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Mornings With Art, Lessons Learned: Feedback Regulation, Restriction Threshold Biology, and Redundancy Govern Molecular Stress Responses

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Work from the laboratory of Dr. Arthur B. Pardee has highlighted basic principles that govern cellular and molecular biological processes in living cells. Among the most important governing principles in cellular and molecular responses are: (i) threshold ''restriction'' responses, wherein a level of response is reached and a ''point of no return'' is achieved; (ii) feedback regulation; and (iii) redundancy. Lessons learned from the molecular biology of cellular stress responses in mammalian cancer versus normal cells after ionizing radiation (IR) or chemotherapeutic agent exposures reveal similar instances of these guiding principles in mammalian cells. Among these are the: (i) induction of cell death responses by β-lapachone (β-lap), a naphthoquinone anti-tumor agent that kills cancer cells via an NQO1 (i.e., X-ray-inducible protein-3, xip3)-dependent mechanism; (ii) induction of secretory clusterin (sCLU) in response to TGF-β1 exposure, and the ability of induced sCLU protein to down-regulate TGF-β1 signaling; and (iii) induction of DNA mismatch repair-dependent G_2 cell cycle checkpoint responses after exposure to alkylating agents. We have learned these lessons and now adopted strategies to exploit them for improved therapy. These examples will be discussed and compared to the pioneering findings of researchers in the Pardee laboratory over the years. J. Cell. Physiol. 209: 604–610, 2006.

LESSONS LEARNED BY DR. BOOTHMAN AND HIS STUDENTS FROM STUDIES DIRECTED BY ARTHUR B. PARDEE, Ph.D.

My time with Dr. Arthur B. Pardee to review my progress and discuss future goals and ideas was on Wednesday mornings, 10 am. Always feeling one-step behind, I was constantly challenged by Dr. Pardee. In one of our meetings at the end of the first month, I proposed to investigate β-lapachone (β-lap), a proposed DNA repair inhibitor (Boorstein and Pardee, 1984), as a radiosensitizer (Boothman et al., 1987). Dr. Pardee proceeded to explain to me that the field of ionizing radiation (IR) studies was not one that he recommended pursuing, since much of the science was not "molecular." I, of course, saw this as an important opportunity to investigate interesting responses in human cancer versus normal cells. Even though Dr. Pardee was apprehensive about my investigating radiation effects research, he still encouraged me to pursue my interests. I proceeded to study IR-related science for the remainder of my time in his lab, much to Dr. Pardee's surprise at the time I am sure, and we were the first to discover X-ray-inducible proteins and transcripts in human cells ((Boothman et al., 1989a, 1993), described below) in the process. The freedom afforded by Dr. Pardee for individuals in his laboratory to investigate their particular interests is (and was) one of the things most cherished by all his students. This attribute of Dr. Pardee's was only surpassed by his ability to continually challenge his students to think, be creative, and perform innovative science. Add unwavering support of his students and it is not surprising that his former colleagues demonstrate an extremely high degree of loyalty; for example, over 100 former students of Dr. Pardee either attended or wished to attend his recent 85th birthday celebration.

BIOLOGICAL PRINCIPLES

Studies from the laboratory of Dr. Arthur B. Pardee over the past six decades (over 350 peer-reviewed papers listed in PUBMED) highlight three basic themes that continue to be rediscovered in all biological processes that govern mammalian cellular and molecular biology and cancer research. These three research themes are:

1. Biological thresholds, highlighted by the discovery of the restriction point (R point, (Campisi et al., 1982; Blagosklonny and Pardee, 2002)) that changed the manner in which we viewed cell cycle checkpoints, as well as processes that controlled cell division in mammalian cells.
2. Feedback repression, highlighted by the discovery by Dr. Pardee's laboratory, along with Drs. Jacob and Monod, of the lac repressor and its operon (i.e., the

Except for Dr. Gao, all authors were students and now colleagues of Dr. Boothman. Dr. Boothman trained with Dr. Arthur B. Pardee from 1986 through 1989.

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PaJaMa Experiments (Pardee, 2002; Pardee et al., 1958)).

3. Redundancy, highlighted by the multiple means of thymidine kinase regulation (Prem veer Reddy and Pardee, 1980; Coppock and Pardee, 1985) in normal cells, and its alterations in cancer cells (Bradley et al., 1990; Dou et al., 1992; Gudas et al., 1992, 1993; Dou and Pardee, 1996).

Molecular stress responses–principles applied

β-Lapachone studies: identifying a cell death restriction point regulator. β-Lapachone is a 1,2-naphthoquinone anti-tumor agent that can radiosensitize all NAD(P)H:quinone oxidoreductase-1 (NQO1)-expressing cancer cells investigated to date (Boothman et al., 1987; Park et al., 2005a,b). Studies from our laboratory demonstrate that β-lapachone kills exclusively through its metabolism by the two-electron oxidoreductase, NQO1 (Pink et al., 2000a; Planchon et al., 2001) that is over-expressed in many human cancer cells, but is also induced after cell stress; NQO1 is also referred to as X-ray-inducible protein-3 (xip3), as isolated by our laboratory (Boothman et al., 1993). Data from our laboratory strongly suggest that β-lapachone kills through a novel cell death pathway outlined in Figure 1. In cells with high levels of NQO1, β-lapachone is metabolized in a futile cycle that generates massive levels of reactive oxygen species (ROS). These ROS cause DNA damage, as well as lead to the rapid release of calcium from endoplasmic reticulum (ER) stores (Tagliarino et al., JBC, 2000). This released calcium, in turn, causes dramatic losses of ATP and NAD^+. It is the loss of ATP and NAD^+ that causes lethality, freezes cells

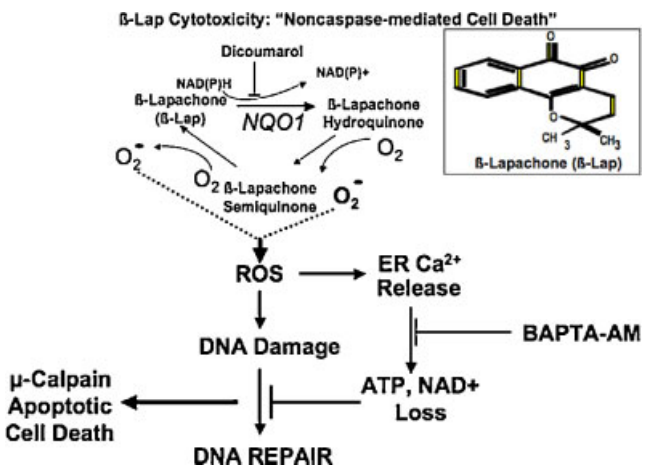

Fig. 1. Pathway of NQO1-dependent, β-lapachone-induced cell death. β-Lapachone (β-lap, see insert for structure) is metabolized in a futile manner by NQO1. For each mole of β-lapachone that enters the reaction with NQO1, over 60 moles of NAD(P)H can be used in 10 min. This reaction uses oxygen and creates a large amount of reactive oxygen species (ROS). ROS formation, in turn, simultaneously damages DNA and causes dramatic release of calcium, first from endoplasmic reticulum (ER) stores followed later by an influx of massive levels of calcium from the outside. Loss of calcium homeostasis is a direct result of ATP loss caused by DNA damage, high levels of intracellular calcium and hyper-activation of PARP-1. Blockage of NQO1 activity, calcium release, or NAD+ and ATP loss by PARP-1 inhibition can prevent β-lapachone-induced apoptosis. Apoptosis induced by β-lapachone is unique due to ATP loss and high levels of calcium that ultimately leads to μ-calpain-induced cell death independent of caspase activation. Knowledge of this novel, well understood and controllable cell death pathway now makes it feasible for the development of β-lapachone as an efficacious anti-tumor agent for the treatment of cancers with endogenous elevations of NQO1, including cancers of the lung (specifically non-small cell lung cancer), prostate, pancreas, breast, and colon.

in their cell cycle positions, and initiates a μ-calpain-dependent cell death pathway. Loss of ATP in cells, as a result of the futile cycling of β-lapachone, appears to explain all of the reported downstream effects of this drug. These include: (i) inhibition or activation of Topoisomerase I (Topo I), since (1) DNA damage could mediate a Topo I-dependent cleavage, uncoiling supercoiled DNA, or (2) when β-lapachone and Topo I are added together, the loss of ATP would explain Topo I inhibition if drug pre-exposures were performed (Boothman et al., 1989b; Li et al., 1993); (ii) inhibition of NF-κB (Manna et al., 1999), since loss of ATP would prevent I-κB loss through proteasome inhibition; (iii) apoptotic cell death in all phases of the cell cycle, including pre-arrested cells treated in G_2/M (Pink et al., 2000b); (iii) lack of caspase activation, since ATP is required for all caspase activation (Tagliarino et al., 2001, 2003); (iv) inhibition of Topoisomerase II-β (Topo II-β), since this enzyme requires ATP (Frydman et al., 1997; Neder et al., 1998); and possibly (v) release of E2F1 and cell cycle checkpoint alterations (Li et al., 2003), although this seems unlikely because loss of ATP would result in hypo-phosphorylation of the retinoblastoma (pRb) protein (Pink et al., 2000b) that should enhance E2F1 binding. It is possible that the subsequent cleavage of pRb (Pink et al., 2000b) could result in E2F1 release. The important issue in understanding these apparently diverse cellular responses caused by β-lapachone in mammalian cells is whether the reported effects are NQO1-dependent or not dependent on redox cycling by this two-electron oxidoreductase. NQO1 polymorphisms that result in lack of enzyme expression occur within the human population at nearly 15%. Therefore, there are many cell lines available (e.g., LNCaP, MDA-MB-231, MDA-MB-468, etc.) that do not express this enzyme. Thus, many of the reported cell responses could be NQO1-independent. NQO1-independent responses require higher doses and/or more prolonged exposures to β-lapachone, under conditions where one-electron reductions (by b5R and P450R oxidoreductases) can metabolize β-lapachone and produce caspase-activating apoptotic responses (Tagliarino et al., 2003). Therefore, all cellular responses to β-lapachone have to be determined as NQO1-dependent or -independent. A rapid way of examining this issue is to apply the 'dicoumarol inhibition' litmus test: does dicoumarol (an effective NQO1 inhibitor) prevent the observed responses to β-lapachone or not? If not, the responses reported are most likely due to non-NQO1 cellular responses to β-lapachone, and these are most likely caused by one-electron reductions of the compound by enzymes such as b5R and P450R, and require supra-lethal doses to achieve the responses.

β-Lapachone as a radiosensitizer. β-Lapachone is not a radiosensitizer in the classic sense of the term. To date, it is the only known compound whereby IR exposure sensitizes cells to the agent, not the other way around. The mechanism can involve the dramatic induction of NQO1 (Boothman et al., 1993; Park et al., 2005a,b), however, induction of NQO1 is not necessary for cells that already express the enzyme (Reinicke et al., in preparation). In cells over-expressing NQO1, exposure to β-lapachone causes dramatic losses of ATP and NAD^+. Since ATP and NAD^+ are both required for DNA repair, it is not surprising that DNA repair is inhibited and cells are radiosensitized.

Lack of cell cycle checkpoint responses after β-lapachone exposure. We extensively examined the ability, or lack thereof, of cells exposed to β-lapachone to

undergo cell cycle checkpoint responses. β-Lapachone did not elicit p53 stabilization responses and, in fact, exposure of cells to this anti-tumor drug resulted in the loss of basal p53 levels (Wuerzberger et al., 1998). To investigate cell cycle checkpoint responses more in-depth, MCF-7 cells were exposed to β-lapachone (β-lap 5 μM, 4 h), camptothecin (CPT, 5 μM, 4 h), or DMSO (0.01%, 4 h) and then analyzed for cell cycle position by BrdUrd labeling for 4 h at various times (shown are analyses at 36 and 96 h, Fig. 2) (Wuerzberger et al., 1998), and analyzed by FACS for cell cycle distributions over the next 96 h (Figs. 2 and 3). Unlike exposure to high dose camptothecin (CPT, 5 μM, 4 h) that caused rapid S-phase and later G_1 and G_2 cell cycle checkpoint responses (Fig. 2), exposure of cells to β-lapachone caused dramatic suppression of all BrdUrd uptake and induced apoptosis in all phases of the cell cycle. The appearance of β-lapachone-exposed cells in between G_1 and G_2 with little or no BrdUrd incorporation (Fig. 3), indicates that cells in G_2/M at the time of exposure underwent apoptosis. This appears on normal flow cytometry histograms as an increase in S-phase, and could be mis-interpreted as an increased S-phase population. However, our data strongly suggest that these are sub-G_2/M apoptotic cells and not cells that were advanced into S-phase. This interpretation of these data is consistent with our previous demonstration that β-lapachone exposure of cells (>80%) arrested in G_2/M by prior colchicine or nocodazole pre-treatment, caused dramatic cell death responses independent of cell cycle status (Pink et al., 2000a,b). These data also ruled out Topoisomerase IIβ as a target of β-lapachone, since expression of this enzyme is low or absent in G_2/M cells, and these cells were as sensitive to β-lapachone as log-phase or G_0-arrested cells (Pink et al., 2000b). We previously demonstrated that G_0/G_1 growth-arrested NQO1-expressing cells can undergo apoptosis after β-lapachone exposure with the same kinetics, IC_{50} and long-term colony forming ability results as β-lapachone-exposed log-phase cells. Thus, to date, NQO1 expression and no other known protein, has been mechanistically demonstrated to be a major determinant in β-lapachone-mediated cell death responses. NQO1 knockdown makes cells resistant to β-lapachone (Ough et al., 2005), expression of NQO1 in cells containing polymorphism-driven deficiencies makes cells sensitive to the anti-tumor agent, while dicoumarol inhibits β-lapachone lethality exclusively in NQO1+ cells (Pink et al., 2000a, Planchon et al., 2001).

β-Lapachone-induced cell death restriction point studies. In studies reminiscent of the Pardee laboratory's delineation of the cell cycle restriction point (above), our lab recently determined the minimal exposure time of β-lapachone treatment required for complete cell death responses of NQO1-expressing cells. In these studies, NQO1 positive cells were exposed to various concentrations of β-lapachone for various lengths of time, cells were washed free of drug, and monitored for apoptosis and long-term survival. Interestingly, we found that all NQO1-expressing cells studied thus far required a minimum time (i.e., 2 h) of β-lapachone exposure to initiate cell death. This time frame was crucial for us to delineate as it coincided with the time where all ATP and NAD^+ levels were depleted from β-lapachone-treated cells. Interestingly, administration of the calcium chelator, BAPTA-AM, early during β-lapachone exposure, spared cells completely from cell death responses, whereas administration of the chelator after the cell death restriction point had little to no affect on β-lapachone-induced cell death responses (Bentle et al., submitted).

Role of PARP-1 hyperactivation. β-Lapachone futile cycling mediated by NQO1 causes the generation of reactive oxygen species (ROS) leading to extensive DNA damage and hyper-activation of poly(ADP-ribose) polymerase-1 (PARP-1). PARP-1 hyperactivation, in turn, depletes essential NAD^+ and ATP pools. Since ATP is required for maintenance of calcium homeostasis, its loss leads to dramatic accumulation of intracellular calcium, causing a μ-calpain-mediated cell death response (Tagliarino et al., 2001, 2003). Consistent with the aforementioned effects of BAPTA-AM on the minimum time to death, β-lapachone-induced PARP-1 hyperactivation and cell death responses were also Ca^{2+}-mediated and -dependent (Bentle et al. submitted). These findings correlate with enhanced DNA repair in the presence of BAPTA-AM, but not in its absence. It is important to note that in these studies, the use of Ca^{2+} chelators had no affect on ROS production or initial DNA damage (Bentle et al. submitted).

Cell death responses induced by β-lapachone. In $NQO1^+$ cells exposed to β-lapachone, μ-calpain activation was observed within 8 h, generating atypical cleavage of PARP-1 and p53 (Pink et al., 2000a,b; Planchon et al., 2001). Caspase activation was not observed, and pan-caspase inhibitors had no affect on β-lapachone-mediated proteolytic events (Pink et al., 2000a; Tagliarino et al., 2001, 2003). To date, this is the only known compound that can cause a tumor-selective (NQO1-mediated), μ-calpain-stimulated cell death response.

Use of β-lapachone for treating human cancers. Insights into the mechanism of β-lapachone cytotoxicity has led to a more well informed use of the compound for cancer therapy. The important aspects of the mechanism are that: (a) cell death is mediated by NQO1 expression, therefore, use of this anti-tumor agent against specific cancers known to over-express NQO1, including prostate, breast, non-small cell lung, pancreas, and colon cancers, is indicated; (b) a minimum of 2 h is required for commitment to cell death and that longer exposure times diminish the therapeutic window of this novel anti-tumor agent, suggesting that bolus doses and not continuous treatments should be applied for optimal advantage of the drug's therapeutic window; and (c) cell death caused by NQO1-induced futile cycling involved reduction of the 1,2-carboxyl sites on β-lapachone, allowing the development of β-lapachone pro-drugs (Reinicke et al., 2005). In the design of β-lapachone pro-drugs, a Schiff's base leaving group was engineered, rendering the compound inactive in NQO1+ cells. However, if the compound was incubated for 1–4 h under acidic conditions the imino leaving group underwent a Schiff's base reaction, converting the initially inert compound to the active β-lapachone anti-tumor agent, as monitored by HPLC-ESI analyses (Reinicke et al., 2005).

Knowledge of the mechanism of β-lapachone cytotoxicity is also being used to pattern cancer therapy strategies against cancers known to have elevated endogenous NQO1 levels. Based on our data and along with the development of β-lapachone prodrugs, we developed strategies that involved short-term and not long-term, in vivo continuous exposures to the drug. Furthermore, these therapeutic strategies are being combined with specific types of drug delivery methodologies. For example, millirods for delivery to treat breast and prostate cancers through brachytherapy regimens

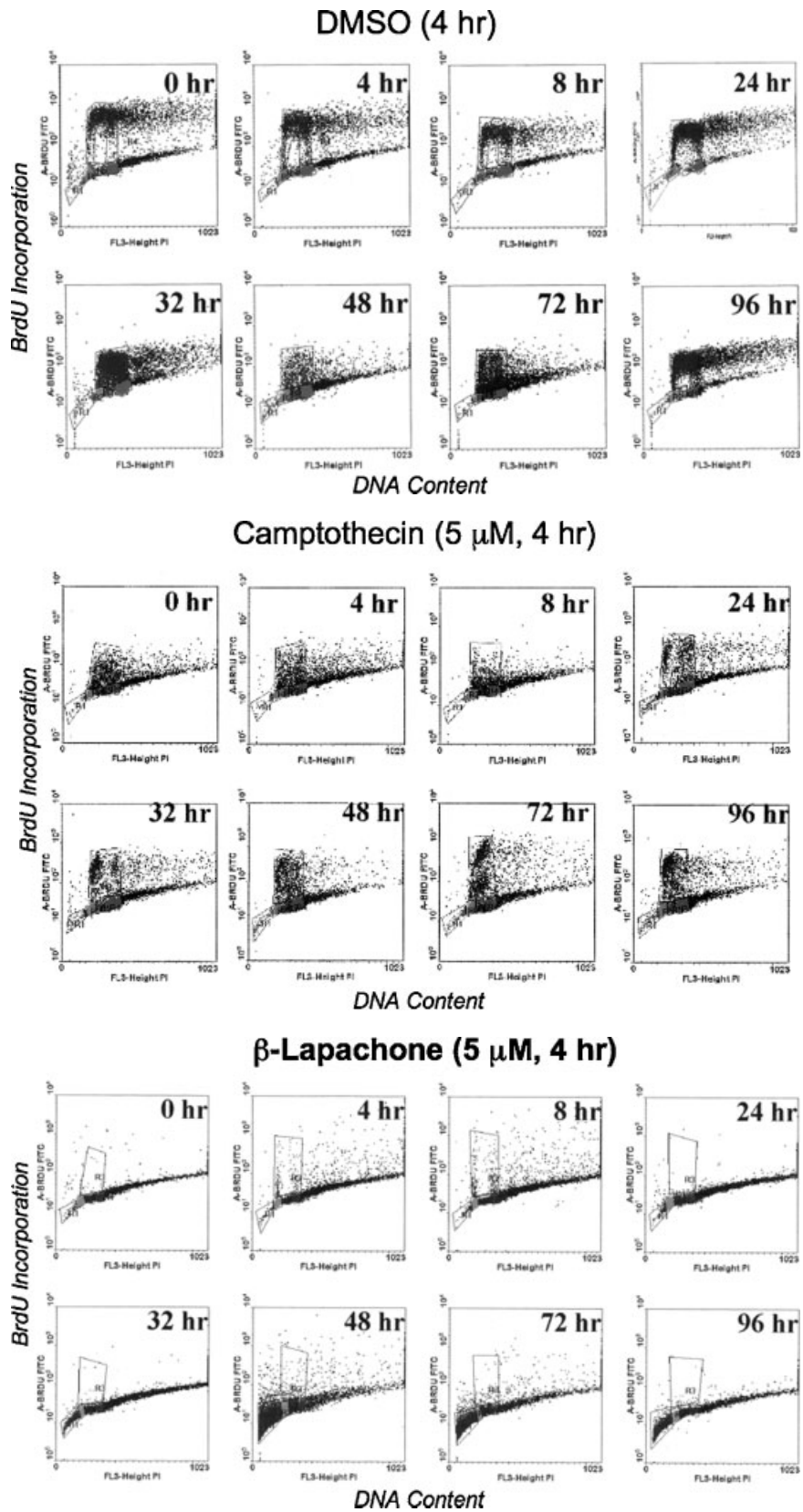

Fig. 2. β-Lapachone causes apoptosis in all phases of the cell cycle. Endogenously expressing NQO1+ log-phase MCF-7 cells were exposed to DMSO alone (**A**), 5 μM camptothecin (CPT) (**B**), or 5 μM β-lapachone (β-lap) (**C**) for 4 h. Cells were allowed to then grow in drug-free complete DMEM medium and at various times labeled with BrdUrd for 4 h and analyzed at various times post-treatment. Note in β-lapachone-exposed cells the absence of BrdUrd containing cells at any stage in the cell cycle. Also, note the appearance of a cell population in between G_1 and G_2 that contained the lowest levels of BrdUrd. These cells represent apoptotic cells from the G_2/M cell population and appear as non-BrdUrd-labeled S-phase cells in standard flow cytometric FACS analyses (not shown). The data are representative of studies performed at least three times in duplicate with identical results.

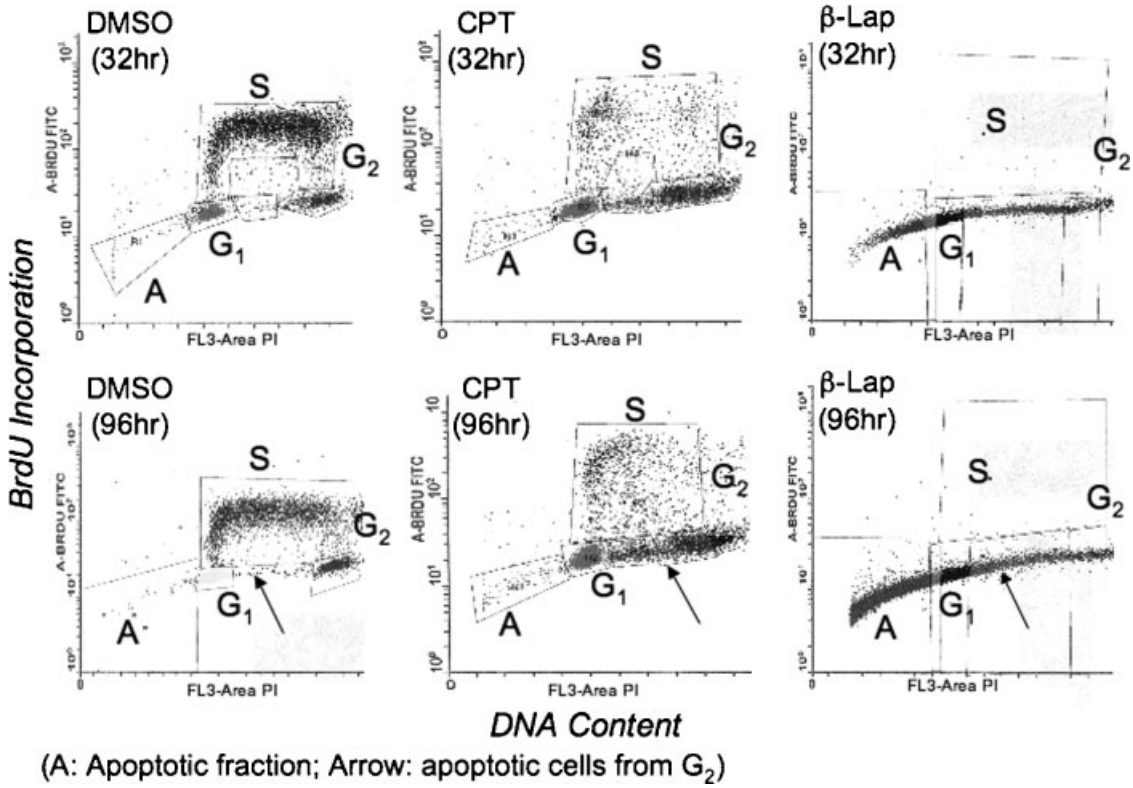

Fig. 3. β-Lapachone-induces apoptosis in a cell cycle independent manner. Composite of studies outlined in Figure 2 at 36 and 96 h. Note the absence of S-phase NQO1+ MCF-7 cells and appearance of apoptotic cells without BrdUrd labeling. Such cells were frozen in their cell cycle, depleted of ATP and NAD+ within 60 min and underwent apoptosis by a μ-calpain-dependent apoptotic pathway. Data are representative of experiments performed three or more times with the same results.

have been developed (Wang et al., in press). Functionalized nanoparticles for systemic and inhalation therapies of breast and non-small cell lung cancer (NSCLC), respectively, have been developed. Thus, by determining the threshold level of β-lapachone required to cause cell death (i.e., "a cell death restriction point") simultaneous with the discovery of the compound's mechanism of action allowed the development of therapeutically useful agents for the specific treatments of human cancers with endogenously elevated NQO1 levels.

Feedback regulation and redundancy principles at work in cell stress responses. Our work on IR-inducible NQO1 expression and the mechanism of β-lapachone lethality was not the only successful exploitation of cell stress responses. The principles of feedback regulation and redundancy also played roles in our studies of IR-inducible proteins (e.g., sCLU regulation and function after IR or TGF-β1 exposures) and DNA mismatch repair-regulated cell cycle G_2/M checkpoint and apoptotic responses (Fig. 4).

Feedback regulation and secretory clusterin (sCLU) transcriptional regulation. As mentioned earlier, my investigations that began in Dr. Pardee's laboratory led to the isolation of X-ray-inducible proteins and transcripts (XIPs and xips, respectively, Boothman et al., 1989a, 1993). These studies are still being pursued in our laboratory. We have been actively investigating the transcriptional regulation of one gene/protein, Clusterin, for the past 5 years. Our recent studies highlight the feedback regulatory nature of this cell stress response protein. Clusterin was isolated as a protein that bound Ku70, a DNA double strand break repair protein, using yeast two-hybrid analyses (Yang et al., 1999). Our studies showed that clusterin was a multi-functional protein, containing both pro-survival (sCLU) as well as pro-death (a splice variant, cytoplasmic/nuclear version of the clusterin protein (i.e., nCLU) (Yang et al., 1999, 2000) functions, whose levels and activation depend on the dose of cytotoxic agents used to treat human cancer cells (Leskov et al., 2001, 2003). nCLU is tightly regulated by nuclear translocation and nuclear localization sites (NLSs), as well as a nuclear export site (NES), demonstrating the tight intracellular regulation of nCLU within nuclei of cells before and after high doses of IR (Leskov et al., submitted). These two proteins, produced by the same gene, act in a Yin-Yang response, counterbalancing cell survival after low doses of IR with a commitment to nCLU-mediated, Bax-dependent cell death responses when cells were stressed with overwhelming damage.

It is clear now that when cells are damaged by cell stress, there are opposing processes in the cell that reach, or fail to reach, threshold levels to yield cell death or pro-survival outcomes. Some of these processes can be permanently changed leading to a growth advantage: either pro-survival processes are permanently expressed, or cell death or growth suppressive processes are permanently turned off, or dramatically suppressed.

The regulation of sCLU by TGF-β1 is an example of feedback regulation. In a series of as yet unpublished experiments, we demonstrated that TGF-β1 exposure caused CLU promoter and sCLU protein induction. Induction of CLU transcription required Smad binding and AP-1 activation, as well as the down-regulation of p53. Interestingly, sCLU protein expression was found, in turn, to bind both TβRI and TβRII receptors and over-

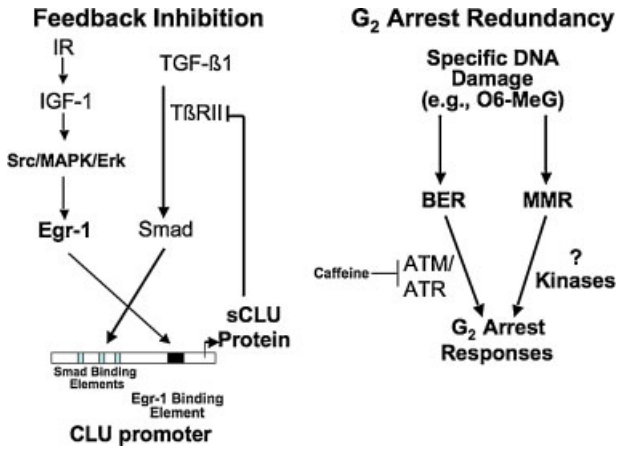

Fig. 4. Pathways of cell stress regulation representing feedback regulation (**A**) and redundancy (**B**). A: the pathway represents a composite of studies on the regulation of x-ray-inducible protein 8 (xip8), also known as secretory clusterin (sCLU), expression following IR (Criswell et al., 2003, 2005) compared to after TGF-β1 exposures (Araki et al., submitted). In particular, induction of sCLU by TGF-β1 exposure results in expression of a secreted protein that, in turn, binds to the TGF-β receptors (TβRI, TβRII) and suppresses subsequent TGF-β1 signaling. Such feedback suppression is reminiscent of the lac repressor discovered by Dr. Pardee over six decades ago. Continuous over-expression of sCLU in colon, breast and prostate cancers can have dramatic bystander effects, greatly repress TGF-β1 signaling and inherently prevent endogenous TGF-β1 growth inhibition. B: our studies on DNA mismatch repair (MMR)-mediated G_2 arrest responses suggest the existence of a MMR-specific and a weaker non-MMR-dependent, ATM/ATR-dependent G_2 cell cycle checkpoint responses. A complete G_2 arrest by cells after many DNA damaging agents relies on both pathways. However, in the absence of MMR, only the ATM/ATR-dependent cell cycle checkpoint arrest pathway is functional after formation of specific lesions detected by MMR.

expression of sCLU in the medium suppressed TGF-β1 signaling and downstream CLU promoter activity induced by TGF-β1 (Fig. 4A). In this manner, much like the *lac* operon discovered in the PaJaMa experiments (noted above), cells effect a response to a signal that is feedback-suppressed in order to properly regulate the response. sCLU is a very important cell stress response regulatory protein, whose expression increases with low doses of stressing agents, such as very low doses of IR (as low as 1 cGy). The functions of the sCLU protein appear to be: (i) suppression of Bax-induced cell death; and (ii) clearance of cell debris caused by trauma. Functionally, sCLU is particularly important for in vivo traumatic responses and for preventing multi-organ dysfunction (MOD) caused by trauma-induced cell debris (Araki et al., 2005).

The role of sCLU protein function as a pro-survival factor is highlighted by its over-expression in numerous cancers, such as prostate, colon and breast. Down-regulation of sCLU protein levels through antisense or siRNA expression strategies have been used to enhance IR or chemotherapeutic agents (Criswell et al., 2005). Clinical trials of sCLU antisense in combination with chemotherapeutic agents are now in progress, and our laboratory recently developed a nanoparticle delivery strategy for sCLU protein knockdown (Sutton et al. Int. J. Nanomed., in press). We are examining its efficacy in preclinical animal trials at this time

DNA mismatch repair (MMR) regulation of G_2 cell cycle checkpoint arrest. As mentioned above, redundancy is a feature of all biological processes. Previous studies from our laboratory confirmed that a deficiency in DNA mismatch repair (MMR) resulted in deficient G_2 cell cycle checkpoint arrest responses, as well as damage tolerance observed as a deficiency in detecting and responding (lack of apoptosis) to alkylating agent or 5-fluorouracil damage (Meyers et al., 2004, 2005). Recent studies from our laboratory have highlighted the redundancy inherent in G_2 arrest responses. Although loss of MMR greatly decreased G_2 arrest, a redundant ATM/ATR-dependent pathway still existed, that was abrogated by caffeine (Wagner et al., submitted), as originally discovered by researchers in the Pardee laboratory (Das et al., 1982; Lau and Pardee, 1982; Fingert et al., 1986; Schlegel and Pardee, 1986; Schlegel et al., 1987). In contrast, the G_2 arrest noted in MMR-proficient cells was not abrogated by caffeine, Chk1 inhibitors or siRNA-mediated knockdown of ATM, or Chk2 proteins. Thus, cells have evolved "redundant coupling responses" between DNA repair and cell cycle checkpoint arrest responses that expend a tremendous amount of energy to stall the cell cycle in order to proof-read DNA before proceeding through cell division (Fig. 4B). Failure to respond or arrest in G_2 by loss of MMR is highlighted by the high rate of mutation and cancer in patients genetically deficient in this repair pathway. In fact, specific loss of expression of two MMR proteins, hMLH1 or hMSH2, account for over 90% of colon cancers, (roughly 8% of all colon cancers) attributed to MMR loss.

SUMMARY AND FUTURE DIRECTIONS

In conclusion, studies from the laboratory of Arthur B. Pardee, Ph.D. have demonstrated and highlighted a number of basic principles that are universal in biological processes. These basic principles include threshold responses (e.g., restriction (R) point control in cell cycle regulation), feedback inhibition, and redundancy. These principles also apply to the molecular biology underlying cell stress responses, and our studies on NQO1 (xip3)-mediated β-lapachone-induced cell death responses, CLU cycle regulation (xip8) gene transcriptional regulation, and MMR-mediated G_2 cell cycle checkpoint arrest responses, respectively, represent examples of these principles in action. Importantly, understanding these processes in the context of these principles can aid our understanding of the processes and reveal targets, that in turn allow for development of targeted novel therapies against specific human cancers. At the University of Texas Southwestern Medical Center at Dallas (UTSW) we have developed an interactive, multidisciplinary program wherein molecular biologists work hand-in-hand with biomedical engineers to develop unique therapeutic agents to exploit molecular targets identified within cancer-selective responses. The principles so well illustrated and highlighted by the past six decades of research performed in the laboratory of Dr. Arthur B. Pardee will be remembered as this program continues to develop therapies that exploit cell stress responses in human cancers, such as stress-induced NQO1 responses.

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