Drug discovery and p53

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In the past two decades, the identification of commonly mutated oncogenes and tumour suppressor genes has driven an unprecedented growth in our understanding of the genetic basis of human cancer. Although oncogenes can clearly serve as classically defined drug targets whose inactivation by small molecules could place a brake on cancer cell proliferation, the restoration of mutated tumour suppressor gene activity by small molecules might appear on the surface to be unrealistic. However, there is a growing realization that many eukaryotic regulatory proteins are partially unfolded and such intrinsically disordered proteins acquire a folded structure after binding to their biological target. Molecular characterization of the p53 protein has shown that its conformational flexibility and intrinsic thermodynamic instability provide a foundation from which its conformation can be quickly post-translationally modified.

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▼ The evolution of the transformed cell involves the sequential mutation in oncogenes and tumour suppressor genes that gives selective growth advantage to populations of cells, thus leading to clonal outgrowth of the tumour. The p53 gene is one of many tumour suppressors identified in recent years (Box 1) but it appears to be relatively unique in its function at a nodal point as a mediator of the cellular response to changes in the microenvironment [1].

The p53 transcriptional pathway

P53 is not required for normal development in mice, but its deletion dramatically increases the risk of cancer incidence [2], suggesting that its function is specialized and/or cryptic. The guardian of the genome model for p53 activation highlighted the latent and inducible nature of the p53 pathway in response to DNA-damage [3], but it is now clear that many distinct, physiological stresses can activate the p53 tumour suppressor to maintain cellular and tissue integrity (Fig. 1). P53 protein activation by these microenvironmental stresses induces its transcription factor activity, which in turn recruits mediators to perform tumour suppressor functions that control apoptosis, growth arrest, kinase signalling, redox status and protein folding (Fig. 1). Upstream effectors in the p53 pathway that respond to DNA damage or oncogene signalling include tumour suppressor genes, such as ATM (ataxia-telangiectasia mutated kinase), CHK2 (checkpoint kinase 2), DAPK, p16 and p14ARF [4]. In this review we will first highlight two p53-inducible genes, p21WAF1 and MDM2, that are the focus of major anti-cancer drug programmes. We will then focus on the prospects of exploiting the conformational flexibility of the p53 protein itself as an avenue for developing anti-cancer drugs.

The p21Waf1 cyclin-dependent inhibitor as a model for anti-cancer drug design

Two p53-inducible gene products, p21WAF1 and MDM2, have been the subject of anti-cancer drug design programmes and these will be reviewed briefly. The most well characterized gene product induced by p53 is the cyclin-dependent kinase inhibitor p21WAF1 [5]. Deletion of the *p21Waf1* gene does not affect cancer incidence like deletion of the p53 gene does, which originally suggested that p21WAF1 was not a major mediator of p53. However, in a Wnt-1 mutant background, *p21Waf1* gene dosage has a significant impact on cancer incidence and p21WAF1 is now defined as a tumour modifier [6]. The steady-state levels of p21WAF1 protein are controlled rapidly in response to signalling changes after phosphorylation via PDK1 (3-phosphoinositide dependent protein kinase) and aPKC (atypical protein kinase C) signalling pathways [7]. Furthermore, the ability of small inhibitory domains of p21WAF1 to function as cell-cycle inhibitors has formed the basis for drug-discovery programmes aimed at replacing p53 function in cancer cells [8]. Synthetic cyclin-dependent kinase inhibitors that mimic p21WAF have been developed [9] and these can activate the wtp53 response [10], as well having independent anti-proliferative effects

1359-6446/03/$ – see front matter PII: S1359-6446(03)02669-2

Box 1. Tumour suppressor genes

Transcription factors	p53, Rb, Wt1, SMAD
Phosphorylation	PTEN, LKB1
Structural	NF2, APC, E-cadherin
Signalling	NF1, INK4a, BRCA1/2
DNA repair	MSH2, MLH1, PMS1/2, MSH6

[11], suggesting that cyclin-dependent kinase inhibitors will prove to be an important portfolio drug for cancer treatment. In effect, therefore, mimetics of p21WAF1 can function as mediators of p53-driven tumour suppression.

A key inhibitor of the p53pathway: MDM2 as a model for anti-cancer drug design

Most human cancers have a defect in the p53 pathway. This inactivation can involve direct mutation and inactivation of p53 itself, mutational inactivation of its upstream-activating enzymes, or amplification of cellular or viral oncogenes that neutralize p53 activity (Box 2). It appears superficially easier to target 'oncogenic' p53-inhibitors for novel anti-cancer drugs than to restore mutant upstream p53-activators like ATM or CHK2, or to reactivate mutant p53 itself. As such, much effort has been placed on identifying cellular proteins, such as viral T-antigen, that inhibit wild-type-(wt)-p53 function [12,13] and some of these proto-oncogenes include the first cellular equivalent of the viral T-antigen, MDM2 [14]. MDM2 has received the most attention as a model for oncogenic drug development. MDM2 protein has a short half-life and its binding to p53 tags the tumour suppressor for ubiquitination, degradation and inactivation by the proteosome. Lead molecules have been designed that can bind MDM2 and activate the wt-p53 response and current reviews highlighting progress in this area extend the drug-development portfolio into ubiquitination-inhibitors, nuclear export inhibitors and proteosome inhibitors [15].

Mutant p53 is an anti-cancer drug target

It is important to note that the p53 protein is not often deleted or truncated in cancers, like many tumour suppressor genes. Selective pressures during cancer progression actually retain a full-length but mutant p53 gene alongside the wild-type gene, suggesting that a gain-of-function oncogenic property is required to promote clonal evolution of the tumour cell [16]. The eventual loss-of heterozygosity by deletion of the wild-type allele is associated with cancers of enhanced metastatic potential and worse prognosis for the patient. The mutant p53 protein is often stabilized in the nucleus of the cancer cell [17] and bound to molecular chaperone complexes [18,19]. The mutant p53 protein is denatured or unfolded in cancers, thus providing the first molecular defect of the p53 pathway in a cancer cell and indicating that p53 is conformationally flexible *in vivo* [20]. There is an ATP-dependent equilibrium between unfolded and folded p53, and co-oligomerization of mutant unfolded p53 drives the wild-type protein into the unfolded conformation [21]. This allosteric nature of the p53 protein provides the only realistic hope that its conformation and activity can be restored by small molecules via stabilization of its oligomeric structure.

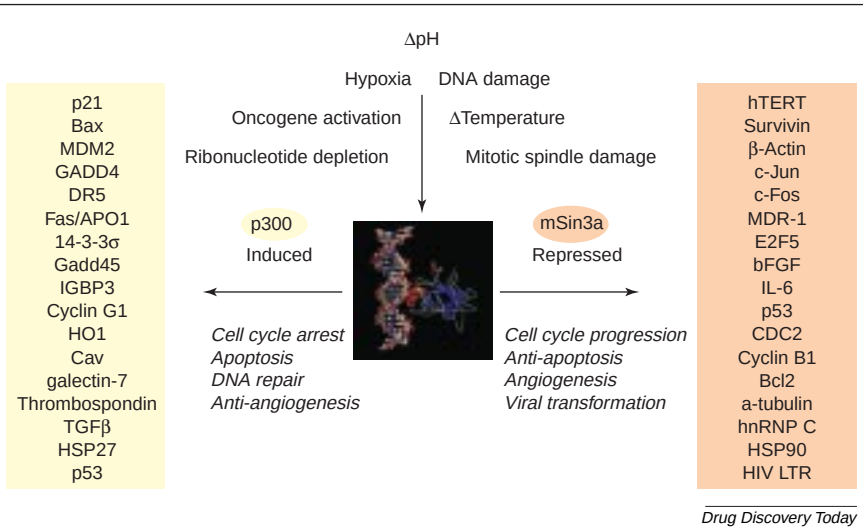

Figure 1. Regulation of p53, a stress-regulated transcription factor that co-ordinately induces or represses sets of gene products in response to changes in the cellular microenvironment. P53 co-operates with the transcriptional activator p300 to induce sets of gene products implicated in growth control (left panel). P53 co-operates with de-acetylases to repress gene expression of proteins implicated in proliferation and survival (right panel). P53 is often mutated in human cancers leading to a protein that can not respond to signalling cues and that can not regulate gene products that orchestrate the stress response. The mutation in p53 unfolds the protein, stabilizes it in the nucleus where it can be bound by molecular chaperones, and creates a protein that often has an oncogenic gain-of-function property.

Reactivation of mutant p53 function

Can the tumour suppressor activity of mutant p53 be restored by controlling protein-folding pathways?

Many human diseases arise, in part, because of the accumulation of misfolded and denatured cellular proteins [22].

Human cancers not only arise as a result of genome instability but also accumulate misfolded proteins during the cellular ageing process that could cause altered signalling required to drive cancer cell proliferation [23]. The cellular 'evolutionary buffer', which permits mutant or misfolded proteins to be stable and to assemble into component pathways *in vivo*, is the chaperone HSP90 [24]. It is encouraging that anti-cancer drugs have been developed that neutralize HSP90 activity and that these drugs can refold mutant p53 into the wild-type conformation [25]. Manipulation of protein conformation in cells by small molecules provides a novel approach that is relatively distinct from classic anti-cancer drugs programmes, which aim to damage DNA and kill cells, to radiosensitize cells or to competitively inhibit engines of the cell-cycle, like mutant *ras* or cyclin-dependent protein kinases.

The p53 protein is tetrameric [26,27], suggesting a relatively complex mechanism of assembly, function and regulation. The tetrameric nature of p53 provides a tetravalent scaffold for the binding of regulatory proteins and expands upon possible regulatory permutations. By characterizing stages in the assembly of a p53 protein transcription complex, various rate-limiting steps have been defined (Fig. 2).

Box 2. Inactivation of the p53 pathway

Dominant-negative missense p53 gene mutation
Upstream tumour suppressor gene mutation (ATM)
Oncogene amplification (MDM2)
Viral oncogene amplification (HPV E6)

These include: (1) p53 monomer assembly into tetramers; (2) equilibrium between the unfolded and folded tetramer, which is controlled by factors such as HSP90; (3) C-terminal kinase phosphorylation that converts p53 protein from a low affinity to high affinity DNA-binding form; and (4) activation domain phosphorylation by CHK2 that promotes p300-catalyzed DNA-dependent acetylation. Data from this last point highlight a conformational constraint that can be placed on p300-catalyzed acetylation [28] and identify a model to begin to study how allosteric changes that are induced upon DNA binding effect core domain-tail interactions [29]. Each of these reactions is regulated by enzymes or modifiers that are potential drug targets and that can either sensitize p53-containing cells to damage-induced death or stimulate the p53 pathway (Fig. 2). In this

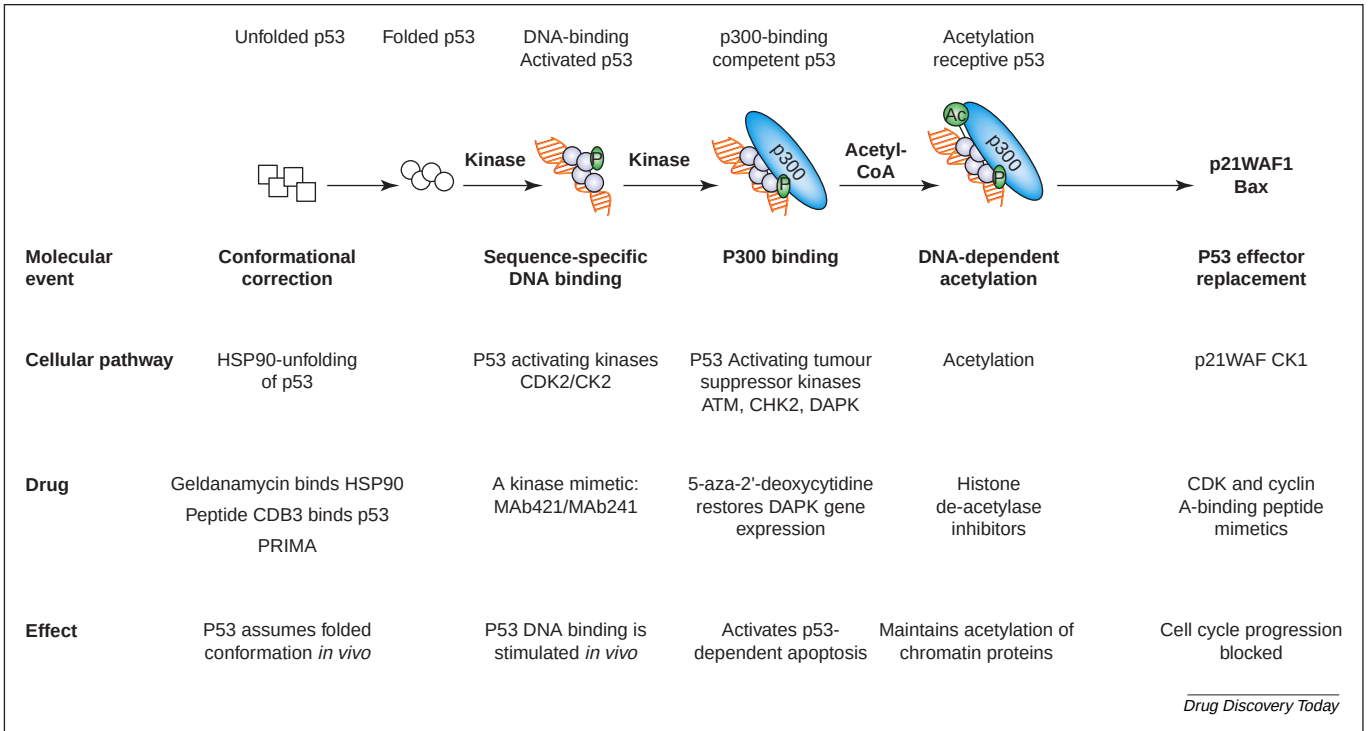

Figure 2. Stages in the assembly of a p53 transcription complex. Dissection of p53 protein post-translational regulation has shown that key steps in its activation include: (1) tetramer assembly controlled by chaperones [66,67]; (2) DNA-binding regulated by protein kinases that neutralize the negative regulatory domain, which normally functions to de-stabilize DNA-binding domain folding [37,38,52]; (3) p300-docking stabilized by p53-kinases [70]; and (4) sequence-specific DNA-dependent acetylation controlled through allosteric effects [28]. Inactivating missense mutations in p53 protein de-stabilize the folding of the DNA-binding domain and compromise tetramer stability [46]. Also, drugs that target specific steps in p53 activation are summarized. The cellular pathway, drug and effect are as indicated.

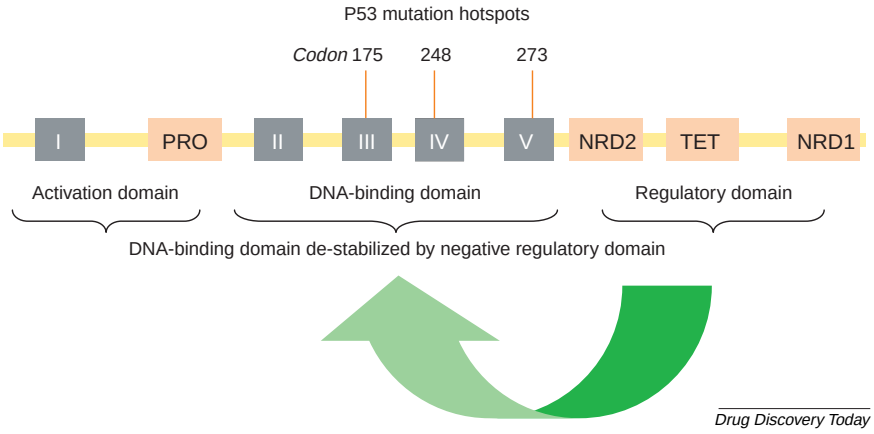

Figure 3. Mutational hotspots in the p53 gene. The domain structure of p53 is indicated and includes the transactivation domain, the DNA-binding domain, and the C-terminal regulatory domain. The C-terminus has two functions: it can destabilize the folding of the DNA-binding domain [38] and is, therefore, a negative regulatory domain. Phosphorylation of the C-terminus relieves the inhibition by the C-terminus and activates DNA-binding [37,49]. By contrast, acetylation of the C-terminus of DNA-bound p53 stabilizes p300-binding [28] and is required for p300-coactivated p53-driven transcription [71]. The C-terminus can therefore also function as a positive regulatory domain. Hot-spot mutations map to the core-DNA binding domain, as indicated. These mutations fall into different functional classes, as indicated in the text.

review, we will focus on the equilibrium that exists between p53 tetramers and prospects for activating the DNA-binding function of mutant p53.

Controversy in p53 allosteric control mechanisms

Mutant forms of p53 protein are defective in sequence-specific DNA-binding because the missense mutation affects one or all of the assembly pathways. An intrinsic conformational or allosteric model for the control of p53 DNA-binding activity permits a mechanism that can alter the equilibrium between conformations, can predict a mechanism for inactivation of p53 by point mutation, and can provide a rationale reactivation of the mutant protein by small molecule binding [30,31]. This conformational or allosteric model has been questioned recently by NMR data showing that the structure of latent and activated-dimeric hybrid p53 proteins are identical [32]. However, this NMR data has not shed light on the dynamic nature of p53 conformational regulation in biochemical assays and a rigid model of p53 conformation can not explain the vast amount of data showing that p53 conformation and activity is regulatable [33]. That is, if p53 tetramers were rigid with no intradomain communication, there could be no peptide activation of mutant p53 by core domain binding (i.e. chaperone rescue of mutant p53 conformation; [34]), no sequence-specific DNA-dependent acetylation by p300 [28], no conformational change detected in p53 after CDK phosphorylation [35], and no destabilizing effects of the core DNA-binding domain by the N-terminal and C-terminal regulatory domains [36–38].

As pointed out previously [39], 'structural information alone is not sufficient evidence for a biologically relevant regulatory mechanism' and other techniques, including partial proteolysis, antibodies directed towards regulatory sites, and truncation mutagenesis, are often required to unmask intrinsic regulatory mechanisms. The allosteric model predicts that an equilibrium exists between two conformations of p53 and also predicts that high concentrations of latent p53 protein, mutations in p53 or phosphorylation, shifts an equilibrium to a different conformation [40]. The high concentrations used in NMR might shift the latent conformation of p53 to an active state and preclude structural insight into the nature of latency. In fact, latent p53 protein can be activated by increasing its concentration in the absence of phosphorylation [41], which is consistent with the allosteric model that there can be an equilibrium between two conformations. Furthermore, the core domain of p53 can be stabilized and stimulated as a DNA-binding protein by a peptide that binds to the core domain, thus indicating that an equilibrium exists between low affinity and high affinity DNA-binding states [34]. The structural basis for this low affinity and high affinity conformation of the core domain are lacking. However, more recent biophysical data have provided a molecular basis for low affinity and high affinity conformations. It has been shown that the C-terminus of p53 can destabilize the core domain of p53 (Fig. 3; [38]) and that, conversely, phospho-mimetic substitution in the C-terminus can stabilize the core domain of p53 [37]. These latter data identify allosteric control mechanisms operating in the p53 tetramer, which appears flexible and not rigid [38].

An additional contradiction to the allosteric model was presented by data showing that p53 protein is not latent on long DNA fragments containing the consensus site and latency is related to non-specific DNA-binding coming from the C-terminal domain [42]. This latter steric model of p53 control predicts, therefore, that p53 is rigid and that phosphorylation activates p53 because it would decrease non-specific DNA-binding. However, phospho-mimetic mutation at the C-terminal phosphorylation site increases non-specific DNA-binding [37] and thus is not consistent

with a steric model of kinase regulation. Furthermore, more recent data has shown that p53 is latent on long DNA containing the consensus site [43,44], indicating that non-specific DNA binding does not have a dominant role in latency.

Additional work by Bakalkin and colleagues have developed a conformational model (two-site model) of p53 regulation that provides an interesting framework from which to study the effects of the C-terminus of p53 in promoting latency of the core DNA-binding domain p53 [33]. The two-site model is also not consistent with a steric model of regulation, but invokes a p53 core domain–C-terminal domain interaction that regulates p53 binding to non-specific and consensus-site DNA. Further, this two-site model is not necessarily inconsistent with recent data showing that sequence-specific DNA acts as an allosteric effector to activate p53 acetylation by p300 [28]: these data indicate that the C-terminus of p53 is constrained as an acetylation substrate in the absence of consensus-site DNA in oligonucleotide or supercoiled plasmid DNA form *in vivo* and identifies an allosteric component to DNA-driven acetylation. Thus, the details of p53 protein allostery and DNA-dependent control of p53 conformation or acetylation are reflected in the dynamic or flexible nature of the p53 tetramer.

Point mutations in the core domain of p53 inactivate the protein by different mechanisms

The study of p53 mutants have shown that a different conformational class of mutants exist and there are at least four different classes of p53 DNA-binding mutants (Fig. 3). The reactivation of each class requires a different strategy depending upon the mechanism of p53 protein misfolding. One class (structural) is thermodynamically unstable as a result of mutations in the core domain of p53, which unfold the tetramer (R175H). A second class has a mutation in the tetramerization domain that destabilizes the tetramer into dimeric or monomeric forms (not shown). A third class (allosteric) has a thermodynamic stability that is similar to wt-p53 (i.e. R273H), but mutations prevent kinase activation [40] and this point mutation in p53 sequesters the activation domain of the mutant p53 [36]. However, the R273H mutant has the intrinsic capacity to bind DNA-sequence specifically because it can be activated by antibodies that bind at the kinase sites [45]. A final class has mutations in the active site (R248Y) and because of the conformational flexibility of the p53 core domain, the active site mutations actually effect folding of the core domain [46]. The R248Y mutation was simply thought to effect p53-DNA contact [47], however, this unexpected influence of the R248Y mutation on core domain stability is consistent with models that p53 is conformationally flexible and not rigid. The prospects for reactivation of two of these classes, allosteric and structural, will be discussed below.

Control of the allosteric class of mutant p53

An 'allosteric' mutant encoded by the His273 allele is one of the common mutations found in cancers and the thermodynamic stability is similar to wt-p53 [46]. The His273 core domain mutation in p53 alters the conformation of p53 at the N-terminal activation domain so that it can not drive transcription in the yeast-two hybrid assay [36], identifying a DNA-binding domain–transactivation domain interaction. This allosteric interaction between the transactivation domain and core DNA-binding domains is seen in many transcription factors [48]. The mutant encoded by the His273 allele has served as a model for reactivation of mutant p53 by modulating the C-terminal regulatory domain of p53 and is reviewed below.

Biochemical studies have shown that the unphosphorylated wt-p53 protein can not bind DNA-sequence specifically when the consensus site is embedded within an oligonucleotide or long DNA fragment [43,44]. Kinase phosphorylation at the CDK site (Ser315) or CK2 site (Ser392) can activate this cryptic function of wt-p53 *in vitro* [49,35] and X-rays or UV irradiation-damage induced kinase pathways phosphorylate these sites *in vivo* [43,50,51]. The discovery of antibodies that can bind to phosphorylation sites that mimic kinases, thereby activating p53 *in vitro* and *in vivo*, gave rise to the allosteric model of p53 activation, whereby four-binding sites need to be occupied to convert the latent p53 tetramer into an activated state [52]. Additional studies have shown that stoichiometrically phosphorylated wt-p53 tetramers (four phosphates/tetramer) can be purified in a latent state, indicating that kinase phosphorylation does not spontaneously activate p53 and, therefore, it does not simply neutralize non-specific DNA binding [49]. In fact, although it was originally postulated that phosphorylation of p53 in its C-terminus would decrease non-specific DNA binding [42], the S392D mutant p53 tetramer paradoxically has an increased affinity for non-specific DNA [37]. Mutant forms of p53 (allosteric class encoded by the His273 and Lys285 alleles) exist that can not be activated by kinase phosphorylation but can be activated by antibody binding *in vitro* and *in vivo* [40,45,53]. These early studies suggested that an equilibrium exists between at least two conformational states in this mutant class of p53. Presumably, phosphate addition can not override the energy barrier to mutant p53 activation, while antibody binding provides the energy to stabilize the active conformation [40]. The equilibrium between states is further supported by data showing that peptides binding to the core DNA-binding domain of mutant p53 can stimulate

DNA-binding by shifting an equilibrium between conformations [34].

More recent biophysical studies on wt-p53 have advanced the molecular basis for p53 latency and have provided a much deeper understanding of these earlier protein structure–function studies on p53 DNA-binding control and explain the allosteric effects of phosphorylation on p53 activity [38]. Many eukaryotic proteins are partially unfolded and such disordered proteins become folded after binding to their respective target [54]. One important new concept to emerge is that wildtype-p53 protein is intrinsically unstable thermodynamically and that some common cancer-derived mutations can destabilize the core domain [31,46]. This is consistent with the models that regulatory proteins are intrinsically disordered and binding and/or modification can induce a folded or stable structure [55]. Thus, p53 mutants can simply be thought of as intrinsically temperature sensitive, thus providing a rational basis for stabilizing mutant conformation with therapeutic effect [56].

Another significant advance in understanding allosteric control of p53 was the demonstration that p53 is an example of a native protein that is partially unfolded and that an equilibrium between 'partially-folded states' can modulate p53 conformation [38]. On the basis of tertiary structure studies using equilibrium unfolding transitions and circular dichroism, several interesting features of p53 protein conformational flexibility were described. Biophysical studies have shown that the C-terminus can induce a significant destabilization in the core domain of wt-p53 [38] and the ability of phosphorylation to neutralize this negative regulatory domain restabilizes the core domain and activates specific DNA-binding. Furthermore, phosphomimetic mutation at the CK2 site in the C-terminus of p53 can significantly stabilize β-sheet structure in the core domain of p53 [37]. These two studies provide the first set of biophysical data that the conformation of the core DNA-binding domain of p53 (not the DNA-binding activity) can be controlled by the C-terminal domain. In essence, the unphosphorylated regulatory domains at the ends of the p53 tetramer behave like missense point mutations in the core domain and decrease core DNA-binding domain p53 stability (Fig. 3).

Recent discussions on protein conformation regulation have highlighted the idea that proteins can be intrinsically unstructured or partially unfolded and that structure can be acquired by an induced-fit mechanism [55]. This allosteric or equilibrium model between unfolded and folded states is significant because it provides a mechanism to control p53 conformation post-translationally. For example, the destabilizing effects of the C-terminus or N-terminus might be neutralized by p53-binding proteins or by modifications such as phosphorylation. The positive cofactor p300 might stimulate p53 activity in part by creating a structure in p53 by an induced-fit mechanism and stabilizing the tetramer. P300 embraces multiple contact points on the p53 tetramer and the sequence-specific DNA-dependence in p300-catalyzed acetylation highlights a novel allosteric effect of DNA on p53 protein conformation and subsequent protein–protein interactions [28,29]. In contrast to p300, negative regulatory cofactors, such as the MDM2 protein, can melt and further destabilize the p53 tetramer (unpublished data), thus indicating that the equilibrium in p53 protein tetramer stability can be driven in two directions.

Refolding of the structural class of mutant p53

The discovery of a mechanism via antibody binding to activate the allosteric class of mutant p53 *in vivo* provides an exciting precedent for targeted activation of classes of p53 mutant [45]. The other common 'structural' mutant p53 encoded by the His175 allele can not be activated by antibody binding and further highlights the existence of different classes of mutant p53 [45]. Four independent programmes of research (see below) have targeted the reactivation of the structural p53 mutant and set a precedent for experimental manipulation of the conformation of a mutant polypeptide.

In vitro *refolding screens*

Most cancer-derived p53 mutants are thermodynamically unstable [31,46] and one of the most oncogenic and unfolded proteins is encoded by the His175 allele. This mutant can not be activated by the same pathway that can be used for the allosteric class of p53 mutant because of the high degree of unfolding at physiological temperatures. Based on the models that the structural mutant p53 protein exists in a folded and unfolded state (both of which can be recognized by a distinct monoclonal antibody) a high throughput screen was used to identify a class of small molecule (CP31398) that could stabilize the conformation of this class of mutant p53 [57]. Although CP31398 can stimulate the p53 pathway in cells, it has been questioned whether this drug actually stabilizes mutant p53 conformation *in vivo* [58]. This study claimed that CP31398 does not bind to the core domain of p53. However, because the regulatory domains of p53 have profound effects on p53 tetramer stability [37,38], it is likely that CP31398 does bind to p53 tetramers by interacting at sites outside the core domain, thus stabilizing core domain conformation by allosteric effects. Furthermore, a recent study has shown that p53 activation *in vivo* by CP31398 actually blocks p53 ubiquitination [59]. Thus, these two studies [57,59] were therefore an important precedent in p53 conformational

control by small molecules since it was established that small molecule screening could identify a compound that prevents unfolding of mutant p53 protein and stabilizes mutant p53 *in vivo*.

In vivo *refolding screens*

A complement to the screening assay of looking for molecules that stabilize the conformation of p53 *in vitro* would be to use a cell-based assay to screen for molecules that activate the transcription function of p53 *in vivo*. Such a screen has recently led to the discovery of a class of small molecule (PRIMA) from the National Cancer Institute (NCI; http://www.nci.nih.gov) library that can refold the mutant conformation of p53 into the wild-type conformation *in vivo* and reactivate mutant p53-dependent transcription and apoptosis *in vivo* [60,61]. Because p53 is so frequently mutated in human cancers, such molecules hold high promise as a precedent for agents that can reactivate mutated p53 in cancers for therapeutic benefit. The disadvantage of that approach is that direct effects of the drug on p53 conformation can not be established. For example, such mutant p53 refolding molecules might act on the molecular chaperone pathways [25].

The chaperones that bind mutant p53 function as a coordinated holoenzyme or protein-folding machine with discreet steps in the protein folding and unfolding process [62,63]. The core polypeptides of the holoenzyme can include HSP90, HSP70, HSP40, $p60^{HOP}$, $p48^{HIP}$, various immunophilins, and p23, with each protein having distinct functions including substrate recognition and release, nucleotide-dependent binding and turnover, and conformational regulation of the holoenzyme complex organization [64]. Chaperones can function as anti-apoptotic effectors in cells that are exposed to otherwise toxic levels of damaging agent, and the ability of anti-tumour antibiotics of the ansamycin class to bind to chaperones, which are overexpressed in tumour cell lines, to induce cell death further highlights their role in cell survival and the attraction for targeting chaperones for therapeutic effect [65]. P53 conformation can be restored *in vivo* using the anti-tumour antibiotic Geldanamycin indicating that the potential exists to manipulate p53 conformation for therapeutic effect [66,67]. Thus, two independent research programmes gave rise to Geldanamycin and PRIMA, both of which can permit refolding of mutant p53 protein *in vivo*.

Rational in vitro *refolding assay: peptide activation by p53 core domain stabilization*

The intrinsic degree of thermoinstability in p53 mutants suggests that a common approach could be used to reactivate the protein [46]. For example, the ability of chemical solvents or hypothermic regimes to refold common p53 mutants *in vivo* [56,68] is consistent with biophysical studies that predicted a mild degree of intrinsic thermodynamic instability in p53 could be overcome to restore conformation. Modelling of the p53-stimulating factor BP2 [69] into a fragment that binds the core domain of p53 has led to the development of small peptides that can stimulate the DNA-binding activity of wt-p53 [34]. The unusual feature of this peptide is that its binding site is adjacent to, or within, the active site for DNA-binding and its ability to stimulate DNA-binding can only be explained by an induced-fit mechanism. This results in the p53 core domain stabilization into an active conformation, followed by peptide dissociation and high affinity DNA-binding. The demonstration that the core domain exists in at least two functional DNA-binding states (low affinity and high affinity) is consistent with the biochemical studies proposing that an equilibrium exists between latent and partially-unfolded native tetramers [38]. The ability of the p53 core-domain binding peptides to stimulate mutant p53 DNA-binding will undoubtedly lead to an expansion of the chaperone-rescue strategy for mutant p53 reactivation. In future, it will be important to further characterize other mutant p53 protein alleles to understand and group them into classes for specific targeted refolding strategies.

Conclusion

Specific degenerative diseases, such as Alzheimers or Huntington's, as well as more general illnesses like cancer, are the result of epigenetic dysregulation of cellular metabolism. The development of many such diseases correlates with the accumulation of malformed proteins during the ageing process. Many regulatory proteins are now believed to be intrinsically unstable or partially unfolded and the control of protein-folding pathways might be a wide-spread therapeutic strategy for disease management. The tumour suppressor p53 is an example of an allosteric protein whose conformation can now be manipulated in model systems and this has provided a precedent that is relevant for diseases that exploit misfolded proteins.

Biological studies on the p53 response have identified its effectors and inhibitors that have key roles in growth control. These effectors have been modelled into synthetic drugs like Roscovitine that inhibit cyclin-dependent kinases and halt cell-cycle progression. The p53-inhibitory pathways involved in ubiquitination or nuclear export have been targeted for drug-development that could form the basis for novel anti-cancer drug treatments. More molecular studies on p53 protein conformation and on the regulation of its DNA-binding activity have indicated that the protein is oligomeric, that p53 protein is in an equilibrium between

different conformational states, that the regulatory domains in p53 control its core DNA-binding domain conformation, and that cancer-prone mutations can alter the normal conformational equilibrium within the tetramer. The ability to exploit these equilibrium or allosteric models and provide a mechanism to reactivate mutant p53 protein has led to the identification of numerous small molecular weight agents that can activate mutant p53 pathway in cancer cells. The accumulation of a battery of small molecules that are capable of reactivating both wild-type and mutant p53 *in vivo* raises our hopes of developing anti-cancer drugs that can be used in combination with sophisticated diagnostics to treat cancer rationally.

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