CHEK2, PCa, and the Cell Cycle

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In castration resistant PCa the impact of the Androgen Receptor ("AR") gene and its expression facilitates proliferation of the aberrant cells. This Note examines some recent work elucidating the effects of the CHK2 gene and its product Chk2 in negatively controlling AR. The result may have clinical importance.

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1 INTRODUCTION

Pathway dynamics in PCa is a complex and somewhat ever increasing set of players and counter-players. The understanding of what may activate a malignant characteristic is slow to unfold but each new piece should be examined and placed on the puzzle table for latter insertion.

In a recent disclosure as reported in Science Daily[1]:

A newly discovered connection between two common prostate cancer treatments may soon make prostate cancer cells easier to destroy. Drugs that could capitalize on the discovery are already in the pipeline, and a clinical trial to test whether the finding could improve treatments for prostate cancer patients could be only a few years away.

The discovery also may allow doctors to better determine which forms of treatment will most benefit individual patients, and there may be implications for other forms of cancer as well.

Frankly statements of this type are a hyperbole at best. The use of the term "may" all too often can be read by those who know as a "wild guess" and by those in despair as to "when". Yet with each new set of connections it is essential to examine them and ascertain if there is some new connection we can put together into the pile of what is there now. They continue:

Prostate cancer is the second-leading cause of cancer death among American men. Common treatments include radiation and androgen ablation, and researchers at the University of Virginia School of Medicine have found an unexpected link between the two.

The researchers determined that a cellular signaling pathway activated by radiation -- to halt cell division and allow repair of damage to DNA -- also controls cells' sensitivity to androgen, a male hormone prostate cancer cells need for growth. Androgen and androgen sensitivity, in turn, can affect how susceptible prostate cancer cells (and possibly other cancer cells) are to the radiation treatment used to kill them.

The statement about "radiation initiation" may be of some merit. We discuss this latter on in this note. However in PCa we are often at a loss as to the cause of the genetic changes and are all too often looking at inflammation responses or oxidation processes.

"Now we have a novel link between two different standards of care for advanced prostate cancer," said UVA researcher Dan Gioeli, PhD, of the Department of Microbiology, Immunology and Cancer Biology and the UVA Cancer Center. "For locally advanced prostate cancer, radiation therapy is one of the standards of care, and that induces DNA damage, which would activate this pathway. Another standard of care for metastatic prostate cancer is androgen ablation, and that acts to inhibit androgen receptor activity. Now we have a new molecular understanding of how those two different standards of care might be connected."

[1] https://www.sciencedaily.com/releases/2015/12/151218084505.htm

With this new information, doctors may be able to manipulate the signaling pathway, Checkpoint Kinase 2, to make it easier to kill prostate cancer cells. By blocking the signaling process, for example, they might sensitize cancer cells to the radiation intended to destroy them. Gioeli and his colleagues believe that this signaling may be lost as prostate cancer advances, helping to explain why the disease inevitably becomes resistant to androgen deprivation therapy.

Manipulating signalling pathways is all too complicated and it is one of those steps which all too often have unintended consequences. Kinase inhibitors have been known now for almost two decades and have been part of the dealing with CML for almost fifteen years. However we know in a similar fashion that the therapeutics for melanoma can work for a short while but all too often find secondary malignancies develop or the genes alter to make the therapeutic obsolete.

Major pharmaceutical companies are already developing drugs to inhibit CHK kinases, and Gioeli hopes that this will speed the clinical trial testing that could lead to better prostate cancer treatments. Testing in people might begin in only three to five years, though it may take longer depending on how the work progresses, he said.

"The next steps are to see whether our predictions about ... targeting this pathway could enhance cancer-killing in response to radiation or androgen ablation," Gioeli said. "Perhaps it would lead to a three-way combination where we would be looking at how androgen withdrawal sensitizes tumor cells to radiation therapy and whether we can further enhance that sensitization by inhibiting this pathway."

From the above mentioned paper by Ta et al we have the following specific claims which we examine in this note:

Prostate cancer is the second leading cause of cancer death in American men, and curing metastatic disease remains a significant challenge. Nearly all patients with disseminated prostate cancer initially respond to androgen deprivation therapy (ADT), but virtually all patients will relapse and develop incurable castration-resistant prostate cancer (CRPC). A high-throughput RNAi screen to identify signaling pathways regulating prostate cancer cell growth led to our discovery that checkpoint kinase 2 (CHK2) knockdowns dramatically increased prostate cancer growth and hypersensitized cells to low androgen levels.

Mechanistic investigations revealed that the effects of CHK2 were dependent on the downstream signaling proteins CDC25C and CDK1. Moreover, CHK2 depletion increased androgen receptor (AR) transcriptional activity on androgen-regulated genes, substantiating the finding that CHK2 affects prostate cancer proliferation, partly, through the AR.

This showing that CHK2 negatively controls AR, or equivalently that CHK2 inhibits AR, does beg the question of what AR was down regulated, residual AR or one of its mutated forms. Notwithstanding AR inhibition most likely would reduce proliferation especially in CRPC.

Remarkably, we further show that CHK2 is a novel AR-repressed gene, suggestive of a negative feedback loop between CHK2 and AR. In addition, we provide evidence that CHK2 physically associates with the AR and that cell-cycle inhibition increased this association. Finally, IHC

analysis of CHK2 in prostate cancer patient samples demonstrated a decrease in CHK2 expression in high-grade tumors.

In conclusion, we propose that CHK2 is a negative regulator of androgen sensitivity and prostate cancer growth, and that CHK2 signaling is lost during prostate cancer progression to castration resistance. Thus, perturbing CHK2 signaling may offer a new therapeutic approach for sensitizing CRPC to ADT and radiation.

Let us examine this summary is a bit of detail. They state:

1. CHK2 silencing, or knockdown, resulted in excess cell growth, namely growth in cancerous cells, and did so in response to low androgen levels.

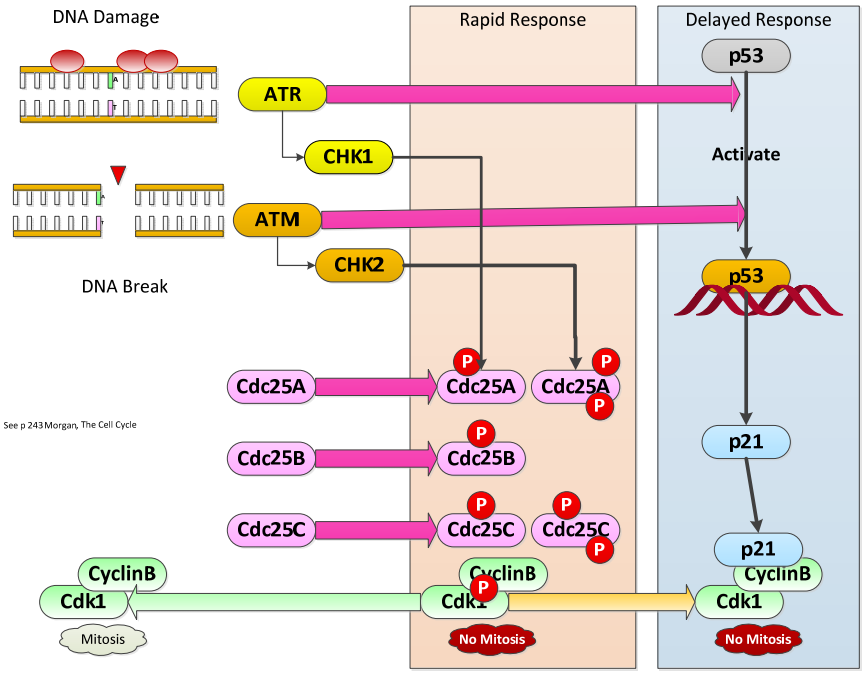
2. The two downstream genes and their expression, namely CDC25c and CDK1, were essential in effecting the outcomes of the CHK2.

3. CHK2 depletion increased AR transcription effects. They conclude a putative relationship between CHK2 control of AR expression and cell growth.

4. CHK2 putatively acts to negatively regulate AR.

5. CHK2 is inactivated or not expressed during CRPCa.

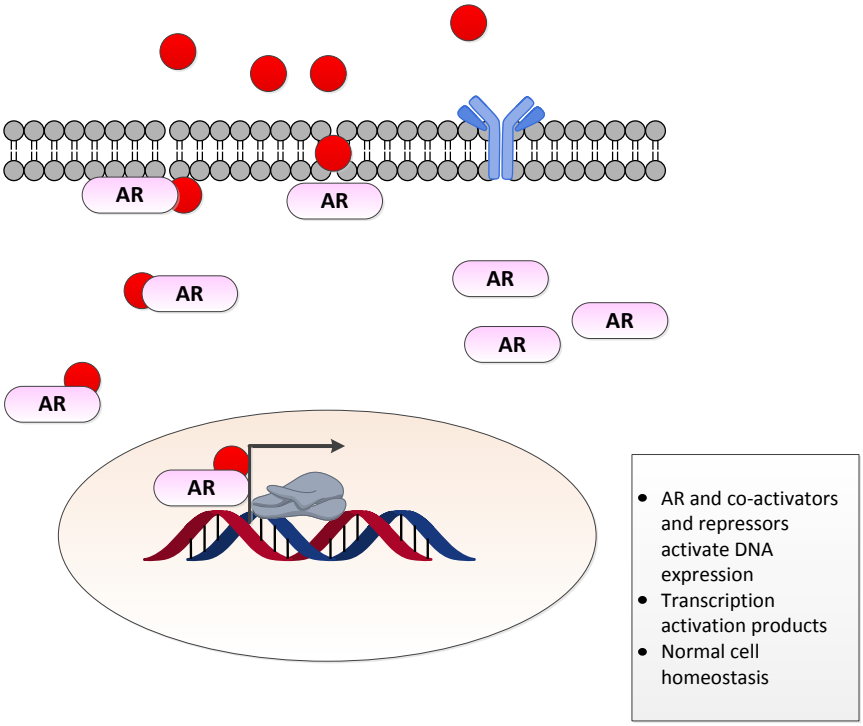
The dynamics of some of these elements are shown below:



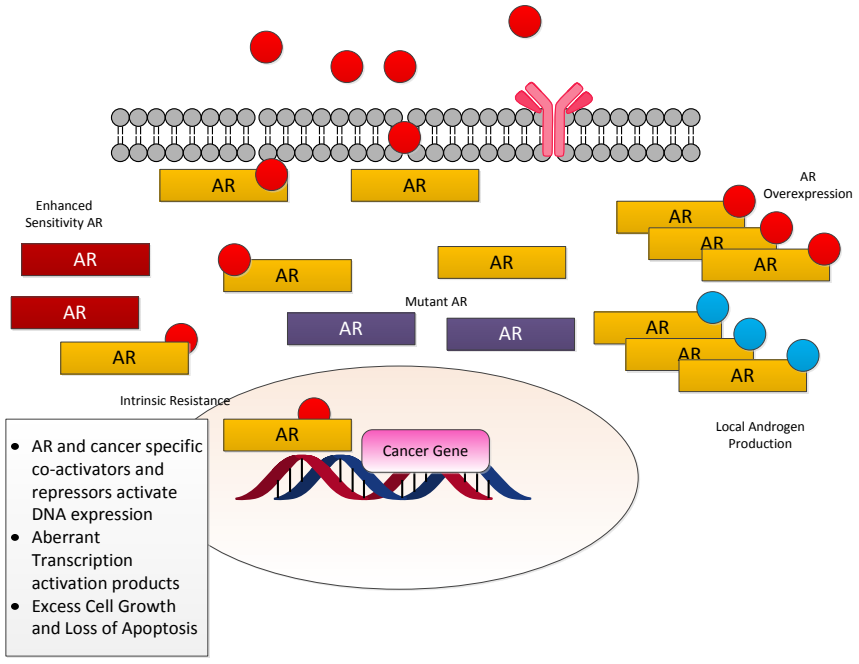
The above depicts the interaction between the Cdk1, Chk2, and Cdc25C. They can together control entry into cell proliferation. We also separately know the dynamics of AR which we shall

discuss latter. However the paper makes the argument for the nexus between AR and Chk2. Namely that Chk2 controls in some manner AR, namely suppressing it.

We know that normal AR action is as below:



We further know that CRC AR action is as below. Here we show mutant AR and enhanced sensitivity AR together increasing the AR functionality on proliferation:



Thus the mechanism for reactivating AR under mutant or otherwise conditions may be related to Chk2. Supposedly Chk2 controls this process and supposedly it is the reduction in CHK2 expression of Chk2 that is one cause of this excess proliferation. As we will note latter there is still the question of the mutant AR seen in many CRPC.

2 THE GENES

We will now briefly go through the specifics on the four genes in question and provide some basic understanding. We can then relate their effects and examine the processes that control metastatic behavior.

2.1 CHK2

We start with CHK2. We will expand on this in a separate section but the critical fact is that CHK2 and its products are essential in DSB repair and apoptosis.

We start with a description from NCBI[2]

In response to DNA damage and replication blocks, cell cycle progression is halted through the control of critical cell cycle regulators. The protein encoded by this gene is a cell cycle checkpoint regulator and putative tumor suppressor. It contains a forkhead-associated protein interaction domain essential for activation in response to DNA damage and is rapidly phosphorylated in response to replication blocks and DNA damage.

When activated, the encoded protein is known to inhibit CDC25C phosphatase, preventing entry into mitosis, and has been shown to stabilize the tumor suppressor protein p53, leading to cell cycle arrest in G1. In addition, this protein interacts with and phosphorylates BRCA1, allowing BRCA1 to restore survival after DNA damage.

Mutations in this gene have been linked with Li-Fraumeni syndrome, a highly penetrant familial cancer phenotype usually associated with inherited mutations in TP53. Also, mutations in this gene are thought to confer a predisposition to sarcomas, breast cancer, and brain tumors. This nuclear protein is a member of the CDS1 subfamily of serine/threonine protein kinases. Several transcript variants encoding different isoforms have been found for this gene.

The inhibition of CDC25C is a key observation which we will note going forward. It is an element of the overall control path. CDC25C can activate CDK1 and this as we will not is a key kinase in the cell cycle. Also note the BRCA1 relationship as well. The phosphorylation of BRCA1 or loss thereof may be another reason for BRCA1 deleted or mutated individuals having a propensity for PCa.

2.2 CDC25C

The next gene works in concert with CHK2.

From NCBI[3]:

[2] http://www.ncbi.nlm.nih.gov/gene/11200

[3] http://www.ncbi.nlm.nih.gov/gene/995

This gene encodes a conserved protein that plays a key role in the regulation of cell division. The encoded protein directs dephosphorylation of cyclin B-bound CDC2 and triggers entry into mitosis. It also suppresses p53-induced growth arrest. Multiple alternatively spliced transcript variants of this gene have been described.

As Chou et al state:

Cdc25C is a cell cycle protein of the dual specificity phosphatase family essential for activating the cdk1/Cyclin B1 complex in cells entering into mitosis. Since altered cell cycle is a hallmark of human cancers, we investigated androgen regulation of Cdc25C protein in human prostate cancer (PCa) cells, including androgen-sensitive (AS) LNCaP C-33 cells and androgen independent (AI) LNCaP C-81 as well as PC-3 cells.

In the regular culture condition containing fetal bovine serum (FBS), Cdc25C protein levels were similar in these PCa cells. In a steroid-reduced condition, Cdc25C protein was greatly decreased in AS C-33 cells but not AI C-81 or PC-3 cells. In androgen-treated C-33 cells, the Cdc25C protein level was greatly elevated, following a dose- and a time-dependent manner, correlating with increased cell proliferation. This androgen effect was blocked by Casodex, an androgen receptor blocker.

Nevertheless, epidermal growth factor (EGF), a growth stimulator of PCa cells, could only increase Cdc25C protein level by about 1.5-fold. Altered expression of Cdc25C in C-33 cells and PC-3 cells by cDNA and/or shRNA transfection is associated with the corresponding changes of cell growth and Cyclin B1 protein level.

Actinomycin D and cycloheximide could only partially block androgen-induced Cdc25C protein level. Treatments with both proteasomal and lysosomal inhibitors resulted in elevated Cdc25C protein levels. Immunoprecipitation revealed that androgens reduced the ubiquitination of Cdc25C proteins.

These results show for the first time that Cdc25C protein plays a role in regulating PCa cell growth, and androgen treatments, but not EGF, greatly increase Cdc25C protein levels in AS PCa cells, which is in part by decreasing its degradation. These results can lead to advanced PCa therapy via up-regulating the degradation pathways of Cdc25C protein.

CDC25C expressions are thus clearly elements in the development of PCa.

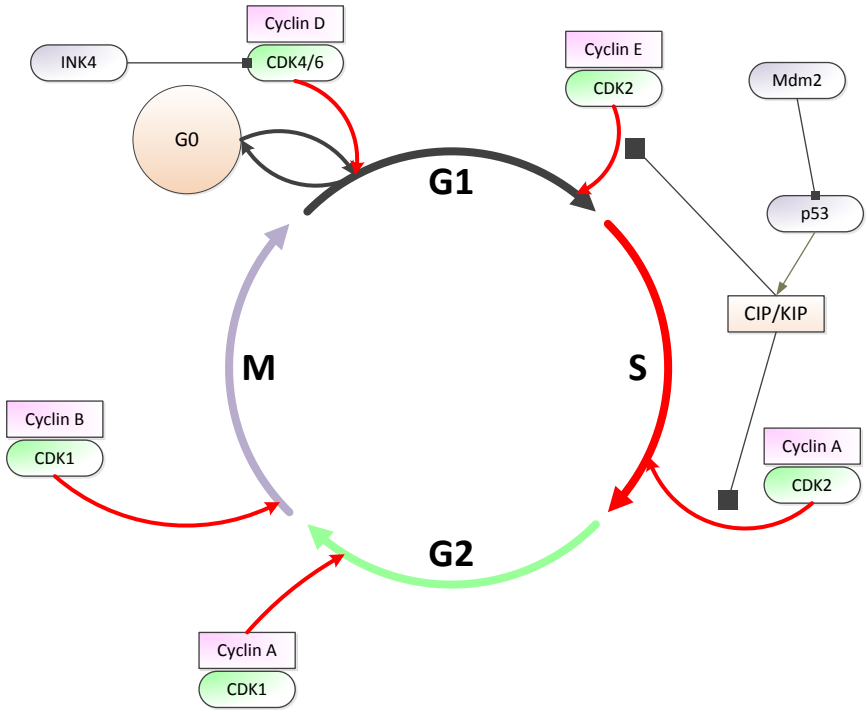
2.3 CDK1

The cyclin dependent kinases, CDKs, are key elements in the operation of the cell cycle and thus key to functional proliferation. Any suppression of them would suppress proliferation and any significant activation would putatively result in excess proliferation. Thus cells have an over activated path controlling these genes would have a proliferation advantage and could arguably be the basis for metastatic growth.

We again use the description from NCBI[4]:

The protein encoded by this gene is a member of the Ser/Thr protein kinase family. This protein is a catalytic subunit of the highly conserved protein kinase complex known as M-phase promoting factor (MPF), which is essential for G1/S and G2/M phase transitions of eukaryotic cell cycle. Mitotic cyclins stably associate with this protein and function as regulatory subunits. The kinase activity of this protein is controlled by cyclin accumulation and destruction through the cell cycle. The phosphorylation and dephosphorylation of this protein also play important regulatory roles in cell cycle control. Alternatively spliced transcript variants encoding different isoforms have been found for this gene.

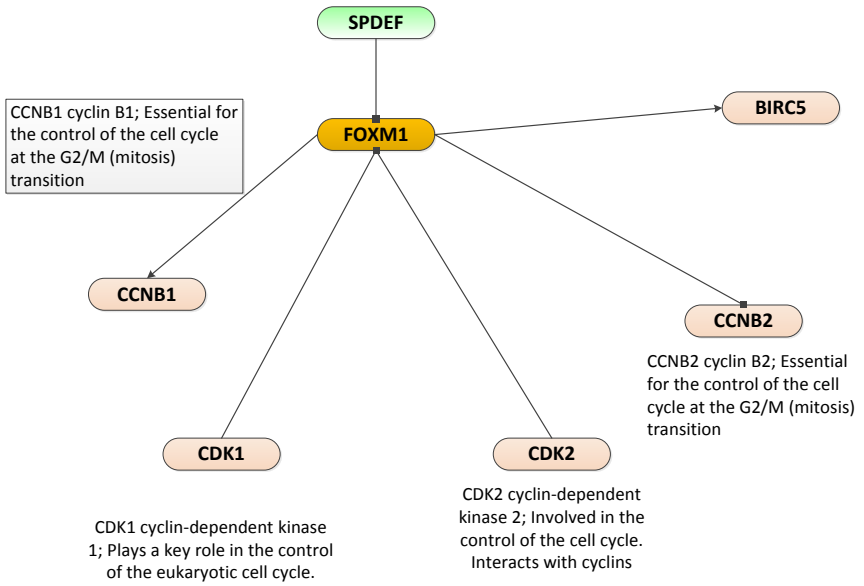
As we have demonstrated previously the cell cycle shown below uses a variety of CDKs in its processing. CDK1 is used in G2 and M and it is essential for the final phase.



It is also useful as shown in the Figure below to understand that CDKs are controlled as well by FOXM and in turn by SPDEF. We have examined this in detail elsewhere[5].

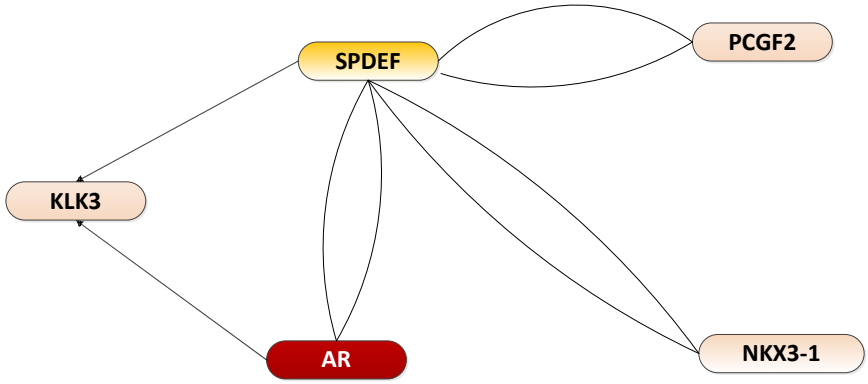
[4] http://www.ncbi.nlm.nih.gov/gene/983

[5] See http://www.telmarc.com/Documents/White%20Papers/117SPDEF.pdf



The SPDEF relationship is of merit but not essential in the current analysis. We also have the following.

The Figure below details a putative pathway element between SPDEF and AR[6]:



http://www.pathwaycommons.org/pc/record2.do?id=64346

There is a connection between SPDEF functioning and AR as well as CDK1. Whether this is a connection of merit between CHK2 functioning and AR is apparently unknown at this time.

2.4 AR

The androgen receptor gene, AR, plays a key role in PCa. AR provides for transcription and proliferation.

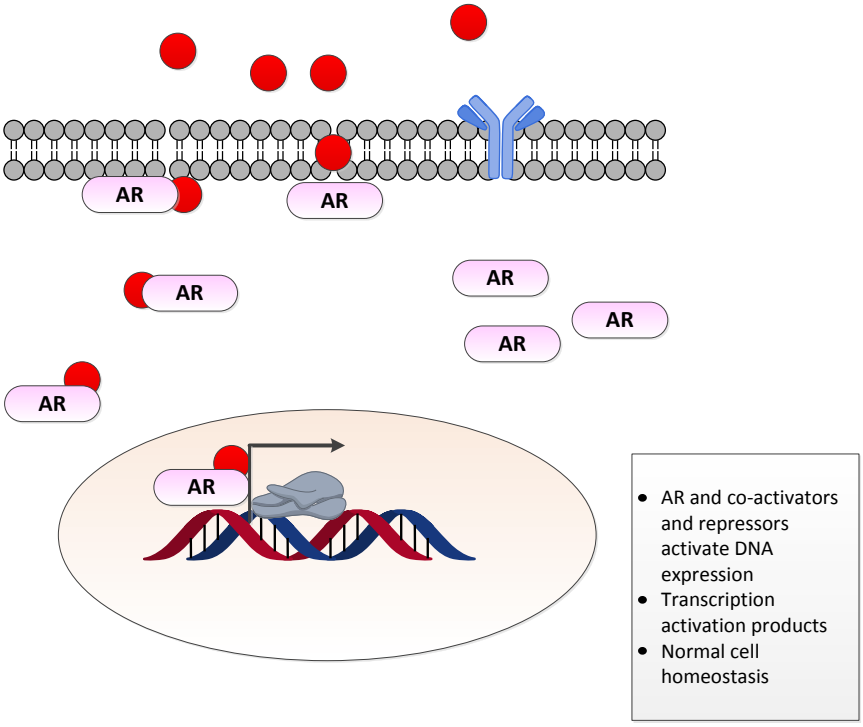
We use the description again from NCBI[7]:

[6] See also: https://targetexplorer.ingenuity.com/gene/EG/25803/pathways

The androgen receptor gene is more than 90 kb long and codes for a protein that has 3 major functional domains: the N-terminal domain, DNA-binding domain, and androgen-binding domain. The protein functions as a steroid-hormone activated transcription factor. Upon binding the hormone ligand, the receptor dissociates from accessory proteins, translocates into the nucleus, dimerizes, and then stimulates transcription of androgen responsive genes. This gene contains 2 polymorphic trinucleotide repeat segments that encode polyglutamine and polyglycine tracts in the N-terminal transactivation domain of its protein.

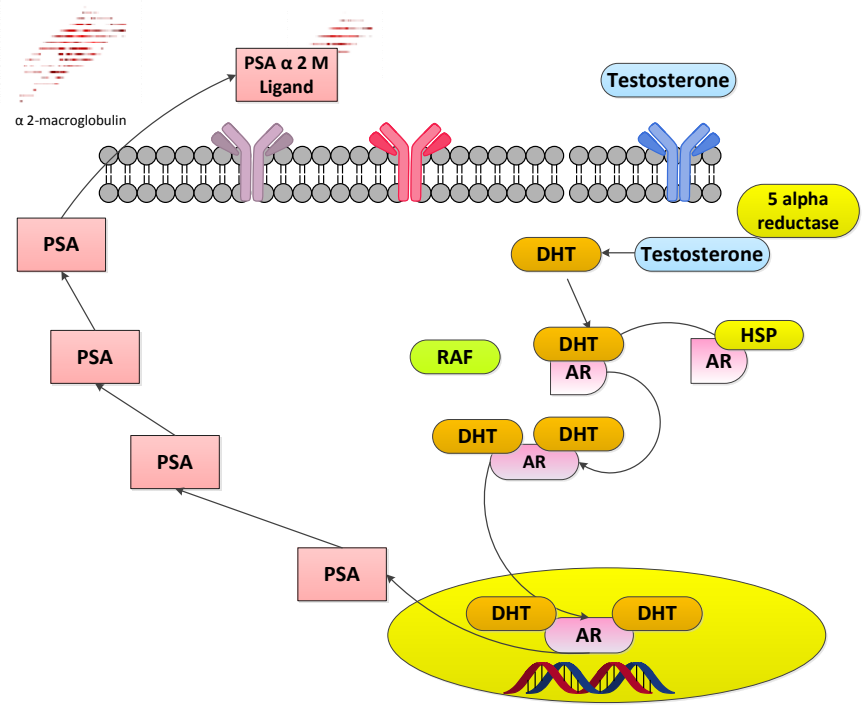
Expansion of the polyglutamine tract causes spinal bulbar muscular atrophy (Kennedy disease). Mutations in this gene are also associated with complete androgen insensitivity (CAIS). Two alternatively spliced variants encoding distinct isoforms have been described.

We demonstrate in the Figure below the normal functioning of the AR gene. AR is involved in normal transcription and cell homeostasis. It receives signals via receptors and responds accordingly. This is a generally well known mechanism.

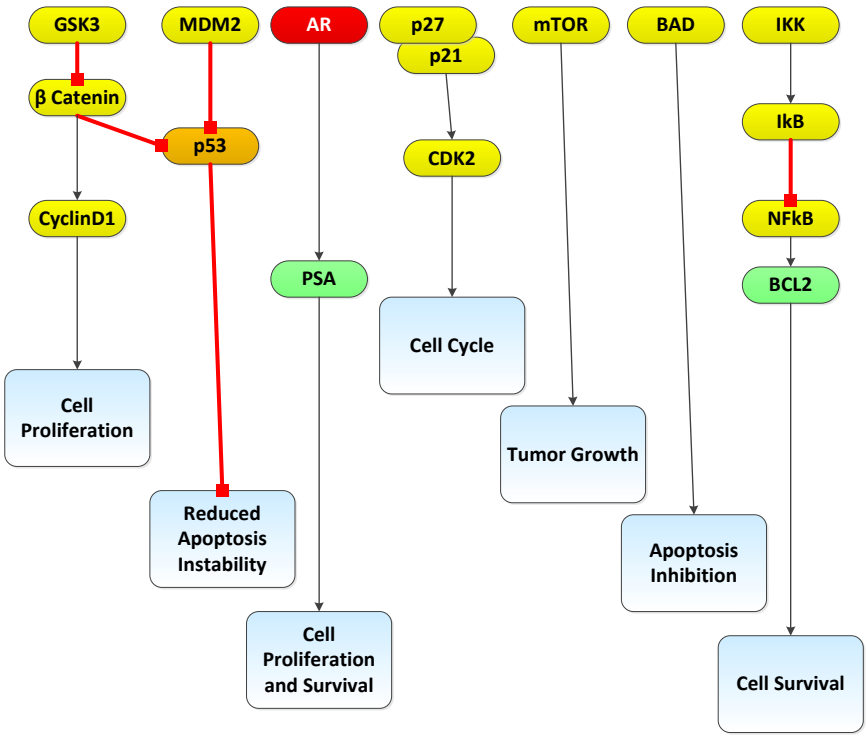


The Figure below demonstrates some of the details in the pathway also focusing on the PSA release which has been the focus of monitoring for potential PCa. As is also well known PSA is not a definitive marker for PCa and it may increase in a variety of circumstances. One of the key points below is the joining of AR in a dimer form which occurs early on and it is this dimer which is moved back into the nucleus for transcription purposes.

[7] http://www.ncbi.nlm.nih.gov/gene/367



The Figure below depicts the many ultimate functions associated with many of these genes and we see the AR function is proliferation and cell survival.

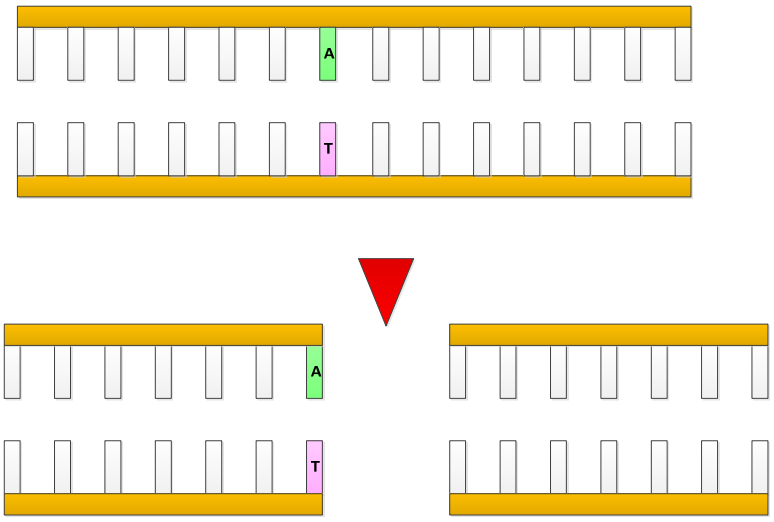


The above is a significant exhibition of cause and effect with regards to the genes and their expression on overall cell stability.

3 DSB REPAIR

The genes we have examined play a key role in DNA repair. Although not specifically focused upon, the implications of CHK2 and its control of CDC25C and in turn CDK1 argue for double stranded breaks, DSB, in DNA as a contributing factor. Thus it is useful to provide a high level review of what we understand at this time. We also not that with the use of CRISPRs, we have another mechanism for DSBs and that the CRISPR approach may be one where the impact of DSBs and their repair may become ever so much more critical.

There are many ways in which DNA can get distorted but we shall examine only the double stranded breaks, DSB, as possibly one of the most significant. We show this example below where we have a break with no sticky ends, just a clean DSB. This is the most complex to deal with.



The simple break above, this specific DSB, is a cut on opposite sides of the DNA. The specific cause and mechanism of this break may not be fully known or understood. However the repair mechanisms are somewhat understood.

As Jackson and Bartek have noted:

Key DDR signalling components in mammalian cells are the protein kinases ATM and ATR, which are recruited to and activated by DSBs and replication protein A (RPA)-coated ssDNA, respectively.

Two of the best studied ATM/ATR targets are the protein kinases CHK1 and CHK2 which, together with ATM and ATR, act to reduce cyclin-dependent kinase (CDK) activity by various mechanisms, some of which are mediated by activation of the p53 transcription factor. Inhibition

of CDKs slows down or arrests cell-cycle progression at the G1–S, intra-S and G2–M cell-cycle checkpoints, which is thought to increase the time available for DNA repair before replication or mitosis ensues.

In parallel, ATM/ATR signalling enhances repair by inducing DNA-repair proteins transcriptionally or post-transcriptionally; by recruiting repair factors to the damage; and by activating DNA-repair proteins by modulating their phosphorylation, acetylation, ubiquitylation or SUMOylation.

To expand the understanding we consider what Valerie and Povirk have noted:

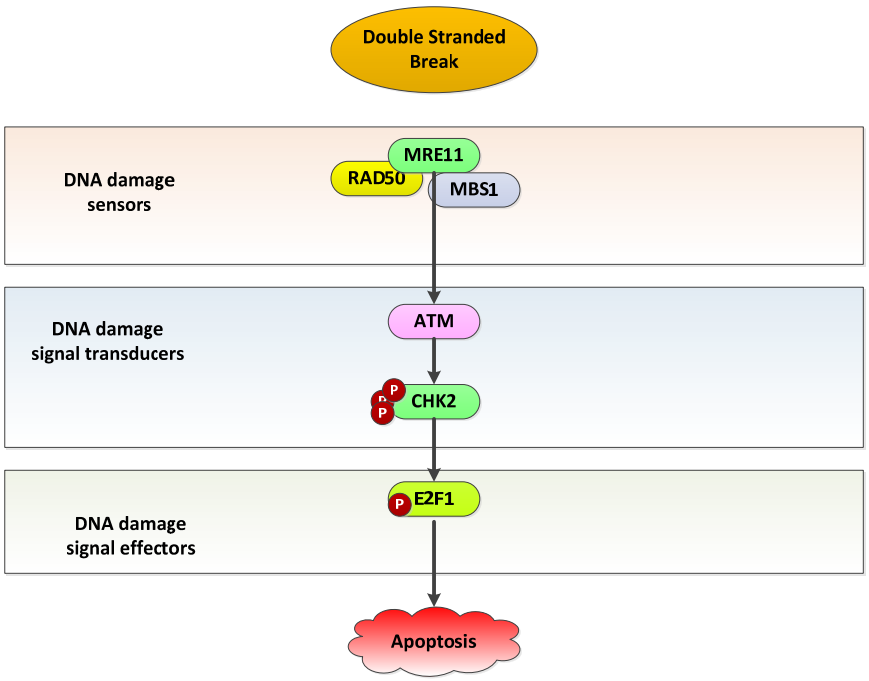
The double-strand break (DSB) is believed to be one of the most severe types of DNA damage, and if left unrepaired is lethal to the cell.

Several different types of repair act on the DSB. The most important in mammalian cells are nonhomologous end-joining (NHEJ) and homologous recombination repair (HRR).

NHEJ is the predominant type of DSB repair in mammalian cells, as opposed to lower eukaryotes, but HRR has recently been implicated in critical cell signaling and regulatory functions that are essential for cell viability.

Whereas NHEJ repair appears constitutive, HRR is regulated by the cell cycle and inducible signal transduction pathways. More is known about the molecular details of NHEJ than HRR in mammalian cells. This review focuses on the mechanisms and regulation of DSB repair in mammalian cells, the signaling pathways that regulate these processes and the potential crosstalk between NHEJ and HRR, and between repair and other stress-induced pathways with emphasis on the regulatory circuitry associated with the ataxia telangiectasia mutated (ATM) protein.

We shall review this in some detail shortly. The above two references lead to the general model depicted below:



There are two methods of repairing DSB, homologous (HEB) and non-homologous (NHEB). As Shrivastav et al state:

NHEJ and HR both contribute to genome stability and both pose risks of large- and small-scale genome rearrangement NHEJ and HR pathways are often described as "error- prone" and "error-free" respectively, but this is an oversimplification. "Clean" DSBs with complementary overhangs, 5' phosphates and 3' hydroxyl groups, such as those produced by nucleases, can be precisely repaired by NHEJ. In yeast and mammalian cells, 25-50% of nuclease DSBs is repaired by precise NHEJ; note that these are minimum estimates because these measurements do not account for multiple cycles of cleavage and precise repair.

When ends cannot be precisely rejoined, NHEJ typically involves alignment of one or a few complementary bases ("microhomology") to direct repair, leading to small deletions and sometimes small insertions. In mammalian cells NHEJ proceeds in a stepwise manner beginning with limited end-processing by the MRE11/RAD50/NBS1 (MRN) complex and perhaps other factors, end-binding by Ku comprising the Ku70 and Ku80 subunits, and recruitment of the DNA-dependent protein kinase catalytic subunit (DNAPKcs), forming the trimeric DNA-PK holoenzyme.

Once bound to broken ends, DNA-PK is activated and it phosphorylates itself and other targets including RPA, WRN, and Artemis; in cells lacking ATM, DNA-PK can also phosphorylate histone H2AX, termed γ-H2AX. In the final step, DNA ligase IV, with its binding partners XRCC4 and XLF (also called Cernunnos), seals the break. The nuclease Artemis helps repair a subset of IR-induced DSBs by NHEJ, and is important for opening hairpins formed during V(D)J recombination.

Ciccia and Elledge have an excellent review article where they also note the likelihood of such damage from various sources. What is striking is the number of lesions per day due to sunlight alone. Compare that to the Hiroshima numbers and one can be surprised.

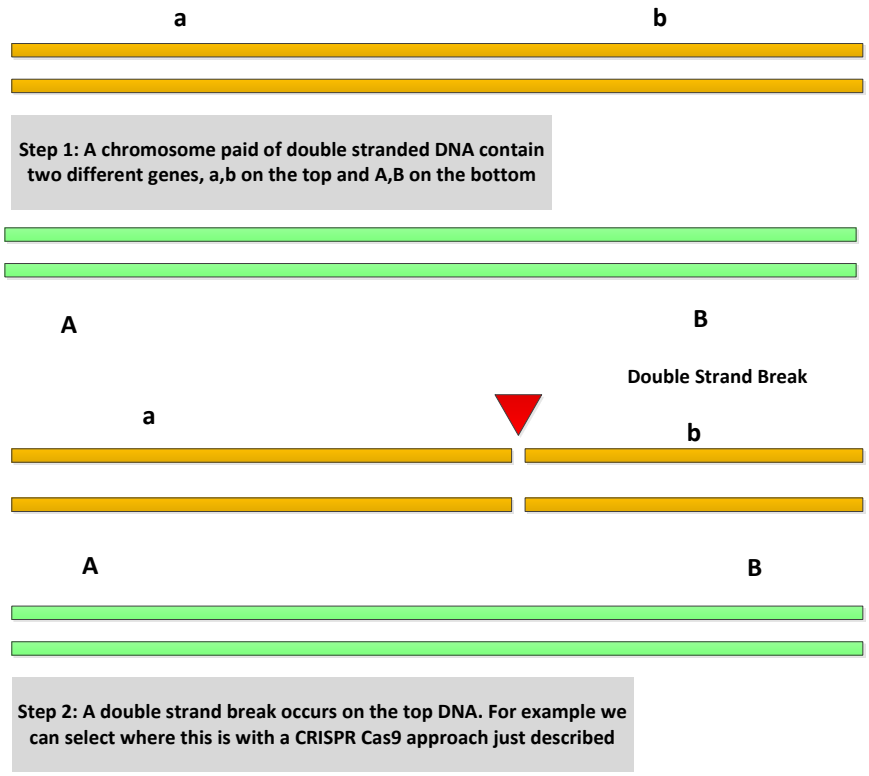
Exogenous DNA Damage	Dose Exposure (mSv)	DNA Lesions Generated	Number Lesions/Cell/Day
Peak hr sunlight	—	Pyrimidine dimers, (6–4) photoproducts	100,000/day
Cigarette smoke	—	aromatic DNA adducts	45–1029
Chest X-rays	0.02f,g,h	DSBs	0.0008
Dental X-rays	0.005f,g,h	DSBs	0.0002
Mammography	0.4f,g,h	DSBs	0.016
Body CT	7f	DSBs	0.28
Head CT	2f,g	DSBs	0.08
Coronary angioplasty	22h	DSBs	0.88
Tumor PET scan (18F)	10h	DSBs	0.4
131I treatment	70–150h	DSBs	2.8–6
External beam therapy	1800–2000j	DSBs	72–80
Airline travel	0.005/hrf	DSBs	0.0002/hr
Space mission (60 days)	50k	DSBs	2
Chernobyl accident	300l	DSBs	12
Hiroshima and Nagasaki atomic bombs	5–4000k	DSBs	0.2–160

3.1 HOMOLOGOUS REPAIR

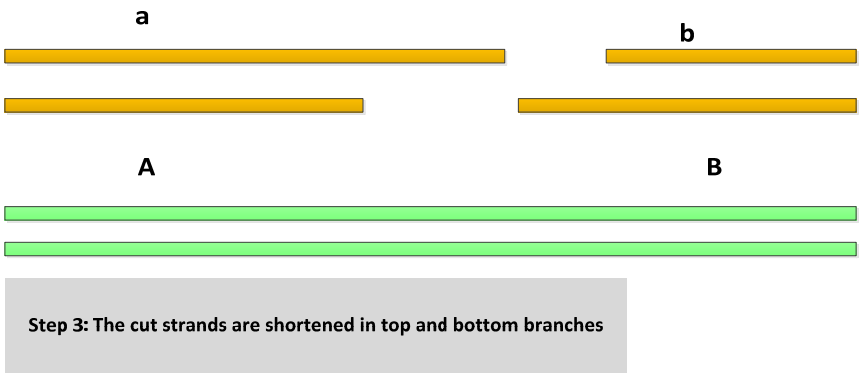
The paper by Chapman et al is a key document presenting many of the details we show herein. We have taken a simplified view so as to focus on the genetic elements of concern herein. Thus there is a significant amount of complexity left aside.

Homologous repair is one where a DSB uses another comparable chromosome or DNA sequence and uses it for a pallet to compare and restructure the broken DNA. The following Figure depicts this process. We have explained this in the applications to CRISPR editing as well.

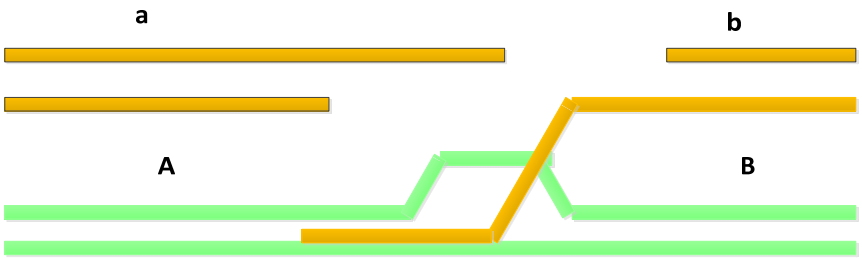
We start with the process and expand it in 3 Figures. The following Figure depicts the beginning.



Now the second phase is shown in the following Figure. Here we show how a sister piece of identical DNA can be used as a repair template. That assumes that such an identical pair exists and is available.

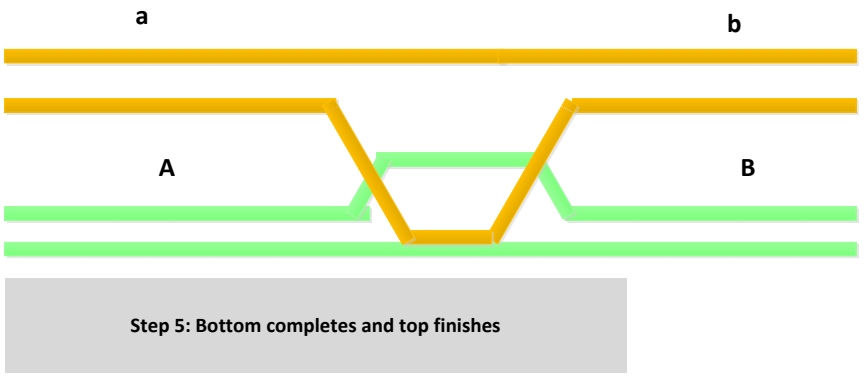


Step 3: The cut strands are shortened in top and bottom branches

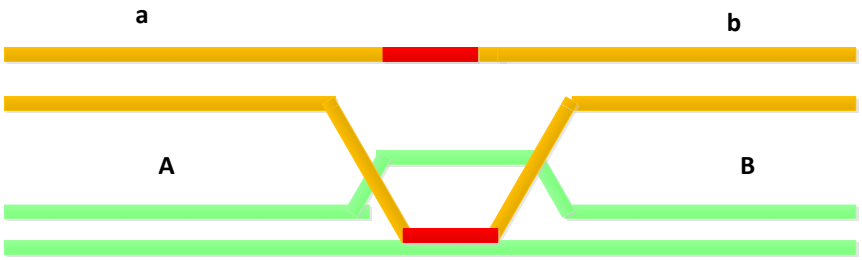


Step 4: The bottom branch uses the bottom branch as a renewal template reconstructing the full strand.

The third step in the process is shown in the Figure below. The sister elements are copied and the final reconstruction is accomplished. Generally this is a fairly accurate process with reasonably good copying.



Step 5: Bottom completes and top finishes



Step 6: A selected gene can be inserted and added, or it can equally well be removed by a two cut process.

The net result of the Homologous repair is almost in all cases a perfect repair. However as noted is presupposes the existence of a sister pallet and a functioning mechanism.

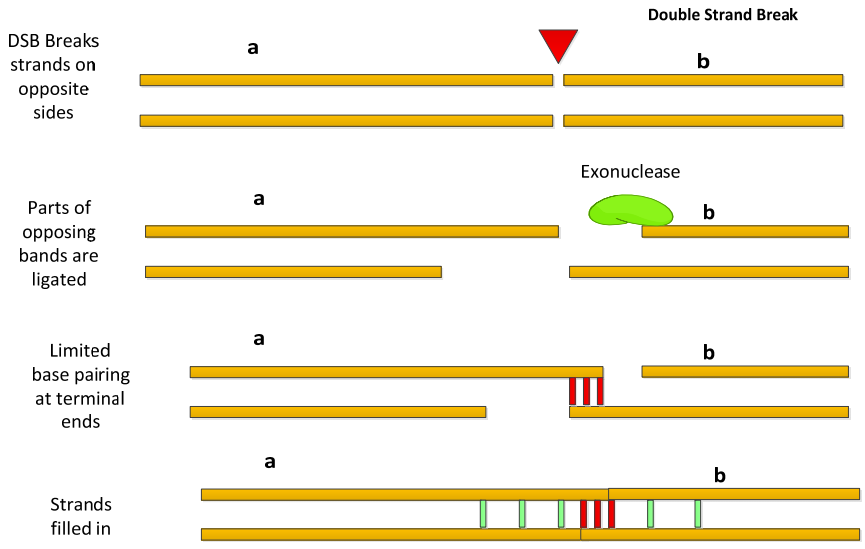
3.2 NON HOMOLOGOUS

The non-homologous mechanism takes the assumptions of the homologous mechanism away and tries to repair by itself. We demonstrate this process below. Basically it does the following:

1. With a clear DSB the ends of the opposing sides are ligated on opposing strands opening what may be “sticky” ends. This is done by an exonuclease.

2. The longer ends try to find a match and begin the process of sticking. This is the more difficult phase since the match finding may result in the ligation of bases.

3. Once the base pairs are matched the complete repair is performed in a standard manner.

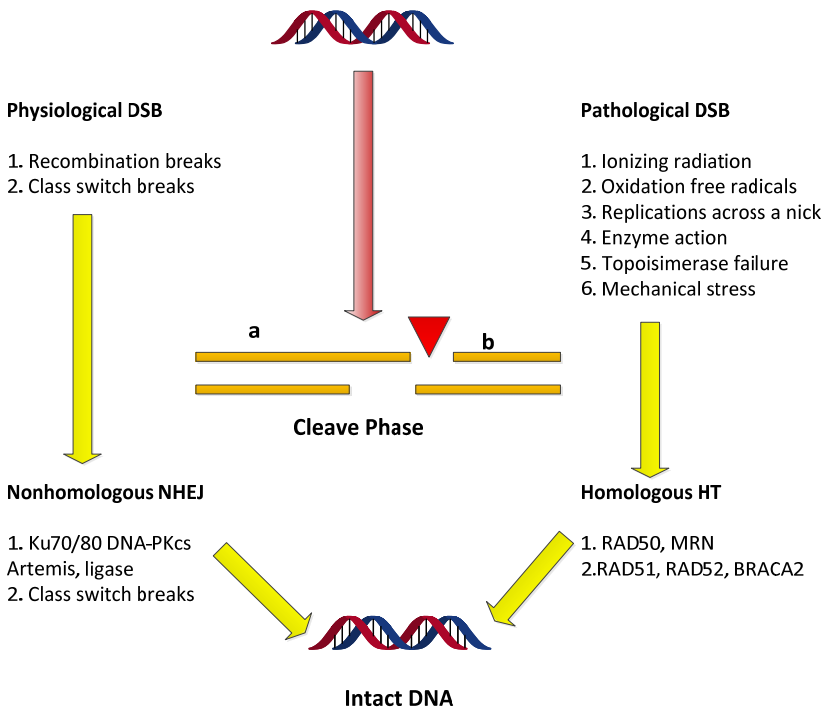


See p 53 Mendelsohn et al

This is not a perfect process. It is prone to a loss of bases and this can create a gene mutation. In fact this may be one of the most imperfect processes around and could very well be the cause for many malignancies.

3.3 RELATIONSHIPS

The Figure below depicts the collection of these processes in toto. There are seen to be two classes of DSB. The physiological class tends to lead to non-homologous repair and those of a pathological basis the homologous.



The DSB repair mechanisms are as we have noted prone to mistakes. That is where we also have certain backup mechanisms as p53 and other similar genes. However if as we have noted the CHK2 process becomes overly active we may have added instability which p53 and its helpers cannot properly control.

4 CHK2

Now let us examine Chk2 in some further detail as the putative therapeutic target.

4.1 CHK2 DETAILS

CHK2 has been studied extensively and especially for its importance in DNA damage control. We briefly state from Antoni et al:

In the past decade, CHK2 has emerged as an important multifunctional player in the DNA-damage response signalling pathway. Parallel studies of the human CHEK2 gene have also highlighted its role as a candidate multiorgan tumour susceptibility gene rather than a highly penetrant predisposition gene for Li–Fraumeni syndrome.

As discussed here, our current understanding of CHK2 function in tumour cells, in both a biological and genetic context, suggests that targeted modulation of the active kinase or exploitation of its loss in tumours could prove to be effective anti-cancer strategies.

From Bohkagi et al we note the specific importance of CHK2 in the ATM and p53 pathway for DNA damage control:

The serine threonine kinase checkpoint kinase 2 (CHK2) is a DNA damage checkpoint protein important for the ATM-p53 signaling pathway. In addition to its phosphorylation, CHK2 is also ubiquitylated, and both post-translational modifications are important for its function. However, although the mechanisms that regulate CHK2 phosphorylation are well established, those that control its ubiquitylation are not fully understood.

In this study, we demonstrate that the ubiquitin E3 ligase PIRH2 (p53-induced protein with a RING (Really Interesting New Gene)-H2 domain) interacts with CHK2 and mediates its polyubiquitylation and proteasomal degradation.

We show that the deubiquitylating enzyme USP28 forms a complex with PIRH2 and CHK2 and antagonizes PIRH2-mediated polyubiquitylation and proteasomal degradation of CHK2. We also provide evidence that CHK2 ubiquitylation by PIRH2 is dependent on its phosphorylation status. Cells deficient in Pirh2 displayed accumulation of Chk2 and enhanced hyperactivation of G1/S and G2/M cell-cycle checkpoints.

This hyperactivation was, however, no longer observed in Pirh2−/−Chk2−/− cells, providing evidence for the importance of Chk2 regulation by Pirh2. These findings indicate that PIRH2 has central roles in the ubiquitylation of Chk2 and its turnover and in the regulation of its function.

It is for these reasons that one should focus on the role of CHK2 as well as its predecessor control in ATM and its subsequent controlled elements CDC25C as well as CDK1.

4.2 ATM AND AN ADJUNCT

The ATM gene is a driver of CHK2 especially in the DSB environment. We examine this briefly as well.

The following Figure is adapted from Khalil et al. They note:

The Ataxia Telangiectasia Mutated gene encodes the ATM protein, a key element in the DNA damage response (DDR) signalling pathway responsible for maintaining genomic integrity within the cell.

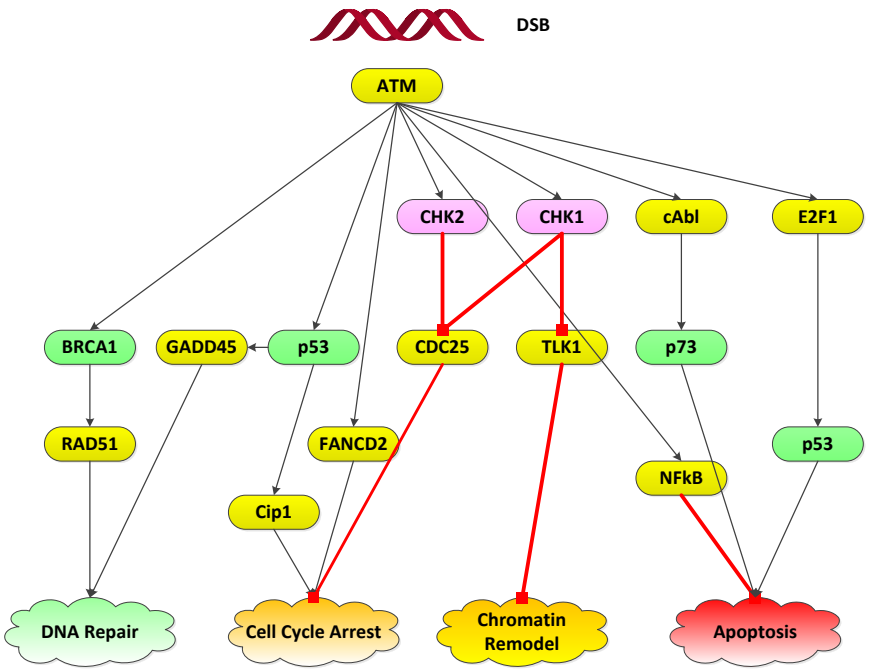
The ATM protein belongs to a family of large protein kinases containing the phosphatidylinositol-3 catalytic domain, including ATM, ATR and PI3K. ATM provides the crucial link between DNA damage, cell cycle progression and cell death by first sensing double stranded DNA breaks and subsequently phosphorylating and activating other downstream proteins functioning in DNA damage repair, cell cycle arrest and apoptotic pathways.

Mammalian cells are constantly challenged by genotoxic agents from a variety of sources and therefore require a robust sensing and repair mechanism to maintain DNA integrity or activate alternative cell fate pathways.

This review covers the role of ATM in DDR signalling and describes the interaction of the ATM kinase with other proteins in order to fulfil its various functions.

Special emphasis is given to how the growing knowledge of the DDR can help identify drug targets for cancer therapy, thus providing a rationale for exploiting the ATM pathway in anticancer drug development. Moreover, we discuss how a network modelling approach can be used to identify and characterise ATM inhibitors and predict their therapeutic potential.

They note the importance of ATM and we have included it in our discussion but have not singled it out. However we believe that it too has significant importance.



The ATM substrates are as follows:

G1	G1/S	S	G2/M
P53 Mdm2 Nbs1	P53 cAbl Rad51	RPA Chk2 FANCD2 H2AX BRCA1 CtIP MRN	Chk1 Chk2 Rad17(RFC)

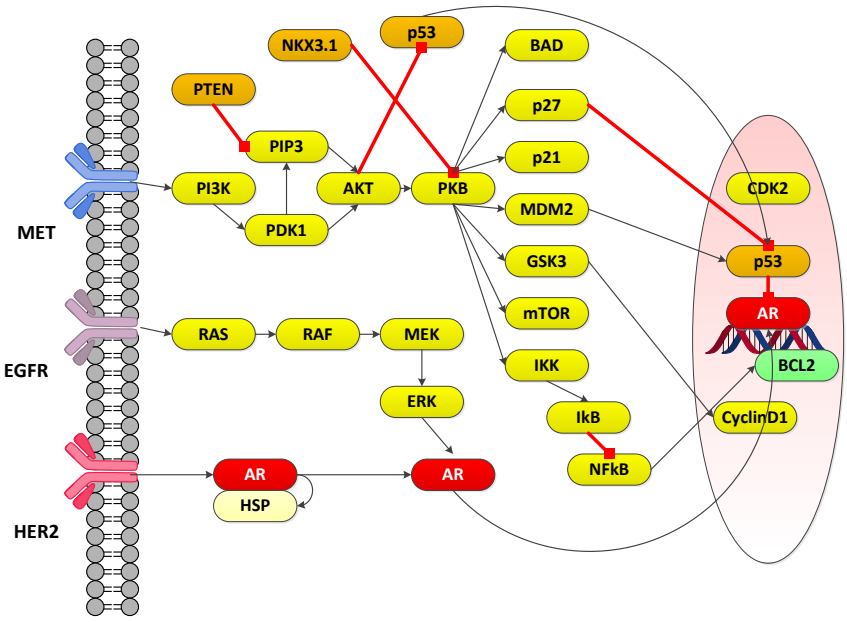
This Table does present the importance of each CDK in the cell cycle process.

5 OBSERVATIONS

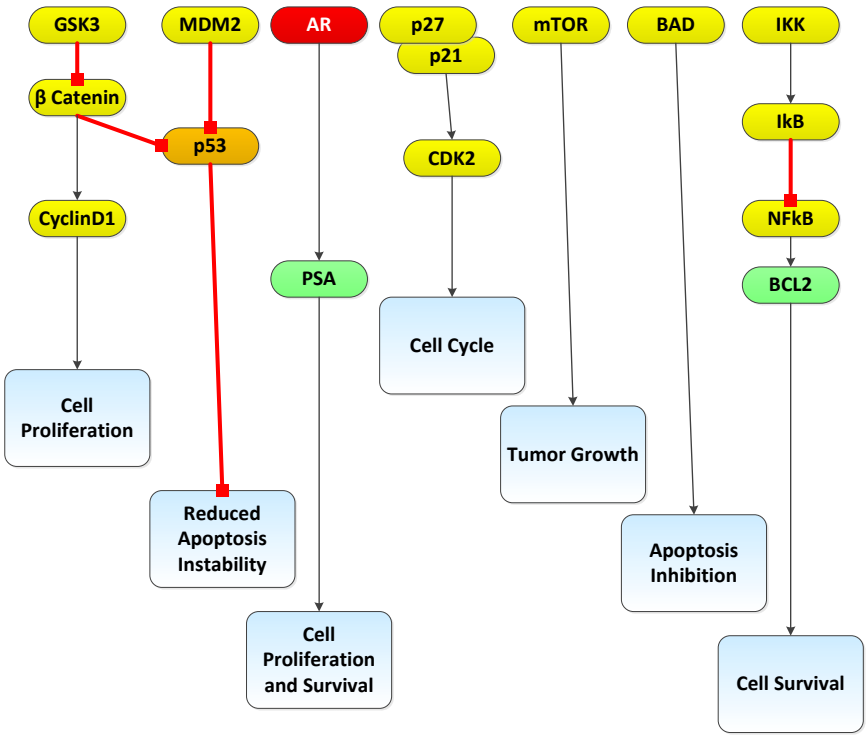
This is an interesting set of observations. It asks the question regarding a specific gene product and how it may be an integral part of the pathways that appear to break down during CRPC. Whether this is a useful target is yet to be explored and further whether this is a therapeutic target is also an unanswered question.

5.1 THE PATHWAYS

The figure below is a KEGG modified diagram of some of the key interactions in PCa. AR is shown as part of the process and especially as part of what it does in the process of gene translation.



We summarize some of these key pathways below and their ultimate impacts. They rand from growth, proliferation, and overall loss of cell cycle control. What is interesting is the impact that CHK2 products have in the DSB domain. That is we know that CHK2 is a DSB repair facilitator but is this process one of prime concern in PCa as well.



Understanding these pathways and their ultimate goals, not in any teleological sense, but in a factual sense, is essential in understanding the importance of this perceived relationship. Focusing on elements involved in proliferation and in reducing apoptosis is a critical set of paths to consider. The work described in the reference paper is an excellent step in that direction.

5.2 DSBs

DSBs are a frequent occurrence and their cause can be many. One we had referred to was radiation events but in PCa this may not be a prime cause. Inflammation and oxidative stress may most likely be a cause but again that is definitively unknown at present. Notwithstanding a definitive cause we do know that DSBs can have significant effects especially when a NH repair is made and parts of the chromosome and putatively a critical gene location is distorted.

As Chapman et al have noted:

DNA double-strand breaks (DSBs) are highly toxic lesions that can drive genetic instability. To preserve genome integrity, organisms have evolved several DSB repair mechanisms, of which nonhomologous end-joining (NHEJ) and homologous recombination (HR) represent the two most prominent. It has recently become apparent that multiple layers of regulation exist to ensure these repair pathways are accurate and restricted to the appropriate cellular contexts.

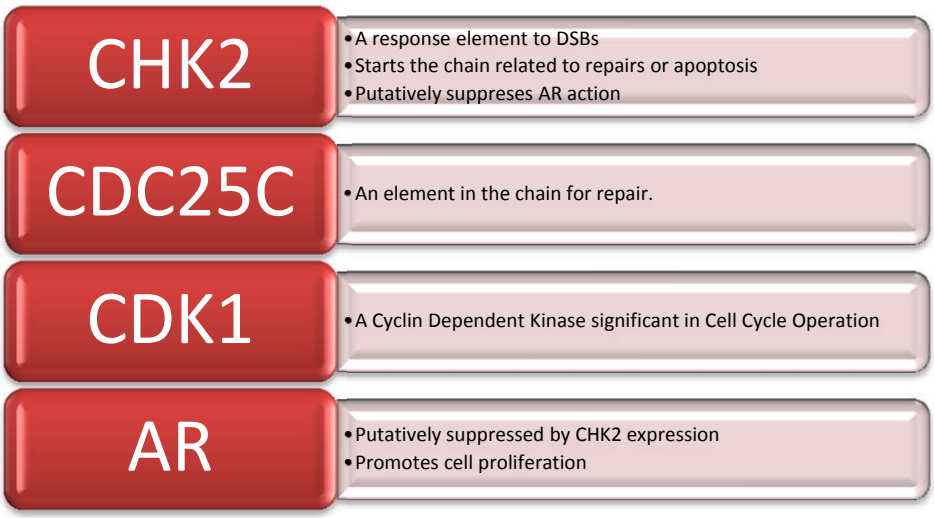
Such regulation is crucial, as failure to properly execute DSB repair is known to accelerate tumorigenesis and is associated with several human genetic syndromes. Here, we review recent insights into the mechanisms that influence the choice between competing DSB repair pathways,

how this is regulated during the cell cycle, and how imbalances in this equilibrium result in genome instability.

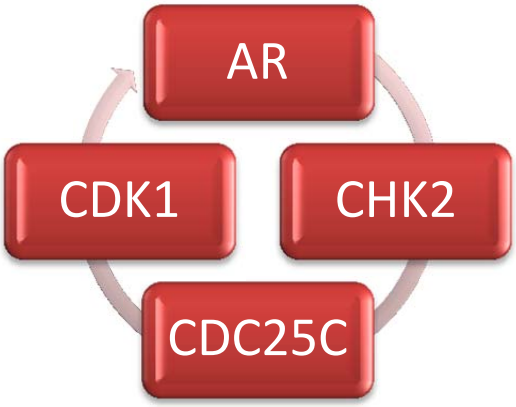
Thus one question is; are the DSBs produced at the heart of this checkpoint process? The CHK2 process we have examined appears to be primarily one focused on repairing DSBs. Why then the importance on AR functions which are proliferation oriented.

5.3 THE PARADIGM

Finally we examine the proposed paradigm. The Figure below is a simplified attempt to articulate what has been proposed.



Clearly in the above we have three well known paths and a separate path. What we do not know is how the CHK2 expression product controls AR. Further we do not understand it appears if this suppression function works when AR becomes an aberrant form found in CRC. Namely is CRC AR a variant of normal AR and if so what is the change?



The new proposed paradigm may also have therapeutic promise but it still requires in our opinion significant investigation. This analysis however does open an important gateway to understanding the control mechanisms involved.

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