at arginine 3 of histone H4 is the Jumonji domain-  
352 containing 6 protein (JMJD6) [69]. However, no publication has confirmed these results and,  
353 very recently, the Bottger's group demonstrated that JMJD6 is a lysine hydroxylase involved  
354 in RNA splicing indicating that demethylation of methylarginine residues is not its major  
355 activity [70]. Altogether, this suggests that additional arginine demethylases remain to be  
356 identified.

357

358



359 **Box 2. Estrogen receptor and coregulator complexes**

360 Estrogens or anti-estrogens induce ER ligand binding domain (LBD) conformational change,  
361 DNA binding to specific response elements in promoter regions of target genes and  
362 recruitment of coregulator complexes [4]. Agonist-induced LBD conformational change  
363 allows recruitment of coactivator complexes composed of p160 coactivators or PGC-1 $\alpha$  and  
364 of histone-modifying enzymes (p300, CARM1 and PRMT1), whereas antagonists permit  
365 recruitment of corepressors complexes containing SMRT, NCoR and HDAC enzymes.  
366 Recruitment of coactivator complexes helps to pull down chromatin remodeling ATPase  
367 complexes (e.g. BRG1, SWI/SNF1), which participate in chromatin remodeling. This event  
368 facilitates the recruitment of the Mediator complex and thereby of the transcription machinery  
369 (which contains among others the RNA Polymerase II and the TATA Binding Protein, TBP)  
370 to the initiation start point [6]. Enzymes associated with coactivator complexes are  
371 acetyltransferases (p300, SRC-1, SRC-3) and methyltransferases (CARM1, PRMT1). They  
372 modify histone tails inducing the open state of chromatin and gene expression. On the  
373 contrary, HDACs deacetylate histones leading to the closed conformation of chromatin and  
374 gene repression. In the presence of estradiol, ER can also bind corepressors, such as RIP140  
375 which interacts with HDACs, leading to gene repression (Figure I).

376

377

378

379 **Box 3. Outstanding questions**

380 **- Arginine methylome and methylarginine target proteins**

381 A fundamental issue is to define the entire arginine methylome in estrogen signaling, i.e. to  
382 set up proteomic approaches with high-performance mass spectrometry methods in order to  
383 describe all the methylated arginine residues in proteins involved in the estrogen pathway. In  
384 addition, although several methyl lysine-binding proteins have been identified [71], effectors  
385 for arginine-methylated proteins remain to be found. Actually, only three mammalian proteins  
386 have been demonstrated to bind methylarginine motifs through their Tudor domains [72, 73].  
387 Defining the proteins which recognize methylated arginines of estrogen receptors and  
388 coregulators will certainly enhance our understanding of the downstream cascades dependent  
389 on arginine methylation in estrogen signaling.

390 **- Regulation of arginine methylation**

391 Studies addressing the integration of signal transduction and arginine methylation pathways  
392 are also needed. Specifically, how are arginine methylation and PRMT activities regulated by  
393 PTMs such as phosphorylation, acetylation, ubiquitination, sumoylation and trans- or  
394 automethylation? How are PRMT activity and expression regulated by estrogens or other  
395 stimuli? Finally, we also clearly need a better characterization of the enzymes that  
396 demethylate arginine residues.

397 **- In vivo function of arginine methylation**

398 Most of the published studies discuss the importance of arginine methylation in a cellular  
399 context. It will be of great interest to elucidate further the *in vivo* function of the modifications  
400 in estrogen target tissues. To achieve this goal, conditional knock-out approaches targeting  
401 specifically breast, ovarian or uterus tissues together with knock-in strategies in mice with  
402 unmethylatable mutants will be of a great help.

403

404 **Acknowledgments**

405

406 This work was supported by a grant from the “Association pour la Recherche sur le Cancer”  
407 (#3169) to L.C. and V.C. and the Ligue Contre le Cancer (L.C.). C.T. is funded by the “Ligue  
408 Contre le Cancer”

409

410

411 **References**

412

- 413 1. Bedford, M.T., and Clarke, S.G. (2009) Protein arginine methylation in mammals: who,  
414 what, and why. *Mol Cell* 33, 1-13
- 415 2. Bedford, M.T., and Richard, S. (2005) Arginine methylation an emerging regulator of  
416 protein function. *Mol Cell* 18, 263-272
- 417 3. Aletta, J.M., and Hu, J.C. (2008) Protein arginine methylation in health and disease.  
418 *Biotechnol Annu Rev* 14, 203-224
- 419 4. O'Malley, B. (2008) The Year in Basic Science: nuclear receptors and coregulators. *Mol*  
420 *Endocrinol* 22, 2751-2758
- 421 5. Lonard, D.M., and O'Malley, B.W. (2006) The expanding cosmos of nuclear receptor  
422 coactivators. *Cell* 125, 411-414
- 423 6. Rosenfeld, M.G. *et al.* (2006) Sensors and signals: a coactivator/corepressor/epigenetic  
424 code for integrating signal-dependent programs of transcriptional response. *Genes Dev* 20,  
425 1405-1428
- 426 7. Lee, D.Y. *et al.* (2005) Role of protein methylation in regulation of transcription.  
427 *Endocr Rev* 26, 147-170
- 428 8. Augereau, P. *et al.* (2006) The nuclear receptor transcriptional coregulator RIP140. *Nucl*  
429 *Recept Signal* 4, e024
- 430 9. Jones, P.L., and Shi, Y.B. (2003) N-CoR-HDAC corepressor complexes: roles in  
431 transcriptional regulation by nuclear hormone receptors. *Curr Top Microbiol Immunol* 274,  
432 237-268
- 433 10. Bjornstrom, L., and Sjoberg, M. (2005) Mechanisms of estrogen receptor signaling:  
434 convergence of genomic and nongenomic actions on target genes. *Mol Endocrinol* 19, 833-  
435 842
- 436 11. Migliaccio, A. *et al.* (2006) Crosstalk between EGFR and extranuclear steroid receptors.  
437 *Ann N Y Acad Sci* 1089, 194-200
- 438 12. Acconcia, F. *et al.* (2005) Palmitoylation-dependent estrogen receptor alpha membrane  
439 localization: regulation by 17beta-estradiol. *Mol Biol Cell* 16, 231-237
- 440 13. Greger, J.G. *et al.* (2007) Phosphorylation of MNAR promotes estrogen activation of  
441 phosphatidylinositol 3-kinase. *Mol Cell Biol* 27, 1904-1913
- 442 14. Azuma, K. *et al.* (2009) Association of estrogen receptor alpha and histone deacetylase  
443 6 causes rapid deacetylation of tubulin in breast cancer cells. *Cancer Res* 69, 2935-2940
- 444 15. Litt, M. *et al.* (2009) Histone arginine methylations: their roles in chromatin dynamics  
445 and transcriptional regulation. *Biosci Rep* 29, 131-141

- 446 16. Wysocka, J. *et al.* (2006) Histone arginine methylation and its dynamic regulation.  
447 *Front Biosci* 11, 344-355
- 448 17. Pal, S., and Sif, S. (2007) Interplay between chromatin remodelers and protein arginine  
449 methyltransferases. *J Cell Physiol* 213, 306-315
- 450 18. Koh, S.S. *et al.* (2001) Synergistic enhancement of nuclear receptor function by p160  
451 coactivators and two coactivators with protein methyltransferase activities. *J Biol Chem* 276,  
452 1089-1098
- 453 19. Chen, D. *et al.* (1999) Regulation of transcription by a protein methyltransferase.  
454 *Science* 284, 2174-2177
- 455 20. Le Romancer, M. *et al.* (2008) Regulation of estrogen rapid signaling through arginine  
456 methylation by PRMT1. *Mol Cell* 31, 212-221
- 457 21. Lahusen, T. *et al.* (2009) The role and regulation of the nuclear receptor co-activator  
458 AIB1 in breast cancer. *Breast Cancer Res Treat* 116, 225-237
- 459 22. Harigopal, M. *et al.* (2009) Estrogen receptor co-activator (AIB1) protein expression by  
460 automated quantitative analysis (AQUA) in a breast cancer tissue microarray and association  
461 with patient outcome. *Breast Cancer Res Treat* 115, 77-85
- 462 23. Torres-Arzuayus, M.I. *et al.* (2004) High tumor incidence and activation of the  
463 PI3K/AKT pathway in transgenic mice define AIB1 as an oncogene. *Cancer Cell* 6, 263-274
- 464 24. Kuang, S.Q. *et al.* (2004) AIB1/SRC-3 deficiency affects insulin-like growth factor I  
465 signaling pathway and suppresses v-Ha-ras-induced breast cancer initiation and progression in  
466 mice. *Cancer Res* 64, 1875-1885
- 467 25. Feng, Q. *et al.* (2006) Signaling within a coactivator complex: methylation of SRC-  
468 3/AIB1 is a molecular switch for complex disassembly. *Mol Cell Biol* 26, 7846-7857
- 469 26. Naeem, H. *et al.* (2007) The activity and stability of the transcriptional coactivator  
470 p/CIP/SRC-3 are regulated by CARM1-dependent methylation. *Mol Cell Biol* 27, 120-134
- 471 27. Lee, Y.H. *et al.* (2005) Regulation of coactivator complex assembly and function by  
472 protein arginine methylation and demethylination. *Proc Natl Acad Sci U S A* 102, 3611-  
473 3616
- 474 28. Teyssier, C. *et al.* (2005) Activation of nuclear receptor coactivator PGC-1alpha by  
475 arginine methylation. *Genes Dev* 19, 1466-1473
- 476 29. Mostaqul Huq, M.D. *et al.* (2006) Suppression of receptor interacting protein 140  
477 repressive activity by protein arginine methylation. *Embo J* 25, 5094-5104
- 478 30. Kouzarides, T. (2007) Chromatin modifications and their function. *Cell* 128, 693-705
- 479 31. Taverna, S.D. *et al.* (2007) How chromatin-binding modules interpret histone  
480 modifications: lessons from professional pocket pickers. *Nat Struct Mol Biol* 14, 1025-1040
- 481 32. O'Malley, B.W. *et al.* (2008) Cracking the coregulator codes. *Curr Opin Cell Biol* 20,  
482 310-315
- 483 33. Mostaqul Huq, M.D. *et al.* (2008) Post-translational modifications of nuclear co-  
484 repressor RIP140: a therapeutic target for metabolic diseases. *Curr Med Chem* 15, 386-392
- 485 34. Huq, M.D. *et al.* (2009) Lysine methylation of nuclear co-repressor receptor interacting  
486 protein 140. *J Proteome Res* 8, 1156-1167
- 487 35. Gupta, P. *et al.* (2008) PKCepsilon stimulated arginine methylation of RIP140 for its  
488 nuclear-cytoplasmic export in adipocyte differentiation. *PLoS ONE* 3, e2658
- 489 36. Berthet, C. *et al.* (2002) Interaction of PRMT1 with BTG/TOB proteins in cell  
490 signalling: molecular analysis and functional aspects. *Genes Cells* 7, 29-39
- 491 37. Lin, W.J. *et al.* (1996) The mammalian immediate-early TIS21 protein and the  
492 leukemia-associated BTG1 protein interact with a protein-arginine N-methyltransferase. *J*  
493 *Biol Chem* 271, 15034-15044

- 494 38. Rouault, J.P. *et al.* (1998) Interaction of BTG1 and p53-regulated BTG2 gene products  
495 with mCaf1, the murine homolog of a component of the yeast CCR4 transcriptional  
496 regulatory complex. *J Biol Chem* 273, 22563-22569
- 497 39. Robin-Lespinasse, Y. *et al.* (2007) hCAF1, a new regulator of PRMT1-dependent  
498 arginine methylation. *J Cell Sci* 120, 638-647
- 499 40. Wang, H. *et al.* (2001) Methylation of histone H4 at arginine 3 facilitating  
500 transcriptional activation by nuclear hormone receptor. *Science* 293, 853-857
- 501 41. Huang, S. *et al.* (2005) Methylation of histone H4 by arginine methyltransferase  
502 PRMT1 is essential in vivo for many subsequent histone modifications. *Genes Dev* 19, 1885-  
503 1893
- 504 42. Prevot, D. *et al.* (2001) Relationships of the antiproliferative proteins BTG1 and BTG2  
505 with CAF1, the human homolog of a component of the yeast CCR4 transcriptional complex:  
506 involvement in estrogen receptor alpha signaling pathway. *J Biol Chem* 276, 9640-9648
- 507 43. Xu, W. *et al.* (2004) A methylation-mediator complex in hormone signaling. *Genes Dev*  
508 18, 144-156
- 509 44. Higashimoto, K. *et al.* (2007) Phosphorylation-mediated inactivation of coactivator-  
510 associated arginine methyltransferase 1. *Proc Natl Acad Sci U S A* 104, 12318-12323
- 511 45. Feng, Q. *et al.* (2009) Biochemical control of CARM1 enzymatic activity by  
512 phosphorylation. *J Biol Chem*
- 513 46. Frietze, S. *et al.* (2008) CARM1 regulates estrogen-stimulated breast cancer growth  
514 through up-regulation of E2F1. *Cancer Res* 68, 301-306
- 515 47. Pawlak, M.R. *et al.* (2000) Arginine N-methyltransferase 1 is required for early  
516 postimplantation mouse development, but cells deficient in the enzyme are viable. *Mol Cell*  
517 *Biol* 20, 4859-4869
- 518 48. Yadav, N. *et al.* (2003) Specific protein methylation defects and gene expression  
519 perturbations in coactivator-associated arginine methyltransferase 1-deficient mice. *Proc Natl*  
520 *Acad Sci U S A* 100, 6464-6468
- 521 49. Miller, L.D. *et al.* (2005) An expression signature for p53 status in human breast cancer  
522 predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci U S*  
523 *A* 102, 13550-13555
- 524 50. van 't Veer, L.J. *et al.* (2002) Gene expression profiling predicts clinical outcome of  
525 breast cancer. *Nature* 415, 530-536
- 526 51. Richardson, A.L. *et al.* (2006) X chromosomal abnormalities in basal-like human breast  
527 cancer. *Cancer Cell* 9, 121-132
- 528 52. Finak, G. *et al.* (2008) Stromal gene expression predicts clinical outcome in breast  
529 cancer. *Nat Med* 14, 518-527
- 530 53. Ivshina, A.V. *et al.* (2006) Genetic reclassification of histologic grade delineates new  
531 clinical subtypes of breast cancer. *Cancer Res* 66, 10292-10301
- 532 54. Hendrix, N.D. *et al.* (2006) Fibroblast growth factor 9 has oncogenic activity and is a  
533 downstream target of Wnt signaling in ovarian endometrioid adenocarcinomas. *Cancer Res*  
534 66, 1354-1362
- 535 55. Goulet, I. *et al.* (2007) Alternative splicing yields protein arginine methyltransferase 1  
536 isoforms with distinct activity, substrate specificity, and subcellular localization. *J Biol Chem*  
537 282, 33009-33021
- 538 56. Chang, X., and Han, J. (2006) Expression of peptidylarginine deiminase type 4 (PAD4)  
539 in various tumors. *Mol Carcinog* 45, 183-196
- 540 57. Chang, X. *et al.* (2009) Increased PADI4 expression in blood and tissues of patients  
541 with malignant tumors. *BMC Cancer* 9, 40

542 58. Dong, S. *et al.* (2007) Estrogen-enhanced peptidylarginine deiminase type IV gene  
543 (PADI4) expression in MCF-7 cells is mediated by estrogen receptor-alpha-promoted  
544 transactors activator protein-1, nuclear factor-Y, and Sp1. *Mol Endocrinol* 21, 1617-1629  
545 59. El Messaoudi, S. *et al.* (2006) Coactivator-associated arginine methyltransferase 1  
546 (CARM1) is a positive regulator of the Cyclin E1 gene. *Proc Natl Acad Sci U S A* 103,  
547 13351-13356  
548 60. Tokunaga, E. *et al.* (2006) The association between Akt activation and resistance to  
549 hormone therapy in metastatic breast cancer. *Eur J Cancer* 42, 629-635  
550 61. Spannhoff, A. *et al.* (2009) Cancer treatment of the future: inhibitors of histone  
551 methyltransferases. *Int J Biochem Cell Biol* 41, 4-11  
552 62. Lee, Y.H., and Stallcup, M.R. (2009) Minireview: protein arginine methylation of  
553 nonhistone proteins in transcriptional regulation. *Mol Endocrinol* 23, 425-433  
554 63. Krause, C.D. *et al.* (2007) Protein arginine methyltransferases: evolution and  
555 assessment of their pharmacological and therapeutic potential. *Pharmacol Ther* 113, 50-87  
556 64. Metivier, R. *et al.* (2003) Estrogen receptor-alpha directs ordered, cyclical, and  
557 combinatorial recruitment of cofactors on a natural target promoter. *Cell* 115, 751-763  
558 65. Cuthbert, G.L. *et al.* (2004) Histone deimination antagonizes arginine methylation. *Cell*  
559 118, 545-553  
560 66. Wang, Y. *et al.* (2004) Human PAD4 regulates histone arginine methylation levels via  
561 demethylination. *Science* 306, 279-283  
562 67. Hidaka, Y. *et al.* (2005) Methylation of the guanidino group of arginine residues  
563 prevents citrullination by peptidylarginine deiminase IV. *FEBS Lett* 579, 4088-4092  
564 68. Raijmakers, R. *et al.* (2007) Methylation of arginine residues interferes with  
565 citrullination by peptidylarginine deiminases in vitro. *J Mol Biol* 367, 1118-1129  
566 69. Chang, B. *et al.* (2007) JMJD6 is a histone arginine demethylase. *Science* 318, 444-447  
567 70. Webby, C.J. *et al.* (2009) Jmjd6 catalyses lysyl-hydroxylation of U2AF65, a protein  
568 associated with RNA splicing. *Science* 325, 90-93  
569 71. Kim, J. *et al.* (2006) Tudor, MBT and chromo domains gauge the degree of lysine  
570 methylation. *EMBO Rep* 7, 397-403  
571 72. Cote, J., and Richard, S. (2005) Tudor domains bind symmetrical dimethylated  
572 arginines. *J Biol Chem* 280, 28476-28483  
573 73. Cheng, D. *et al.* (2007) The arginine methyltransferase CARM1 regulates the coupling  
574 of transcription and mRNA processing. *Mol Cell* 25, 71-83  
575 74. Bauer, U.M. *et al.* (2002) Methylation at arginine 17 of histone H3 is linked to gene  
576 activation. *EMBO Rep* 3, 39-44  
577 75. Saal, L.H. *et al.* (2007) Poor prognosis in carcinoma is associated with a gene  
578 expression signature of aberrant PTEN tumor suppressor pathway activity. *Proc Natl Acad*  
579 *Sci U S A* 104, 7564-7569  
580 76. Cook, J.R. *et al.* (2006) FBXO11/PRMT9, a new protein arginine methyltransferase,  
581 symmetrically dimethylates arginine residues. *Biochem Biophys Res Commun* 342, 472-481  
582 77. Troffer-Charlier, N. *et al.* (2007) Functional insights from structures of coactivator-  
583 associated arginine methyltransferase 1 domains. *Embo J* 26, 4391-4401  
584 78. Teyssier, C. *et al.* (2002) Requirement for multiple domains of the protein arginine  
585 methyltransferase CARM1 in its transcriptional coactivator function. *J Biol Chem* 277,  
586 46066-46072  
587  
588  
589  
590  
591

592 **Figure legends**

593 **Figures inside Boxes:**

594 **Box 1. Figure I– The PRMT family**

595 The nine members of the mammalian PRMT family are shown. Recently, two related  
596 members, FBXO11 (also called PRMT9) and FBXO10, have been added to the family [63,  
597 76]. The common methyltransferase domain consists of a series of short conserved motifs  
598 (blue bars) that are important for binding the methyl donor and for catalysis. This catalytic  
599 core region is formed by a Rossmann fold and two  $\alpha$  helices (black boxes). The less conserved  
600  $\beta$ -barrel structure (gray boxes) folds against the catalytic region to form the protein substrate  
601 binding cleft [77]. Additional specific motifs, such as the SH3 domain (SH3), the zinc finger  
602 (ZnF), the myristylation motif (Myr), the FBox motif and the tetratricopeptide repeat motif  
603 (TPR) are represented in green boxes. CARM1 uniquely contains a substantial C-terminal  
604 region which contains an autonomous transcriptional activation domain [78].

605

606 **Box 2. Figure I- Gene regulation mechanism by estrogen receptor and its coregulators**

607 Estrogen receptor (ER) binds to specific regions called estrogen response element (ERE)  
608 represented by a pink box on the DNA target gene drawn as a thick black line wrapped  
609 around histones symbolized by yellow plots. The initiation start point is represented by a  
610 black arrow. The green and red arrows represent the impacts of coactivator and corepressor  
611 complexes on chromatin state and gene transcription.

612

613 **Figure 1 –Arginine methylation and estrogen signaling**

614 Methylarginine proteins are involved in genomic and non-genomic estrogen actions.

615 **(a)** In estrogen genomic action, histones and numerous estrogen receptor  $\alpha$  (ER) coregulators  
616 are substrates for PRMTs. Arginine methylation regulates their transcriptional activity (PGC-

617 1 $\alpha$ ), their subcellular localization (RIP140), their stability (SRC-3) and their complex  
618 assembly (p300-GRIP1). The removing methyl mark enzymatic function of PADI4 regulates  
619 the assembly of the complex containing GRIP1, CARM1 and p300. This action is represented  
620 by a yellow dotted line.

621 **(b)** In non-genomic estrogen action, induction of arginine methylation of cytoplasmic ER $\alpha$  by  
622 estradiol leads to activation of downstream kinase cascades and corresponding target gene  
623 activation. The role of methylated RIP140 in this non-genomic pathway remains to be  
624 demonstrated. In both figures, the methyl mark is represented by a pink circle.

625

626

627



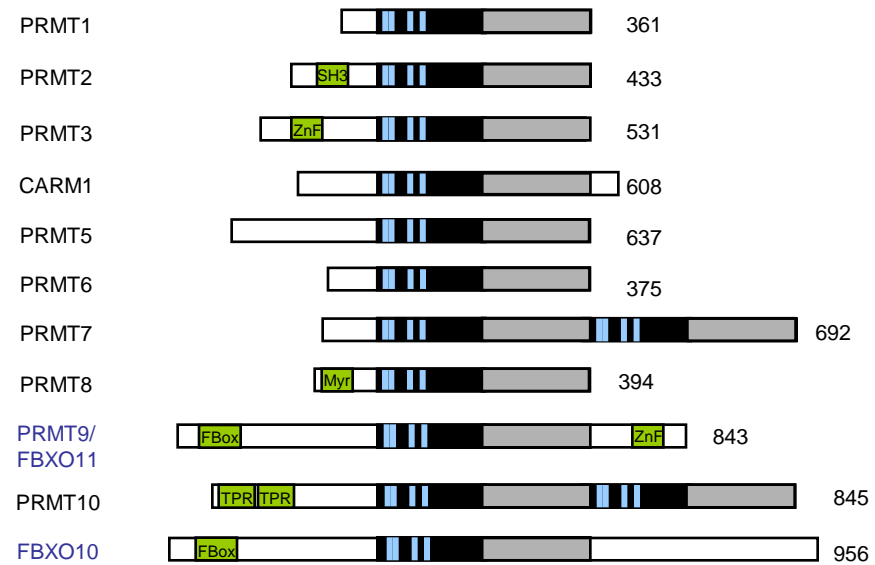


Figure I

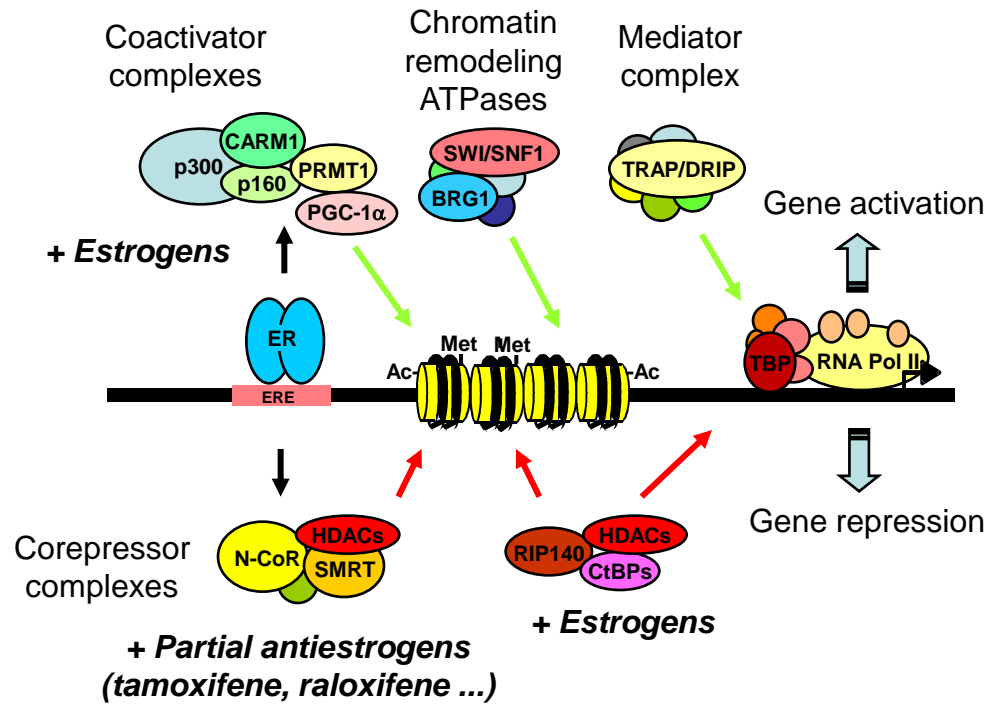
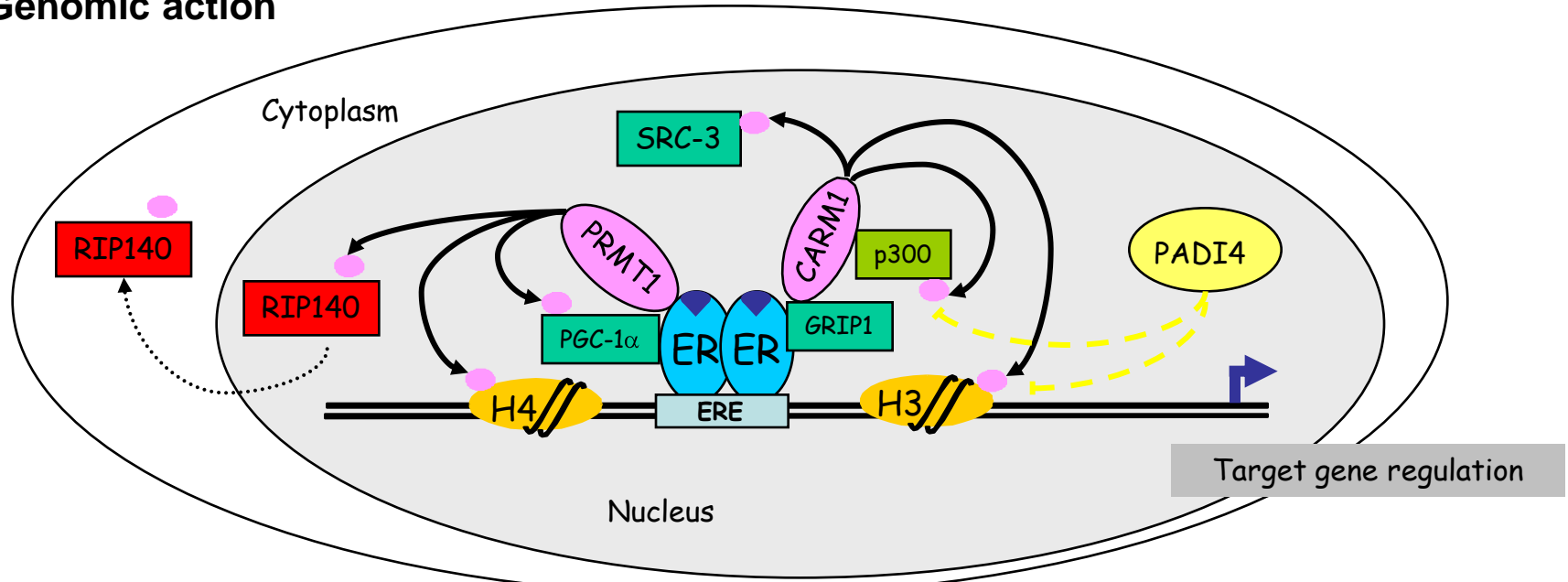


Figure I

## (a) Genomic action



## (b) Non-genomic action

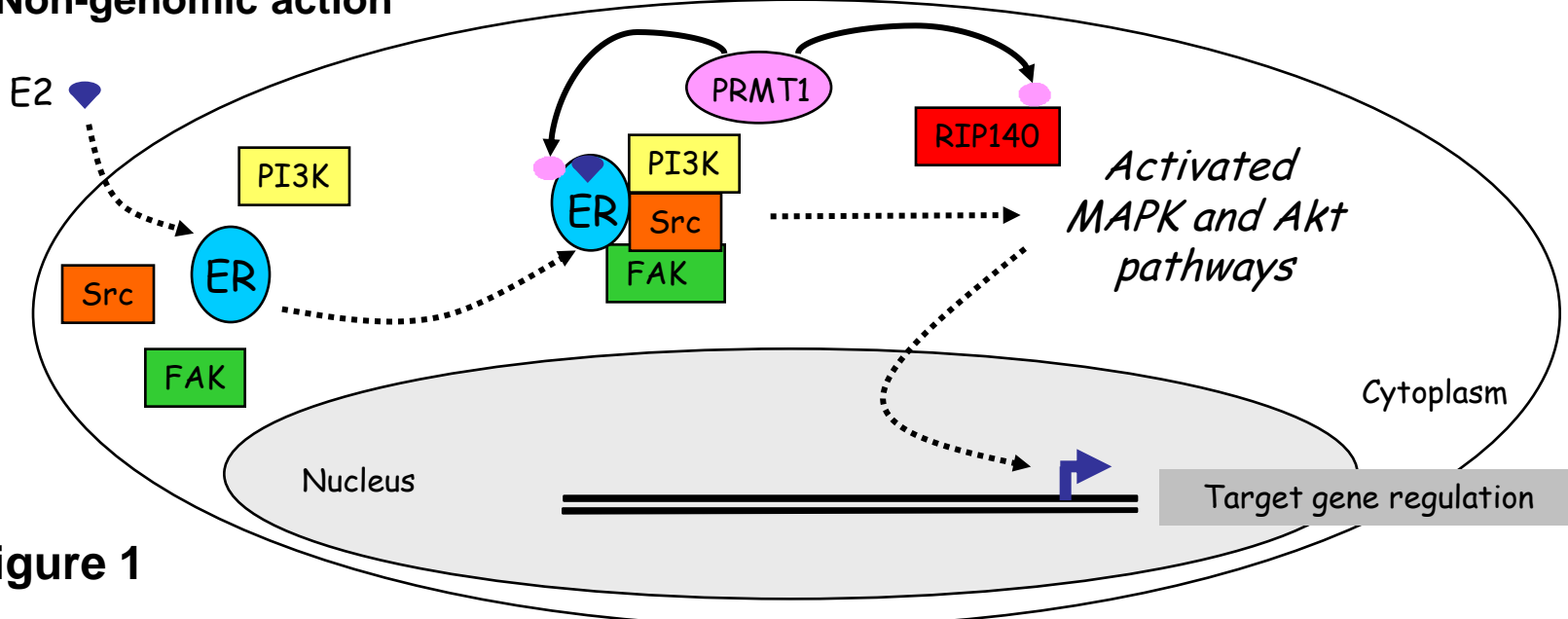


Figure 1

**Table 1. Methylarginine proteins involved in estrogen pathway**

<b>Enzyme</b>	<b>Substrates</b>	<b>Arginine (R)</b>	<b>Impact</b>	<b>Refs</b>
PRMT1	Histone H4	R3	Gene activation	[40]
	ER $\alpha$	R260	Interaction with Src and PI3K	[20]
	PGC-1 $\alpha$	R665, 667, 669	Stimulation of coactivator activity	[28]
	RIP140	R240, 650, 948	Inhibition of repressive activity	[29]
CARM1	Histone H3	R2, 17, 26	Gene activation	[19, 74]
	p300/CBP	R2142	Complex assembly regulation	[27]
	SRC-3	R1171	Complex assembly regulation	[25, 26]
		R1178, 1184, 1195	Activity and stability regulation	

**Table 2. Arginine methylation enzymes in estrogen-dependent cancers**

<b>Enzyme (Locus)</b>	<b>Expression levels</b>	<b>Refs</b>
PRMT1 (19q13.3)	Lower levels in OC Lower levels in p53 mutant BC Expression increases in high tumor grade BC Splicing variants expression altered in BC <sup>b</sup>	[53] [54] [49] [55]
PRMT2 (21q22.3)	Lower levels in ER negative BC Lower levels in OC	[51] [54]
PRMT3 (11p15.1)		
PRMT4 (19p13.2)	Higher levels in aggressive BC <sup>b</sup>	[59]
PRMT5 (14q11.2)	Lower levels in BC Lower levels in OC	[52] [54]
PRMT6 (1p13.3)		
PRMT7 (16q22.1)		
PRMT8 (12p13.3)		
FBXO11 (2p16.3)	Expression increases in high grade BC	[50]
PRMT10 (9p13.2)	Lower levels in BC Lower levels in ER negative and PR negative BC	[52] [52]
PADI4 (1p36.13)	Lower levels in ER negative BC Expression increases in BC <sup>b</sup> Expression increases in MCF-7 cells <sup>b</sup>	[75] [57] [58]
JMJD6 (17q25)	Expression increases in high tumor grade BC	[49, 53]
<sup>a</sup> Abbreviations: OC: ovarian cancer; BC: breast cancer; ER: estrogen receptor All studies were found in the OncoPrint database ( <a href="http://www.oncoPrint.org">www.oncoPrint.org</a> ) but <sup>b</sup> .		