The Androgen Receptor and Signal Transduction Pathways in Hormone Refractory Prostate Cancer: Androgen receptor co-factors and bypass pathways (part 2).

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Abstract: Prostate cancer is the second leading cause of cancer related deaths in men from the western world. Treatment of prostate cancer has relied on androgen deprivation therapy for the past 50 years. Response rates are initially high (70-80%), however almost all patients develop androgen escape and subsequently die within 1-2 years. Unlike breast cancer, alternative approaches (chemotherapy and radiotherapy) do not increase survival time. The high rate of prostate cancer mortality is therefore strongly linked to both development of androgen escape and the lack of alternate therapies.

AR mutations and amplifications can not explain all cases of androgen escape and post-translational modification of the AR has become an alternative theory. However recently it has been suggested that AR co-activators e.g. SRC-1 or pathways the bypass the AR (Ras/MAP kinase or PI3K/Akt) may stimulated prostate cancer progression independent of the AR. This review will focus on how AR coactivators may act to increase AR transactivation during sub-optimal DHT concentrations and also how signal transduction pathways may promote androgen escape via activation of transcription factors, e.g. AP-1, c-Myc and Myb, that induce cell proliferation or inhibit apoptosis.

Keywords: androgen receptor, hormone resistance, MAP kinase, PI3K, SRC-1, IL-6R, JAK and STAT3.

INTRODUCTION

In 2001 prostate cancer was responsible for approximately 10,000 deaths in the UK, making it the second most common cause of male cancer related deaths [1]. Treatment for advanced or metastatic prostate cancer has relied on androgen deprivation therapy for the past 50 years [2]. At present, few treatment options offer effective relief for patients who develop resistance to androgen deprivation. The lack of novel and effective therapies to treat this disease reflects a poor understanding of the mechanisms underlying development of both the primary disease and more particularly those events, which drive resistance. In part 1 of this review we have described how prostate cancer growth is stimulated in response to androgens and consequently how androgen deprivation therapy acts to combat this. We have then continued by explaining how modifications to the AR itself via mutations, amplification and phosphorylation may impact the development of androgen escape. However in recent years it has became increasingly apparent that androgen escape may also involve mechanisms that do not directly modify the AR. It is these mechanisms that we are going to concentrate on in this part of the review, these include AR co-factors and how they may influence AR transactivation and also how signal transduction pathways can act independent of the AR to influence prostate cancer cell growth and survival.

ACTIVATION OF THE ANDROGEN RECEPTOR

Androgen dependent transcription as described in the previous review (part 1) may be significantly enhanced by interactions between the AR and “co-activators” [3]. AR co-activators are proteins that generally do not themselves bind DNA, but are recruited to gene promoter regions through protein-protein interactions with AR, usually in a ligand dependent manner. A comprehensive list of currently known

proteins that interact with the AR is available in the appendix of a recent review by Lee and Chan, 2003 [4]. Co-activators function to facilitate assembly of transcription factors into a stable pre-initiation complex. In addition, some co-activators including steroid receptor coactivator-1 (SRC-1), cAMP response element binding protein binding protein (CBP), and p300 can also remodel chromatin by acetylating histones and recruiting the p300/CBP associated factor which harbours intrinsic histone acetyltransferase activity. When the ligand bound AR dimer binds to AREs, co-activators and p300/CBP associated factor are recruited. This loosens the nucleosomal structure of the gene, by targeted histone acetylation, and initiates the stable assembly of the pre initiation complex via their bridging function. The end result is an enhanced rate of transcription initiation by RNA polymerase II.

HORMONE RESISTANT PROSTATE CANCER

As discussed previously in part 1 the function of androgen deprivation therapy is to prevent the activation of AR mediated gene transcription. Recently it has been demonstrated that androgen escape may note only be due to modification of the AR, but may also involved the action of AR co-activators or pathways independent of AR. In the remainder of this review we summarise our current understanding of the molecular mechanisms underlying androgen escape with particular emphasis on AR co-activators and AR bypass pathways.

ANDROGEN RECEPTOR COFACTORS

AR co-factors by definition are proteins that, through binding directly or in a multi-protein complex to the AR, increase or inhibit the transcriptional activity of the AR. It is most likely that AR co-activators contribute to the development of AIPC by increasing AR transcriptional activity in the presence of low ligand concentrations or

by altering the ligand specificity of the AR, allowing antiandrogens and oestrogens to act as agonists [5].

Co-activators known to alter ligand specify of the AR include AR-associated proteins CBP, β catenin, ARA55 and ARA70 [6-8]. These co-activators can change the action of antagonists to agonists or allow other steroids to activate the AR, and may thus be important in the development of clinical androgen escape.

CBP is overexpressed in hormone refractory prostate cancer and allows hydroxyflutamide to function as an agonist in vitro [5]. The AR co-activators ARA55 and β catenin/S33F, alter AR ligand specificity and enhance AR transactivation in response to estradiol [5]. Phosphorylation of ARA55 by proline rich tyrosine kinase 2 (PYK2) decreases AR transcriptional activity as phosphorylated ARA55 cannot interact with AR. Both PYK2 and ARA55 are expressed in normal prostate epithelium, however as prostrate cancer progresses the expression of PYK2 is reduced resulting in decreased ARA55 phosphorylation and increased AR/ARA55 interaction [9]. This ultimately results in an increase in AR mediated transcription, and increased PSA expression.

ARA70 may also modify AR ligand specificity in the development of hormone refractory cancer. Yeh et al. [6] first reported ARA70 as an AR specific co-activator in 1996, and ARA70 overexpression occurs in prostate cancer and hormone refractory CWR22 xeonografts [7]. ARA70 interacts primarily with the AR ligand-binding domain, and enables antiandrogens hydroxyflutamide and bicalutamide to function as AR ligands, increasing transcriptional activity [10]. Elevated ARA70 expression in hormone refractory prostate cancer promoting AR activation by antiandrogens may contribute toward the failure of maximum androgen blockade even in the presence of wild type AR. In addition to the action that ARA70 has on anti androgens, *in vitro*

experiments demonstrate that increased ARA70 expression allows low concentrations of adrenal androgens (similar to those found in serum during maximum androgen blockade) or estradiol to activate AR [11].

However the function of ARA70 as a specific AR co-activator is disputed by two groups who demonstrated that ARA70 binds to other nuclear receptors and that up-regulation of AR activity by ARA70 does not add to the enhancement of activity caused by other co-activators [12,13]. In addition Alen et al. 1999, reported that *in vitro* mutations in the ligand binding domain of the AR that impaired the interaction with ARA70 and AR only moderately decreased AR transcriptional activity [13]. However the weight of evidence supports the role of ARA70, interacting with both wild type and mutated AR, in development of hormone refractory disease [14].

Co-activators that influence development of androgen escape by activating the AR in the absence of ligand (or at low ligand concentrations) include SRC-1, p300, Tip60, SRC-3 and c-Jun.

C-Jun functions as an AR co-activator by binding to the N-terminal binding domain at amino acids 503-555 [15]. This region contains many phosphorylation consensus sites and is critical for ligand independent transactivation of the AR [16], however, the role of phosphorylation in promoting or inhibiting AR/C-Jun interaction remains unclear at this time. Binding of c-Jun to the N-terminal binding domain promotes AR homodimerisation (via an AR N-C domain interaction) allowing AR to bind to DNA in a sequence specific manner and act as a transcription factor even in the absence of ligand [17]. However, *in vivo*, either in normal physiological or androgen depleted states, it is likely that this interaction serves to potentiate the action of AR in the present of low concentrations of ligand. It is thought that c-Jun can act in conjunction with the co-activator TIF2 (SRC-2) which is also overexpressed in

hormone refractory tumours to potentiate AR transactivation. The effect of c-Jun and TIF2 binding on AR transactivation is additive [17].

TIF-2 is a member of the steroid receptor cofactor family (SRC1, TIF2(SRC2) and AIB1 (SRC3/RAC3)). This family is commonly overexpressed in hormone refractory prostate cancer and is known to potentiate AR transcriptional activity in the presence of androgens [18]. The formation of AR homodimers may be mediated by SRC-1 which targets both the N-terminal domain and the ligand binding domain [15]. It has also been reported that the MAP kinase may increase AR activity by phosphorylating SRC-1, independent of AR phosphorylation (Fig. 1) [14,19]. This offers an alternative route for MAP kinase signal transduction to influence the development of androgen escape [3]. SRC-1 is increased in a large proportion of recurrent prostate tumours and in LNCaP cells it enhances ligand independent activation of the AR by binding to of the N-terminal binding domain. Physical interaction between the N-terminal binding domain of AR and SRC-1 is critical for androgen independent AR signalling in LNCaP cells [19]. Although such physical AR/SRC-1 interaction does not require phosphorylation of SRC-1 by MAP kinase, it is only when SRC-1 is phosphorylated by MAP kinase that the AR is activated in the absence of androgens [3]. The interaction of phosphorylated SRC-1 with the AR results in activation of the AR to the same magnitude as that obtained by DHT [20]. In the physiological situation it may be a combination of MAP kinase phosphorylating the AR to sensitise it to DHT, allowing it to enter the nucleus and MAP kinase phosphorylating SRC-1 to increase transcriptional activity. SRC3 expression correlates with decreased disease free survival and facilitates RNA polymerase II recruitment to a distant enhancer element of the PSA gene resulting in increased PSA levels in response to very low level adrenal androgens [18,21].

AR co-activator Tat interactive protein, 60 kDa (Tip60) expression and nuclear localisation increases in response to androgen withdrawal in both CWR22 prostate xenografts and LNCaP prostate cancel cells [22]. In hormone refractory tumours Tip60s exclusive nuclear localisation may mediate increased AR sensitivity to low concentrations of androgens as Tip60 is linked with transcription of PSA gene in hormone refractory cells lines and is thought to influence transcription of other AR genes by inducing changes to the acetylation status of AR [23].

Expression of the AR co-activator p300 correlations with high Gleason score and is associated with prostate cancer progression [24]. P300 is associated with proliferation of prostate cancer cells both *in vitro* and *in vivo*, and is thought to be involved with the cell cycle. In prostate cancer cell line models, IL-6 stimulated growth in the absence of androgens requires p300 and early apoptosis is not detected following p300 silencing. Therefore p300 may be important in the development of hormone refractory prostate cancer [24].

The balance of co-activators to co-repressors has also been demonstrated to influence the development of androgen escape, especially in the presence of AR antagonists. Cell line studies demonstrate that addition of bicalutamide to cell culture medium results in a slight reduction in the interaction of SRC-1 with AR. However, more significantly, when biclutamide is added to the cell culture medium a large increase in the interaction between the AR and the co-repressors SMRT is noted. However if when SRC-1 is over expressed in this cell line, this interacts with the receptor in preference to the co-repressor. Therefore, as with breast cancer resistance, androgen independence may be a combination of the association of co-activators with AR as well as recruitment of co-repressors. This balance should be investigated in the clinical situation in more detail [25]. It has been suggested that hormone independent

transcriptional activity of the AR and may be mediated solely through interactions with co-activator such as the SRC family and p300 [25].

The third mode by which AR cofactors can influence the development of androgen escape is not as well established and involves binding of co-factors to the AR resulting in AR translocation to the nucleus. An example of this is STAT3, which is a member of the JAK/STAT3 pathway. *In vitro* studies demonstrate that IL-6 activation of the JAK/STAT3 pathway is accompanied with transition from androgen sensitive to androgen insensitive prostate cancer cell growth [26](Fig. 2). Levels of activated STAT3 are significantly higher in the hormone refractory prostate cancer cell lines (DU145 and PC3) than in hormone sensitive cell lines (LNCaP cells) [27]. In LNCaP cells the activated dimer of STAT3 binds ligand free AR before entering the nucleus therefore facilitating the translocation of AR to the nucleus in the absence of androgens [28]. The AR/STAT-3 complex can activate androgen regulated gene transcription and PSA expression is elevated even in the absence of androgens [28,29]. This mechanism is supported by data that demonstrates IL-6 can activate the AR in a ligand independent manner [30]. However, it should also be noted that the oncogenic role of STAT3 in prostate cancer is not clearly established and STAT3 has also been correlated with IL-6 induced growth arrest in cell lines including LnCaP cells [31,32].

In summary, there is now strong *in vitro* evidence that implicates AR co-factors in the development of androgen escape via three routes:- altering ligand specificity, activation in the presence of low levels of androgens and translocation to the nucleus. However these have not all been demonstrated *in vivo*. The clinical evidence that is available to supports the role of co-factors in altering the specificity of AR to surrogate ligands (e.g. flutamide), may explain why PSA levels fall in some patients

following anti-androgen withdrawal. As in breast cancer, this may indicate a degree of anti-steroid therapy dependence in these tumours. These patients may respond well to removal of anti-androgen therapy. In the light of strong *in vitro* evidence for the involvement of AR co-factors in the promotion of steroid resistance, studies seeking to identify the *in vivo* significant of these findings are urgently required. Only once such evidence is available will we be able to determine the potential of these pathways as therapeutic targets in hormone insensitive prostate cancers.

ANDROGEN RECEPTOR BYPASS PATHWAYS.

As previously discussed in part 1, the MAP kinase (Fig. 1), PI3K (Fig. 3) and PKC (Fig. 4) cascades may be involved in the development of androgen escape via activation of the AR. These pathways may also however be involved in the development of androgen escape by increasing cell proliferation and decreasing apoptosis completely independently of the AR. The Ras/Raf/MAP kinase cascade may influence cell cycle regulation and/or increase cell proliferation via AP-1, c-MYC and NF-κB transcription factors [33-35] (Fig. 1). Members on the MAP kinase cascade are amplified in hormone refractory prostate cancer [36] and cell line studies demonstrate that androgen escape may be induced by transfection with Ras, resulting in increased expression and activation of MAP kinase [33]. Weber et al. 2004 demonstrated that following castration, prostate cancer recurrence in mice correlated with up-regulation of phosphorylated and hence activated MAP kinase [37]. It has also been demonstrated in human tissue that an increase in Raf expression in the transition from hormone sensitive to hormone refractory prostate cancer is associated with time to relapse and expression of activated MAP kinase increases with Gleason

score, tumour grade and androgen resistance [37,38]. Hence it is evidence that the MAP kinase cascade is associated with the development of hormone refractory cancer, however the down stream events remain to be clarified. We have recently demonstrated that those patients who express high levels of phosphorylated c-Jun survive for a significantly shorter period than those who express low levels of phosphorylated c-Jun [39]. This data supports a role for AP-1 activation possibly via the MAP kinase cascade in the development of hormone refractory prostate cancer. AP-1 is involved in control of cell growth and differentiation, and is composed of the nuclear proteins c-Jun and c-Fos, encoded by *c-jun* and *c-fos* proto oncogenes. AP-1 can either be a c-Jun/c-Jun homodimer or a c-Jun/c-Fos heterodimer, the latter being the most stable [40]. Formation of either dimer requires c-Jun phosphorylation at serine residues 63 and 73 by c-Jun N-terminus kinase (JNK)[40]. AP-1 induces transcriptional activation by binding to the TPA responsive element (TRE) [40]. TREs are recognised by both AP-1 c-Jun homodimers and c-Jun/c-Fos heterodimers [40]. AP-1 is thought to influence the development of androgen escape by competing with the AR to alter expression of androgen regulated genes (Fig. 1)[40]. AR and AP-1 are capable of binding to each other, this protein/protein interaction prevents either from being able to bind to DNA and hence results in a decrease in gene transcription [40]. However evidence also suggests that AP-1 can increase expression of androgen regulated genes by binding to a TRE domain within the promoter region [40]. Therefore the effect of AP-1 on androgen regulated gene expression could be dependent on the ratio of AR to AP-1 and the ability of free AP-1 or AR to bind to specific promoter regions within the androgen regulated gene [41]. Such competition could influence the ability of AP-1 to increase androgen regulated genes in the absence of androgens and hence might influence the development of androgen escape

[42]. This is especially important in androgen regulated genes which contain multiple TREs in the promoter region such as PSA and PSMA [43]. In a situation where the ratio of AP-1 to AR is high (e.g. in the absence of androgens), there would be less AR available to initiate transcription by binding to the ARE [40]. However there would be excess AP-1 available for binding to an alternative TRE, resulting in an increase in androgen regulated gene expression. Therefore it is conceivable that such a situation could influence the development of androgen escape i.e. increase androgen regulated gene expression in the absence of androgens [40,43]. *In vitro* work has demonstrated that in PC3 cells (prostate cancer cells which have progressed to androgen independence), the intracellular concentration of c-Jun and c-Fos is 7 fold greater than in LNCaP cells (androgen sensitive prostate cancer cells)[40]. This suggests that AP-1 influences androgen escape in the PC-3 cell line [41]. Our work *in vivo* substantiates that found in cell line studies that AP-1 is involved in the development of hormone refractory prostate cancer[39].

Similarly Akt may influence the development of androgen escape independent of AR phosphorylation. Akt has been demonstrated to have roles in control of cell apoptosis and proliferation in prostate cancer cell lines. Akt may inhibit apoptosis by suppressing the pro-apoptotic functions of BAD, via Ser^{136} phosphorylation and caspase 9 (Fig. 3) [44]. Akt may also signal for G1 cell cycle progression by mTOR and $p70^{S6K}$ which act via $p21^{CIP/WAF1}$ and hence CDK4 and cyclin D1 (Fig. 3) [45]. In addition Akt may inactivate the Forkhead family of transcription factors to decrease protein expression of $p27^{KIP1}$, a cell cycle regulator [46]. Therefore there are multiple routes by which Akt may influence the development of hormone refractory prostate cancer and all of these mechanisms have been demonstrated to function in prostate cancer cell lines [45,46].

PKC has also been demonstrated to influence the development of androgen escape in cell line studies. We have recently demonstrated that those patients who have an increase in PKC expression with the development of hormone refractory prostate cancer survive for a significantly shorter period that those who PKC expression remains unchanged or falls [39]. This does not, in contrast to data from *in vitro* studies, appear to be mediated via AP-1 activation [41, 42. Fig 4]. PKC is widely expressed in tissue and abnormal levels have been found in many transformed cell lines and tumours [47]. The PKC family consists of at least 12 isoforms that have been reported to have different and occasionally opposing roles in cell growth and differentiation [48]. The diversity of PKC isoforms was highlighted in a recent review by Mackay and Twelves[48]. They reported that PKC α, δ and ε may activate the Raf-1/MAP kinase pathway via Raf phosphorylation, PKC θ may activate the Rac1/JNK pathway via Rac-1 and PKC α, $\beta 1$ and γ may specifically inactivate GSK-3β by phosphorylation, leading to activation of the c-Jun transcription factor [48]. There is therefore significant potential for PKC to interact with many of the pathways described above. Data on the *in vivo* expression of specific PKC isoforms in hormone resistant prostate cancer is currently lacking, therefore it is difficult to speculate at present which of the above mechanisms may be responsible for the association between PKC expression and hormone escape.

In conclusion; there is strong *in vitro* signal transduction mechanisms may promote androgen escape independent of the AR via regulation of apoptosis and cell proliferation. However, as discussed below, these pathways interact significantly with AR co-activators and co-repressors as well as directly modifying the AR itself. The pluripotant nature of these signalling pathways, linked to a growing body of evidence that they provide effective therapeutic targets, suggests that future therapies for

hormone resistant prostate cancer may well be directed against specific targets within these pathways.

CONCLUSION

It is likely that prostate cancer cells achieve the transition to from androgen sensitive to androgen independent by different multistep routes, including adapting the androgen receptor pathway via the MAPK, PI3K, JAK/STAT pathways or by bypassing the androgen receptor via inhibition of apoptosis or increased cellular proliferation. In order to develop future therapies it is crucial that the molecular alterations underlying the development of androgen escape are fully understood.

As previously discussed in part 1 is now apparent that the control of AR function involves interaction of the receptor with multiple co-activators and co-repressors and that these interactions, and the function of the AR itself, are modified significantly by post-translational modification (generally via phosphorylation). The signalling pathways which mediate these modifications can also promote tumour growth by bypassing the AR completely. Given the complexity of these pathways, it is likely that prostate cancer cells achieve the transition to androgen independent growth by different multistep routes. These include co-factors, adapting the AR pathway via the MAPK, PI3K and PKC pathways or by bypassing the AR via inhibition of apoptosis or increased cellular proliferation. Further, multiple mechanisms may be active within a single cell population. However, despite this complexity, significant progress has been made and it may now be possible to predict the most fruitful avenues for future therapeutic studies.

The dominant pathways involved in the development of androgen escape both via AR modifications (as described in part 1) and independent of the AR are the MAP kinase and PI3K pathways. These may act by altering AR sensitivity to androgens

and altering expression of genes responsible for promotion of tumour growth and inhibition of apoptosis (Figs. 1 and 3). Already there seems to be sufficient evidence to begin early clinical trials of drugs that inhibit these pathways with agents such as farnesyl transferase inhibitors or Akt/mTOR based inhibitors

Current evidence suggests that in the future the most effective way of treating prostate cancer would involve profiling of individual tumours in order to identify appropriate therapies. It appears that it is only by matching therapies to individual tumours that we are going to offer significant improvement on current approach to treatment of prostate cancer. By using this approach we believe that we will begin to solve the problem of androgen escape.

REFERENCES

1 www.prostate-research.org.uk. 2003.

2 Arnold JT, Isaacs JT. Mechanisms involved in the progression of androgen-independent prostate cancers: it is not only the cancer cell's fault. *Endocrine-Related Cancer* 2002: **9**(1):61-73.

3 Ueda T, Max DT, Bruchovsky N, Sadar MD. Ligand-independent activatin of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells. *The Journal of Biological Chemistry* 2002: **277**:38087-38094.

4 Lee HJ, Chang C. Recent advances in androgen receptor action. *Cellular and Molecular Life Sciences* 2003: **60**(8):1613-1622.

5 Gelfanov VM, Burgess GS, Litz-Jackson S, King AJ, Marshall MS, Nakshatri H et al. Transformation of interleukin-3-dependent cells without participation of Stat5/bcl-xL: cooperation of akt with raf/erk leads to p65 nuclear factor kappa B-mediated antiapoptosis involving c-IAP2. *Blood* 2001: **98**(8):2508-2517.

6 Yeh S, Chang C. Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. *Proc Natl Acad Sci U S A* 1996: **93**(11):5517-5521.

7 Culig Z, Klocker H, Bartsch G, Hobisch A. Androgen receptors in prostate cancer. *Endocrine-Related Cancer* 2002: **9**(3):155-170.

8 Heinlein CA, Chang C. Androgen receptor in prostate cancer. *Endocr Rev* 2004: **25**(2):276-308.

9 Wang X, Yang Y, Guo X, Sampson ER, Hsu CL, Tsai MY et al. Suppression of androgen receptor transactivation by Pyk2 via interaction and

phosphorylation of the ARA55 coregulator. *J Biol Chem* 2002: **277**(18):15426-15431.

10 Miyamoto H, Yeh S, Wilding G, Chang C. 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* 1998: **95**(13):7379-7384.

11 Miyamoto H, Yeh S, Lardy H, Messing E, Chang C. Delta5-androstenediol is a natural hormone with androgenic activity in human prostate cancer cells. *Proc Natl Acad Sci U S A* 1998: **95**(19):11083-11088.

12 Gao T, Brantley K, Bolu E, McPhaul MJ. RFG (ARA70, ELE1) interacts with the human androgen receptor in a ligand-dependent fashion, but functions only weakly as a coactivator in cotransfection assays. *Mol Endocrinol* 1999: **13**(10):1645-1656.

13 Alen P, Claessens F, Schoenmakers E, Swinnen JV, Verhoeven G, Rombauts W et al. Interaction of the putative androgen receptor-specific coactivator ARA70/ELE1alpha with multiple steroid receptors and identification of an internally deleted ELE1beta isoform. *Mol Endocrinol* 1999: **13**(1):117-128.

14 Powell SM, Christiaens V, Voulgaraki D, Waxman J, Claessens F, Bevan CL. Mechanisms of androgen receptor signalling via steroid receptor coactivator-1 in prostate. *Endocrine-Related Cancer* 2004: **11**(1):117-130.

15 Wise SC, Burmeister LA, Zhou XF, Bubulya A, Oberfield JL, Birrer MJ et al. Identification of domains of c-Jun mediating androgen receptor transactivation. *Oncogene* 1998: **16**(15):2001-2009.

16 Rochette-Egly C. Nuclear receptors: integration of multiple signalling pathways through phosphorylation. *Cellular Signalling* 2003: **15**(4):355-366.

17 Bubulya A, Chen SY, Fisher CJ, Zheng Z, Shen XQ, Shemshedini L. c-Jun potentiates the functional interaction between the amino and carboxyl termini of the androgen receptor. *J Biol Chem* 2001: **276**(48):44704-44711.

18 Linja MJ, Porkka KP, Kang ZK, Savinainen KJ, Janne OA, Tammela TLJ et al. Expression of androgen receptor coregulators in prostate cancer. *Clinical Cancer Research* 2004: **10**(3):1032-1040.

19 Suzuki H, Ueda T, Ichikawa T, Ito H. Androgen receptor involvement in the progression of prostate cancer. *Endocrine-Related Cancer* 2003: **10**(2):209-216.

20 Ueda T, Bruchovsky N, Sadar MD. Activation of the androgen receptor N-terminal domain by interleukin-6 via MAPK and STAT3 signal transduction pathways. *The Journal of Biological Chemistry* 2002: **277**:7076-7085.

21 Louie MC, Yang HQ, Ma AH, Xu W, Zou JX, Kung HJ et al. Androgen-induced recruitment of RNA polymerase II to a nuclear receptor-p160 coactivator complex. *Proceedings of the National Academy of Sciences of the United States of America* 2003: **100**(5):2226-2230.

22 Halkidou K, Gnanapragasam VJ, Mehta PB, Logan IR, Brady ME, Cook S et al. Expression of Tip60, an androgen receptor coactivator, and its role in prostate cancer development. *Oncogene* 2003: **22**(16):2466-2477.

23 Gaughan L, Logan IR, Cook S, Neal DE, Robson CN. Tip60 and histone deacetylase 1 regulate androgen receptor activity through changes to the acetylation status of the receptor. *J Biol Chem* 2002: **277**(29):25904-25913.

24 Debes JD, Sebo TJ, Lohse CM, Murphy LM, Haugen dA, Tindall DJ. p300 in prostate cancer proliferation and progression. *Cancer Res* 2003: **63**(22):7638-7640.

25 Huang ZQ, Li JW, Wong JM. AR possesses an intrinsic hormone-independent transcriptional activity. *Molecular Endocrinology* 2002: **16**(5):924-937.

26 Lee SO, Lou W, Hou M, de Miguel F, Gerber L, Gao AC. Interleukin-6 promotes androgen-independent growth in LNCaP human prostate cancer cells. *Clinical Cancer Research* 2003: **9**(1):370-376.

27 Mora LB, Buettner R, Seigne J, Diaz J, Ahmad N, Garcia R et al. Constitutive activation of Stat3 in human prostate tumors and cell lines: Direct inhibition of Stat3 signaling induces apoptosis of prostate cancer cells. *Cancer Res* 2002: **62**(22):6659-6666.

28 Chen T, Wang LH, Farrar WL. Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. *Cancer Res* 2002: **60**(8):2132-2135.

29 Trachtenberg J, Blackledge G. Looking to the future: Advances in the management of hormone- refractory prostate cancer. *European Urology Supplements* 2002: **1**(7):44-53.

30 Corcoran NM, Costello AJ. Interleukin-6: minor player or starring role in the development of hormone-refractory prostate cancer? *BJU International* 2003: **91**(6):545-553.

31 Sanford DC, Dewille JW. C/EBPdelta is a downstream mediator of IL-6 induced growth inhibition of prostate cancer cells. *Prostate* 2004.

32 Spiotto MT, Chung TD. STAT3 mediates IL-6-induced growth inhibition in the human prostate cancer cell line LNCaP. *Prostate* 2000: **42**(2):88-98.

33 Bakin RE, Gioeli D, Sikes RA, Bissonette EA, Weber MJ. Constitutive activation of the Ras/mitogen-activated protein kinase signaling pathway promotes androgen hypersensitivity in LNCaP prostate cancer cells. *Cancer Res* 2003: **63**(8):1981-1989.

34 Bell WC, Myers RB, Hosein TO, Oelschlager DK, Grizzle WE. The response of extracellular signal-regulated kinase (ERK) to androgen-induced proliferation in the androgen-sensitive prostate cancer cell line, LNCaP. *Biotechnic & Histochemistry* 2003: **78**(1):11-16.

35 Fu Z, Smith PC, Zhang LZ, Rubin MA, Dunn RL, Yao Z et al. Effects of Raf kinase inhibitor protein expression on suppression of prostate cancer metastasis. *Journal of the National Cancer Institute* 2003: **95**(12):878-889.

36 Edwards J, Krishna NS, Witton CJ, Bartlett JMS. Gene Amplifications Associated with the Development of hormone resistant prostate cancer. *Clinical Cancer Research* 2003: **9**(14):5271-5281.

37 Weber MJ, Gioeli D. Ras signaling in prostate cancer progression. *Journal of Cellular Biochemistry* 2004: **91**(1):13-25.

38 Gioeli D, Mandell JW, Petroni GR, Frierson HF, Weber MJ. Activation of mitogen-activated protein kinase associated with prostate cancer progression. *Cancer Res* 1999: **59**(2):279-284.

39 Edwards J, Krishna NS, Mukherjee R, Bartlett JMS. The role of c-Jun and c-Fos expression in Androgen Independent Prostate Cancer. *J Pathol* 2004: **204**:153-158.

40 Sato N, Sadar MD, Bruchovsky N, Saatcioglu F, Rennie PS, Sato S et al. Androgenic induction of prostate-specific antigen gene is repressed by protein-protein interaction between the androgen receptor and AP-1/c-jun in the human prostate cancer cell line LNCaP. *J Biol Chem* 1997: **272**(28):17485-17494.

41 Bonaccorsi L, Muratori M, Carloni V, Zecchi S, Formigli L, Forti G et al. Androgen receptor and prostate cancer invasion. *International Journal of Andrology* 2003: **26**(1):21-25.

42 Sadar MD, Hussain M, Bruchovsky N. Prostate cancer ; Molecdular biology of early progression to androgen indjependence. *Endocrine related cancer* 1999: **6**:487-502.

43 Sadar MD. Androgen independent induction of prostate specific antigen gene expression via cross talk between the androgen receptor and protein kinase A signal transduction pathways. *J Biol Chem* 1999: **274**(12):7777-7783.

44 Sun M, Yang L, Feldman RI, Sun XM, Bhalla KN, Jove R et al. Activation of phosphatidylinositol 3-kinase/Akt pathway by androgen through interaction of p85 alpha, androgen receptor, and Src. *J Biol Chem* 2003: **278**(44):42992-43000.

45 Gao N, Zhang Z, Jiang BH, Shi XL. Role of PI3K/AKT/mTOR signaling in the cell cycle progression of human prostate cancer. *Biochemical And Biophysical Research Communications* 2003: **310**(4):1124-1132.

46 Li PF, Lee H, Guo SD, Unterman TG, Jenster G, Bai WL. AKT-independent protection of prostate cancer cells from apoptosis mediated through

complex formation between the androgen receptor and FKHR. *Molecular And Cellular Biology* 2003: **23**(1):104-118.

47 lahn M, Sundel K, Gleave M, Ladan F, Su C, Li S et al. Protein kinase C-alpha in prostate cancer. BJU International 93[8], 1076-1081. 2004.

48 Mackay HJ, Twelves CJ. Protein kinase C: a target for anticancer drugs? *Endocrine-Related Cancer* 2003: **10**(3):389-396.

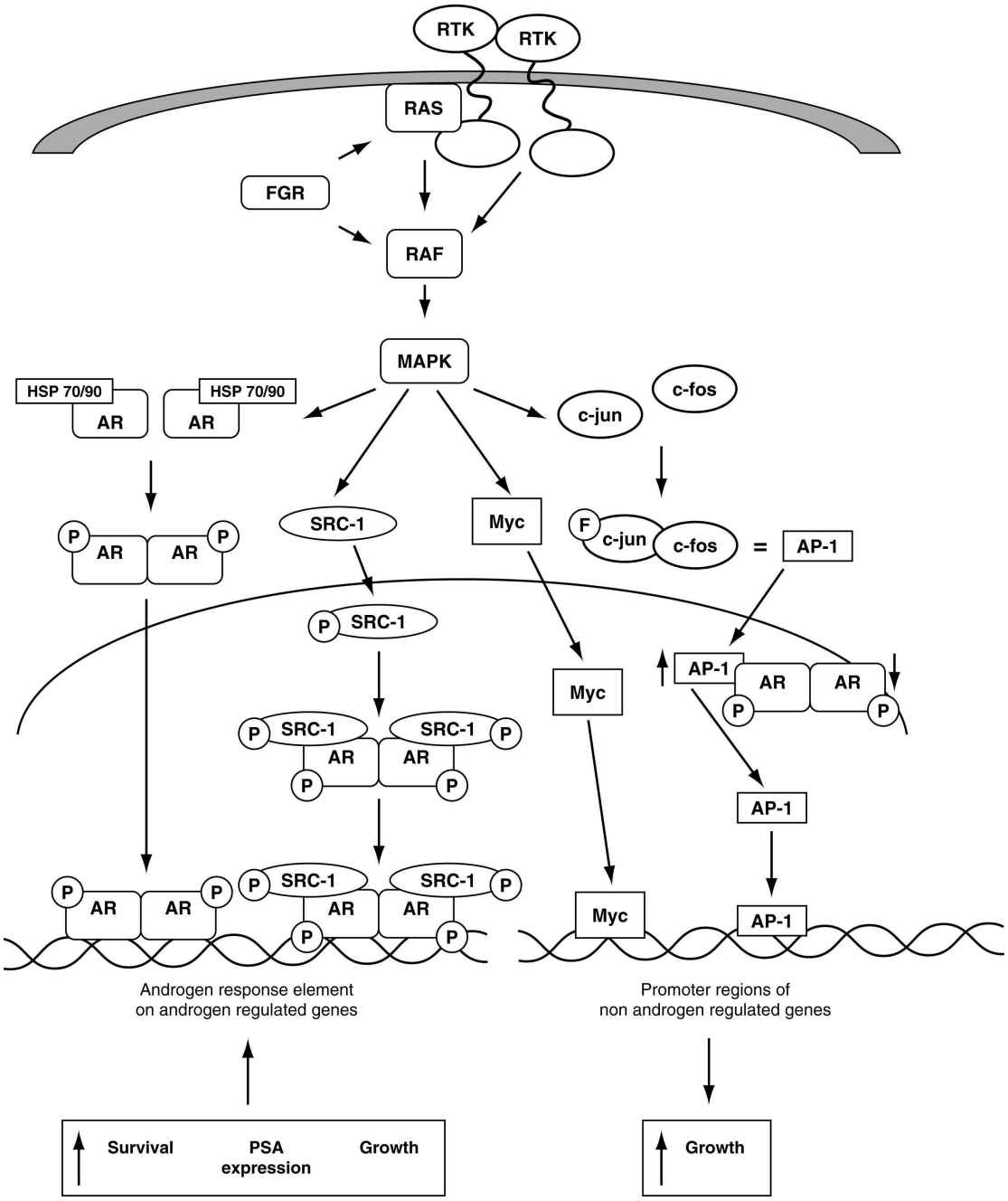
FIGURE LEGENDS

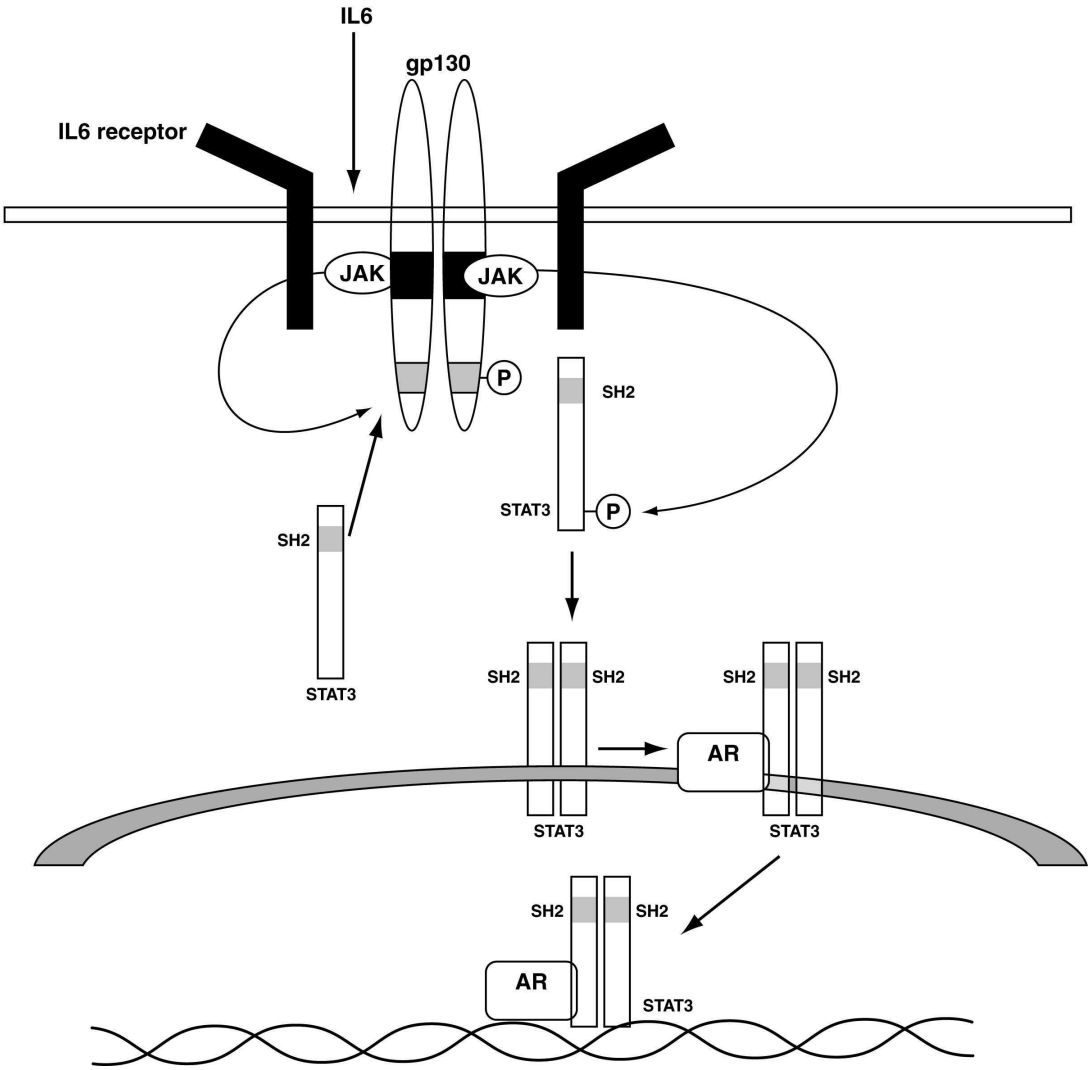
Figure 1 shows how the MAP kinase pathway impacts prostate cancer growth. AR denotes androgen receptor, HSP denotes heat shock proteins, SRC denotes steroid receptor cofactor and P denotes phosphorylation.

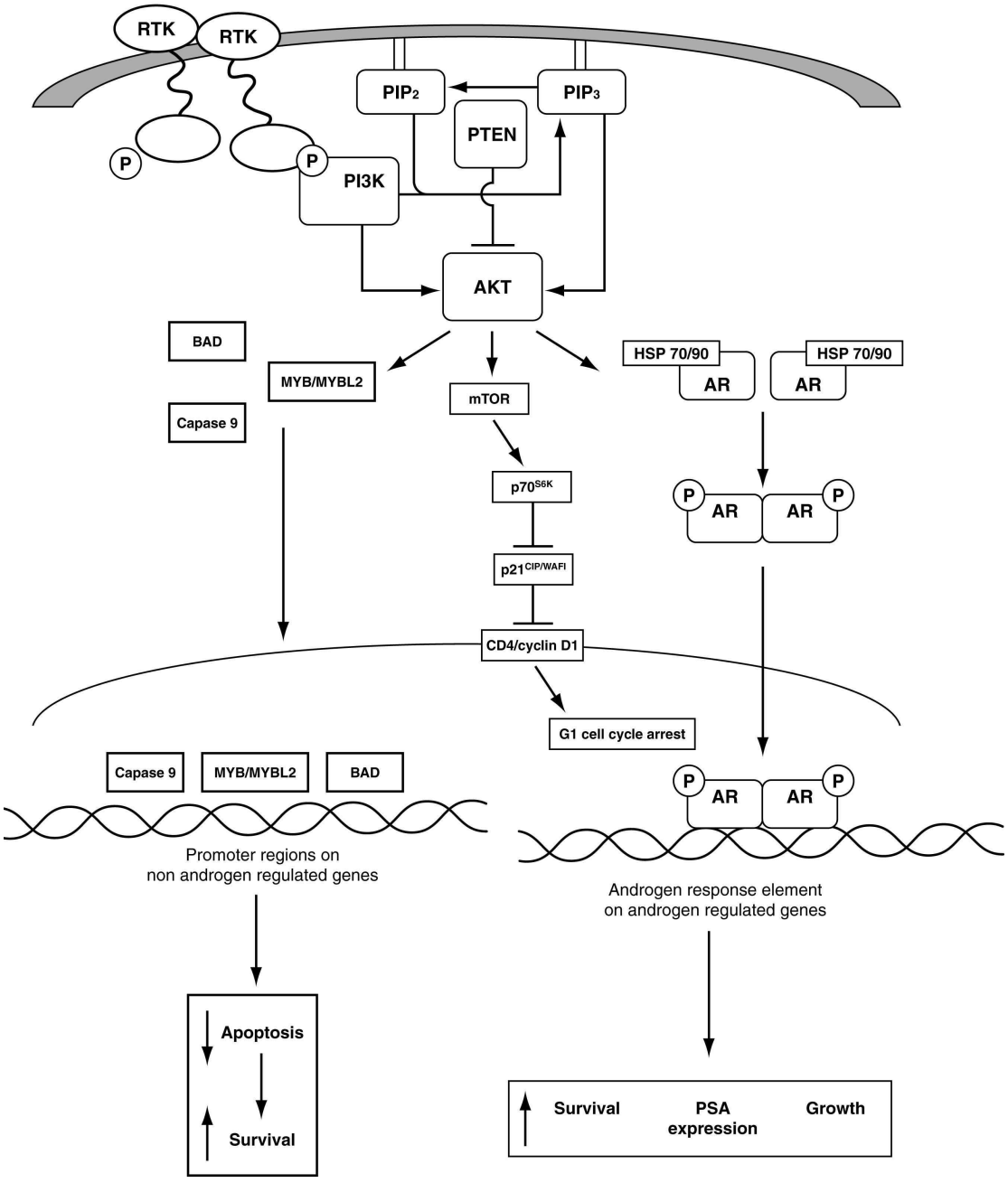
Figure 2 shows how the JAK/STAT pathways impacts prostate cancer growth. AR denotes androgen receptor, and P denotes phosphorylation

Figure 3 shows how the PI3K pathway impacts prostate cancer growth. AR denotes androgen receptor, HSP denotes heat shock proteins and P denotes phosphorylation.

Figure 4 shows how PKC impacts prostate cancer growth. AR denotes androgen receptor, HSP denotes heat shock proteins, DAG denotes diacylglycerol, PMA denotes phorbol 12-myristate 13-acetate and P denotes phosphorylation.


RTK
RTK
RAS
FGR
RAF
MAPK
HSP 70/90
AR
HSP 70/90
AR
c-jun
c-fos
P
AR
AR
P
SRC-1
Myc
F
c-jun
c-fos
=
AP-1
P
SRC-1
Myc
AP-1
AR
AR
P
P
SRC-1
SRC-1
P
AR
AR
P
P
AP-1
P
SRC-1
SRC-1
P
P
AR
AR
P
P
Myc
AP-1
Androgen response element
on androgen regulated genes
Promoter regions of
non androgen regulated genes
Survival
PSA
expression
Growth
Growth


IL6
gp130
IL6 receptor
JAK
JAK
P
SH2
STAT3
P
SH2
STAT3
SH2
SH2
SH2
SH2
AR
STAT3
STAT3
SH2
SH2
AR
STAT3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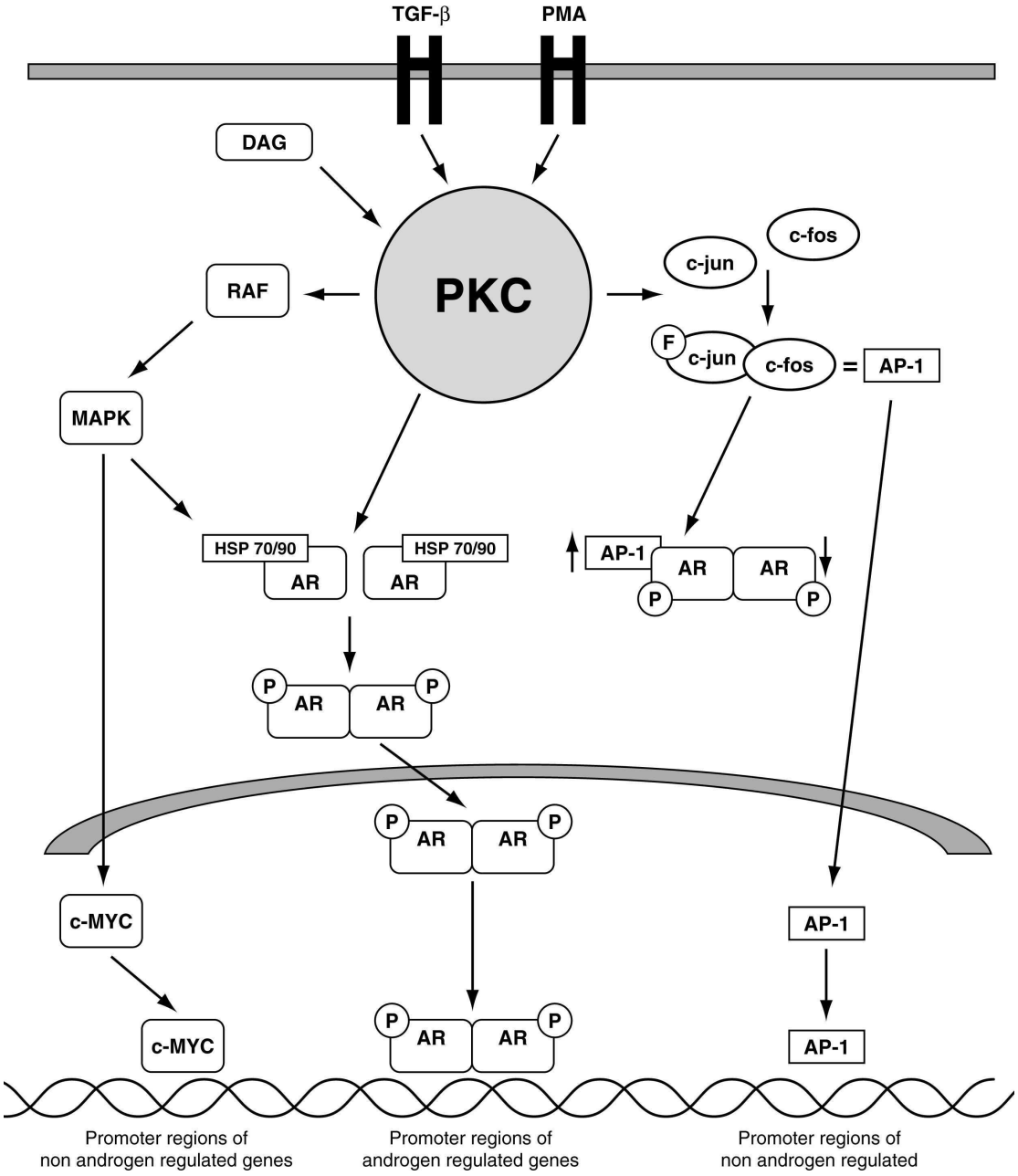
RTK
P
P
PI3K
PIP2
PIP3
PTEN
AKT
BAD
Capase 9
MYB/MYBL2
mTOR
p70S6K
p21CIP/WAFI
CD4/cyclin D1
G1 cell cycle arrest
HSP 70/90
AR
HSP 70/90
AR
P
AR
AR
P
Capase 9
MYB/MYBL2
BAD
Promoter regions on
non androgen regulated genes
P
AR
AR
P
Androgen response element
on androgen regulated genes
Apoptosis
Survival
Survival
PSA
expression
Growth


TGF-β
PMA
DAG
PKC
RAF
MAPK
c-jun
c-fos
F
c-jun
c-fos
=
AP-1
HSP 70/90
AR
HSP 70/90
AR
AP-1
AR
AR
P
P
P
AR
AR
P
P
AR
AR
P
c-MYC
c-MYC
P
AR
AR
P
AP-1
AP-1
Promoter regions of
non androgen regulated genes
Promoter regions of
androgen regulated genes
Promoter regions of
non androgen regulated