

Misfolding, aggregation, and disordered segments in c-Abl and p53 in human cancer

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The current understanding of the molecular mechanisms that lead to cancer is not sufficient to explain the loss or gain of function in proteins related to tumorigenic processes. Among them, more than 100 oncogenes, 20–30 tumor-suppressor genes, and hundreds of genes participating in DNA repair and replication have been found to play a role in the origins of cancer over the last 25 years. The phosphorylation of serine, threonine, or tyrosine residues is a critical step in cellular growth and development and is achieved through the tight regulation of protein kinases. Phosphorylation plays a major role in eukaryotic signaling as kinase domains are found in 2% of our genes. The deregulation of kinase control mechanisms has disastrous consequences, often leading to gains of function, cell transformation, and cancer. The c-Abl kinase protein is one of the most studied targets in the fight against cancer and is a hotspot for drug development because it participates in several solid tumors and is the hallmark of chronic myelogenous leukemia. Tumor suppressors have the opposite effects. Their fundamental role in the maintenance of genomic integrity has awarded them a role as the guardians of DNA. Among the tumor suppressors, p53 is the most studied. The p53 protein has been shown to be a transcription factor that recognizes and binds to specific DNA response elements and activates gene transcription. Stress triggered by ionizing radiation or other mutagenic events leads to p53 phosphorylation and cell-cycle arrest, senescence, or programmed cell death. The p53 gene is the most frequently mutated gene in cancer. Mutations in the DNA-binding domain are classified as class I or class II depending on whether substitutions occur in the DNA contact sites or in the protein core, respectively. Tumor-associated p53 mutations often lead to the loss of protein function, but recent investigations have also indicated gain-of-function mutations. The prion-like aggregation of mutant p53 is associated with loss-of-function, dominant-negative, and gain-of-function effects. In the current review, we focused on the most recent insights into the protein structure and function of the c-Abl and p53 proteins that will provide us guidance to understand the loss and gain of function of these misfolded tumor-associated proteins.

Keywords: misfolding, tumor suppressor, kinases, signaling, cancer

The Folding Perspective of Misfolding

The fundamental dogma in biology for all living organisms dictates that DNA becomes RNA through the transcriptional machinery, and this step is followed by the translation of RNA to specific proteins. From the molecular point of view and using the most recent DNA technologies, it may seem easy to decode our genes to get a picture of the entire organism. However, the actual situation is a more complex and challenging scenario considering the hierarchical perspective of cells, tissues, organs, and the entire organismal network.

The next step to be elucidated in the “DNA-to-RNA-to-protein cascade” and the current challenges faced by researchers include how a linear strand of amino acids is able to minimize its free energy and conformational entropy and, ultimately, collapse into a functional architecture. To assess the reversibility of the folding/unfolding equilibrium (F–U) of a stretched amino acid sequence, several spectroscopic approaches coupled to physical perturbations are available (1–6). Depending on the sequence and length, a pure protein solution is able to shift the F–U equilibrium in a reversible manner. Uncovering this intrinsic feature of proteins to fold independently gives rise to an astonishing hypothesis: the assembly of functional protein architectures in the conformational space proceeds with precision and fidelity by itself and depends exclusively on the amino acid sequence.

From the cellular perspective, the concept of an energy landscape, in which intramolecular forces are mandatory to condense the most thermodynamically stable ensemble of conformations in a stochastic search, must now pay attention to influences from the crowded cellular milieu. The pattern of hydrophobic and polar residues in a specific amino acid sequence dictates the preferential contacts and the fingerprint for protein folding and dynamics. These key elements are likely selected and optimized during protein evolution to ensure sampling of a restricted number of conformations and ensure that proteins are “minimally frustrated” (7). The evolution of protein folding, particularly the folding of proteins containing multiple domains, provides an exquisite understanding of highly energetic substates, also known as protein intermediates. Depending on the protein architecture and secondary content, small proteins (60–100 residues) may be converted from their unfolded to their native states without populating long-lived intermediate states (two-state equilibrium). In contrast, those with more than approximately 100 residues or that are composed of at least two globular domains commonly populate intermediate states during the folding process.

Based on these assumptions, an ongoing puzzle in modern science concerns the involvement of such high-energy intermediates as driving forces to initiate pathological processes, such as several amyloidogenic disorders and cancer. During a “protein’s life” within a crowded cell, from the moment at which a nascent chain is still attached to the ribosome to the moment of death in proteasomes, amino acid chains suffer high environmental pressure to guarantee that folding takes place in a precise manner. Conversely, even considering the strictly evolutionary checkpoints, i.e., key amino acid interactions to avoid misfolding and a specialized endoplasmic reticulum (ER) compartment, folding is not always an infallible process.

In this review, we describe the current understanding in the field of protein misfolding and consider the formation of misfolded species, disordered segments, and aggregation and their involvement in physiological function and cancer development. We focus on the c-Abl and p53 proteins and shed light on the involvement of incorrect conformations in amyloid formation and the triggering of cell malignancy and cancer progression.

The Cell Against Misfolding

Cell survival and proliferation are strictly dependent on multiple hierarchic pathways and a highly orchestrated network of thousands of biomolecules and cofactors. To ensure homeostasis, the building blocks of cells, i.e., protein molecules, require proper folding and dynamics to ensure their ability to work correctly and avoid cellular stress and ultimately malignant transformation. During evolution, cells acquired specialized machinery and a major housing organelle called the ER to regulate protein folding, post-translational modifications, lipid and steroid synthesis, gene expression, cellular metabolism, and calcium signaling.

The ER compartment is a “safe place” responsible for regulating the quality, folding, maturation, and trafficking of newly synthesized proteins. Features of the ER lumen are a high calcium concentration and an oxidizing environment (8) as well as a complete “army” of resident chaperones and enzymes (9, 10) to guarantee the proper folding and maturation of client proteins. The best-understood molecular chaperones are the heat shock proteins Hsp70 and Hsp90, which aid conformational maturation and target misfolded proteins for ubiquitination and proteolysis, and the chaperonins, which sequester newly synthesized proteins or misfolded ones within their structural environment for proper folding. Up to one-third of cellular proteins are synthesized within the ER (10), and most of those destined for the extracellular space are scrutinized for potential toxicity. The most common modifications in the ER factory, such as signal sequence cleavage, N-linked glycosylation, disulfite-bond formation, and glycosylphosphatidylinositol and membrane protein reshuffling and anchoring, make the process from a naive to a mature polypeptide chain slow and not always efficient. Nevertheless, secretory cells, such as hepatocytes, plasma cells, pancreatic β -islet cells, and several exocrine gland cells, manage millimolar amounts of nascent proteins at different stages of folding and assembly with extraordinary efficiency.

Interestingly, after appropriate folding and modifications, mature polypeptides are somehow sorted from misfolded species in the ER and sent to the Golgi apparatus and along the secretory pathway. Despite extended investigations to uncover the mechanism through which resident vs. client and folded vs. misfolded species are sorted in the ER for subsequent trafficking to the Golgi apparatus, this process remains obscure. Sorting models that attempt to explain this phenomenon are classified as follows: (i) receptor-mediated transport, (ii) aggregation of misfolded proteins, which restricts their ability to be transferred from the ER to the Golgi apparatus through small transport vesicles, (iii) ER retention by Golgi retrieval and (iv) the attachment of misfolded proteins to an ER matrix (11).

Dynamic control of protein synthesis, degradation, and repair dictates cell homeostasis. When the protein folding efficiency is threatened due to protein overload within the ER, cells start to experience ER stress and activate the “unfolded-protein response” (UPR). There are three well-characterized signaling sensors triggered by the UPR to overcome ER stress, i.e., inositol-requiring protein-1 α (IRE-1 α), activating transcription factor 6 (ATF6), and protein kinase RNA-like ER kinase (PERK). In this process, the aim is to eliminate misfolded proteins and to reduce the load of newly synthesized polypeptides within the ER. This is accomplished by decreasing the amount of mRNA available for protein synthesis, slowing the transcription/translation machinery for new mRNAs, and increasing the concentration of molecular chaperones and foldases to process accumulated proteins within the ER. Of note, the PERK branch of the UPR response was recently linked to hematopoietic stem cell (HSC) clonal integrity, in which the clearance of individual HSCs after stress prevents the propagation of these damaged progenitors (12). The remaining misfolded proteins not recovered from UPR mechanisms are sent to the ER-associated degradation (ERAD) pathways known as the ubiquitin/proteasome pathway (ERAD I) and the autophagic/lysosomal pathway (ERAD II). UPR and ERAD sensors fight to keep the cell alive and overcome cellular stress, and if these processes are unsuccessful, specialized machinery initiates programmed cell death (PCD) pathways, such as apoptosis (PCD1), autophagy (PCD2), or necrosis (PCD3). A cell's decision to live or die during cellular stress is a complex, fine-tuned mechanism that is not fully understood. For more in-depth information on UPR and ERAD mechanisms, please refer to more specialized literature (13–20).

Although evolutionary mechanisms have developed to guarantee the quality control of native protein conformations, abnormal protein synthesis is common and harmful to cells and is involved in more than 40 protein-misfolding diseases (21), including cancer, as was recently demonstrated (22, 23). The concept of a prion-like seeding mechanism is now behind the most common amyloidoses and neurodegenerative diseases (24). The tumor-suppressor p53 is a transcriptional factor that exerts broad anti-proliferative effects, including growth arrest, apoptosis, and cell senescence after cellular stress, and has been described as the most frequently mutated gene in cancer cells (25). It was recently demonstrated that subunits of the cytosolic group II chaperonin (CCT) are part of the p53 interactome (26). The correct folding of wild-type (wt) p53 requires CCT interaction, and the failure to interact with this molecular chaperone can promote the oncogenic functions of p53, even in the absence of typical DNA-binding domain (DBD) mutations (27). The correct folding of wt p53 in CCT is not a guarantee that it will safely exert its functions because mutated p53 (R248Q) aggregates into a mixture of oligomers and fibrils and sequesters the wt protein into an inactive conformation, explaining the prion-like behavior of this protein (22, 28). Breast cancer cells carrying mutated p53 exhibit a massive expression of aggregated p53 in the nucleus compared with breast cancer cells carrying wt p53, a condition that has also been shown in biopsies of breast cancer tissue (22, 29). The mechanism through which mutated p53, in association with different types of cancer, escapes the ER quality control mechanisms and triggers the

dominant-negative effect of its wt counterpart is a matter of debate and is awaiting further exploration.

The Adaptive Response of Cancer to Misfolding

The reality of cancer is that these cells exhibit genetic plasticity and adaptive advantages to survive in harmful environments. Although cancer cells adapt to trigger angiogenesis, during the growth of solid tumors, the nutrient and oxygen requirements exceed those in the surrounded vascular network. Thus, the highly proliferative and less vascularized environment of several types of cancers generates low pH (lactic acidosis), low oxygen (hypoxia), oxidative stress, and low supplies of glucose and amino acids. A small decrease in pH leads to changes in protein conformation; e.g., p53 tends to adopt a molten-globule conformation at slightly lowered pH values (30). It has also been shown that client p53 assumes a molten-globule-like state in the presence of Hsp90 (31). Depleted glucose affects protein glycosylation and ATP production, and a lack of oxygen, as an electron carrier, impairs disulfide bond formation (32). All of these factors contribute to the accumulation of misfolded proteins within the ER and the UPR. In normal cells, the stress amplitude triggers pro-survival or pro-death UPR signaling, but cancer cells escape pro-death signaling and adapt to grow under these unpleasant conditions.

The binding immunoglobulin protein (BiP), which is also known as 78-kD glucose-regulated protein (GRP78), is a chaperone and the main regulator of the UPR sensors IRE-1 α , PERK, and ATF6. GRP78 inhibits the homodimerization and activity of PERK and IRE-1 α (33, 34) and blocks Golgi-localization signals and further processing of ATF6 to its active conformation (35, 36). This protein was first discovered due to its upregulation in response to glucose depletion (37), a common adaptive condition known as the Warburg effect (aerobic glycolysis) (38, 39). During ER stress, increased levels of misfolded proteins bind to GRP78 in a competitive manner, leading to its dissociation from the UPR regulators IRE-1 α , PERK, and ATF6. The dissociation of these UPR sensors activates ER stress signaling and culminates in the regulation of gene expression to overcome the stress condition. In tumorigenic development, downstream signaling triggered by GRP78 promotes an increase in cell proliferation, protection against apoptotic events, and the activation of tumor angiogenesis (40). Indeed, this chaperone plays a central role in the cancer adaptive response to ER stress.

An increased level of GRP78 has been reported in several solid tumors, including breast, melanoma, lung, brain, and colon (41–43), and is also associated with cancer metastasis (44). It plays a dualistic role: it can control the induction of dormancy at the beginning of tumor development but also promote pro-survival (45) and pro-metastatic functions in advanced stages (44). The localization of GRP78 to the cell surface has been shown to regulate proliferation and apoptosis in neoplastic and endothelial cells under severe ER stress (46). In prostate cancer cells, GRP78-binding partners at the cell surface, such as α_2 -macroglobulin, have been shown to increase cell proliferation (47). The use of antibodies to avoid the interaction of GRP78 with Cripto, a tumor cell-surface protein involved in the regulation of tumor

progression, is sufficient to inhibit oncogenic signaling (48). In addition, ER stress has been shown to accelerate the neovascularization associated with GRP78/T-cadherin complexes (49). In contrast, the binding of GRP78 at the cell surface with Kringle-5, an angiogenesis inhibitor, is required for its anti-angiogenic and pro-apoptotic activities in stressed tumors (50). The overexpression of GRP78 protects human breast cancer cells from estrogen-starvation-induced apoptosis (51), and the binding of GRP78 with caspase-7 prevents apoptotic induction by topoisomerase inhibitors (52). Uncovering the interactome of this important chaperone associated with ER stress and the neoplastic adaptive response may provide insights for targeting cancer cells against tumorigenic development.

Heat shock protein 90 (Hsp90) is another important molecular chaperone that participates in the adaptive response of cancer cells. Together with Hsp70 and other co-chaperones, the Hsp90 complex stabilizes and activates more than 200 client proteins (53). This task is accomplished by several transient low-affinity protein-protein interactions that help Hsp90 client proteins be correctly folded or stabilized. The Hsp90 machinery is used by cancer cells to protect several mutated and overexpressed oncoproteins, such as mutated p53 and Bcr-Abl, from misfolding and degradation. However, it is also involved in normal cellular physiology, including nuclear processes such as those involved in transcription, chromatin remodeling, and DNA damage-induced mutation (54).

The transcriptional repressor BCL-6 regulates ataxia telangiectasia and Rad3-related (ATR) and TP53 gene expression (55). The Hsp90 and BCL-6 protein complex represses ATR and TP53 expression in diffuse large B cell lymphomas. Hsp90 inhibition has been shown to decrease BCL-6 levels due to protein instability, leading to the activation of target genes (ATR and TP53) and the apoptosis of lymphoma cells, showing that the Hsp90-BCL-6 interaction is crucial for lymphoma survival (56). Wt p53 is a short-lived protein that turns over through the ERAD I machinery (57). Hsp90 has been shown to bind wt p53 (31, 58) and is necessary for protein stability and proper DNA-binding ability at physiological temperatures (59, 60). However, Hsp70 is also required to support p53 activity under stress (61). Surprisingly, Mdm2, a negative regulator of p53, has been shown to chaperone against p53 (62). When mutated, however, p53 has an increased intracellular half-life due to impaired ERAD I degradation processing (63). Part of this impaired mechanism occurs because of the aberrant physical association of mutated p53 with the Hsp70 and Hsp90 molecular chaperones, which may protect the protein from ERAD I processing (64, 65). Geldanamycin (GA), a selective Hsp90 inhibitor, is able to restore the ERAD I processing of mutated p53 in tumor cells but is not effective at restoring its transcriptional factor function (63, 65).

Geldanamycin and other benzoquinone ansamycins not only target mutated p53 through Hsp90 inhibition but are also effective at dissociating several kinase-Hsp90 complexes and thereby alleviating downstream signaling pathways and kinase-induced oncogenic transformation (66–68). In chronic myeloid leukemia (CML) or acute lymphoblastic leukemia (ALL), a reciprocal translocation between the *bcr* and *c-abl* genes produces the unregulated kinases p210^{Bcr-Abl} and p185^{Bcr-Abl} (69). In this type of

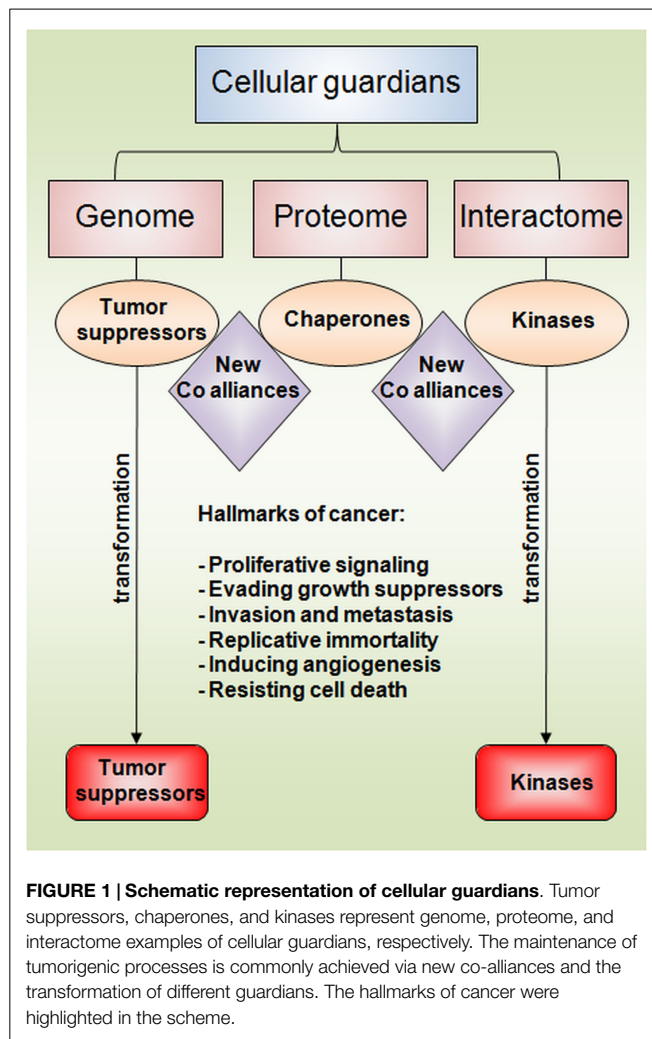
cancer, GA has been shown to sensitize Bcr-Abl-positive cells to cytotoxic chemotherapy (70). Several other drugs, including novel oxime derivatives of radicicol (71) and novobiocin (72), have been shown to be effective for the therapeutic intervention of CML by disrupting the Bcr-Abl-Hsp90 complex. Because Bcr-Abl is destabilized and degraded upon Hsp90 inhibition, it may represent a new opportunity for blocking CML progression in Bcr-Abl mutations associated with a drug-resistant phenotype (73). T315I p210^{Bcr-Abl}, the most aggressive and insensitive mutation to the first and second generation of tyrosine kinase inhibitors, has been shown to remain sensitive to Hsp90 inhibition and to suppress leukemic stem cells in a mouse model (74). Although Hsp90 participates in the protein stability of several oncogenic kinases, the molecular mechanisms underlying these interactions have not been fully elucidated. Recently, a kinase inhibition study contributed to uncovering the conformational plasticity of kinases during Hsp90 interaction. For Bcr-Abl, the disruption of the kinase-Hsp90 complex has been shown to be independent of whether the chimeric protein was in an active or inactive conformation, but this was not true for other kinases (75).

Using an analogy from the medieval era in which guardians from different clans worked together to defend the empires of their kings, cellular homeostasis and survival are maintained under the control of genomic, proteomic, and interactomic guardians (Figure 1). The opportunist behavior of cancer cells to make new guardian co-alliances and to transform and manipulate them to their own benefit may provide an explanation for the maintenance and progression of these neoplastic diseases and their hallmarks (76).

The Impact of Unfolded p53 Segments on its Functions and Cancer Development

Tumor suppressors are complex macromolecules normally occurring as multi-domain proteins flanked by disordered segments. The impact of this architecture on protein activity and cellular function is beyond our current understanding, even using the most recent state-of-the-art methods in structural biology. Three well-organized domains have been recognized in p53: an N-terminal transactivation domain (TAD, spanning residues 1–70), a sequence-specific DBD (residues 94–293), and an oligomerization domain (OD, residues 324–355). Flanking these regions, there are two disordered polyproline regions (PPRs): the first is composed of residues 71–93, which link the TAD to the DBD, and a second spans residues 294–323 and links the DBD to the OD. At the most extreme C-terminal region (residues 356–393), an unstructured basic region ends p53.

The TAD region does not fold independently (77, 78) but acquires a pair of helices upon binding to the nuclear coactivator binding domain of the CREB-binding protein (CBP) (79, 80). The p53 activity and stability are regulated depending on the TAD phosphorylation of specific serine and threonine residues. The transcriptional factors p300/CBP and the ubiquitin protein ligase Mdm2 (Hdm2 for the human ortholog) have overlapping binding sites within this N-terminal region. Upon DNA damage, phosphorylation at Ser15, Thr18, and Ser20 results in the dissociation of Mdm2 from TAD and an increase in p300/CTB affinity,



thus facilitating p53 transcriptional activity (81, 82). Conflicting data exist concerning the participation of phosphorylated Ser15 in regulating the ability of p53 to complex with Mdm2 or to mediate p53 degradation (83–85). A direct effect of Mdm2 binding to TAD is the inhibition of p53 transcriptional function. However, the major effect on p53 occurs through its E3-ubiquitin ligase activity (86). The p53–Mdm2 interaction has been shown to be critical when the lethality of *mdm2*-null mice was rescued by simultaneous deletion of the TP53 gene (87, 88). The nuclear export signaling (NES) of p53 is triggered by the mono-ubiquitination of several lysine residues, which results in a blockade of its transcriptional activity. Otherwise, poly-ubiquitination occurs, which acts as a signal for degradation in the nucleus or cytoplasm (89). In addition to binding to the TAD, a second Mdm2-binding site was identified in the p53 core domain and has been speculated to stabilize the Mdm2–p53 interaction during degradation (90). The incorporation of specific modifications to p53, such as NEDDylation, is also dependent on Mdm2 expression levels and the direct binding of Mdm2 to p53. The Mdm2 RING finger E3-ubiquitin ligase has been shown to be NEDDylated, and after this step, NEDD8 can be conjugated to the C-terminus of p53 to inhibit its activity (91). The status of ubiquitin binding to

p53 is also determined by Mdm2 protein levels. The p53–Mdm2 binding is quite enigmatic and works through negative feedback. The activation of p53 induces the transcription of Mdm2, and the accumulation of Mdm2 inhibits p53 activity. Additionally, Mdm2 is able to block its own transcription (92).

Because a complete loss of transcriptional activity has only been observed with additional substitutions targeting residues Trp53 and Phe54 (93), it has been suggested that the p53 TAD has multiple binding elements, which are able to interact with transcription factors and other regulators. The presence of multiple post-translational modifications and the flexibility of this region were likely selected during protein evolution to serve as multiple binding sites and consequently provide a fine-tuned mechanism for the regulation of p53 activity and stability (94). The exposure of normal cells to genotoxic agents or non-genotoxic stresses leads to p53 phosphorylation at approximately 15 serines or threonines in both the N and C-termini and to the acetylation of approximately six lysines in the C-terminus (95, 96). Therefore, the end regions of p53 act as molecular antennas for the proper activity and interactome signaling of this tumor suppressor.

MdmX (HdmX for the human ortholog) also participates in p53 signaling and inhibits p53 transcriptional activity (97–99). MdmX and Mdm2 may work as partners (100, 101) and directly contribute to tumor formation, as observed by the immortalization and neoplastic transformation of retrovirus-mediated MdmX overexpression in primary mouse embryonic fibroblasts (102). Several tumor cell lines express increased levels of HdmX compared with normal cells (103). The systematic screening of HdmX expression in more than 500 human tumors of different origins has revealed HdmX overexpression in a wide range of these tumors (102), suggesting that HdmX may function as an oncogene. Moreover, MdmX has been shown to block the p300/CBP-mediated acetylation of p53 (104), a modification involved in the tumor-suppressor functions of p53 (105).

The first disordered PPR of p53, which spans residues 71–93, was demonstrated to bear five partially conserved PxxP motifs and to participate in protein activity and regulation. The depletion of this PPR does not influence p53 transcriptional transactivation but severely affects growth suppression (106). This finding shows that p53 transcriptional activity and growth suppression are uncoupled events and that the first PPR region mediates a critical activity in p53-dependent tumor suppression. Further studies have identified this region as crucial for p53-mediated apoptosis but dispensable for cell growth arrest and the suppression of cell transformation (107). The absence of the first PPR in p53 has been shown to decrease both the specificity of target promoters and the induction of apoptotic genes, such as *pig3*, *pig6*, *pig11*, *p85*, and *btg2* (108). This region is also important for p53 regulation because the lack of PPR increases the Mdm2 affinity for p53 and makes it more susceptible to the negative regulation of Mdm2, facilitating protein ubiquitination and nuclear export (109). Further explorations have clarified the mechanism through which PPR renders p53 sensitivity to Mdm2 inhibition. The Pro82 located in the first PPR is required for p53/Chk2 interaction in response to DNA damage and subsequent Ser20 phosphorylation (110). Germline substitutions (e.g., P82L) and somatic mutations (e.g., P85S and P89S) in bladder tumors (111) in subjects with

Li–Fraumeni syndrome and ovarian carcinoma (112) have shown that the first PPR of p53 plays an important role in regulating protein activity. Although classified as a transcription factor, p53 can also mediate apoptosis without new RNA and protein synthesis. This transcription-independent mechanism has been observed in human vascular smooth muscle cells in which p53 activation is able to increase surface Fas (CD95) expression by transport from the Golgi complex (113).

The second PPR located between the DBD and OD contains seven proline residues. As indicated by the presence of several prolines within the first and second PPRs, a cautionary note about these segments concerns the effect of the number of prolines on the kinetics of p53 folding. Proline regions have conformationally constrained backbones that may not only interfere with proper p53 folding but also allow multiple symmetrical orientations among the TAD, DBD, and OD to enhance the induced fit conformations upon protein or DNA binding.

The last disordered segment of p53 concerns the basic C-terminal region (BR) that is also prone to multiple post-translational modifications. The presence of multiple acetylated lysines within the p53 BR makes it similar to histone tails, not only physically but also functionally. The binding of p300/CBP acetyltransferase to p53 TAD is able to acetylate not only histones but also p53 itself (114). A series of studies have been conducted in an attempt to uncover the complex role and regulation of p53 acetylation. Two groundbreaking studies have shown conflicting results concerning the involvement of p53 acetylation in triggering its DNA-binding function. First, Gu and Roeder concluded that p53 acetylation by p300 occurs in the C-terminal domain and is critical for stimulating the DNA-binding function of p53, likely due to an acetylation-induced conformational change (114). Later, Espinosa and Emerson showed that the acetylation of the C-terminus by p300 is not necessary for p53 DNA binding or promoter activation (115). Further exploration revealed the key steps in the mechanism of p300–p53–DNA transactivation. Phosphorylation by Chk2 at Thr18 and Ser20 in the p53 TAD stabilizes p300 docking to the TAD domain (116). This docking was shown to be essential for the DNA-dependent acetylation of p53, suggesting that the acetylation sites within the p53 tetramer are occluded in the absence of DNA (117). Furthermore, the role of acetylation as a post-DNA-binding process is important for clamping and stabilizing the p300–p53 acetylated complex (117). Finally, to identify the p300-docking motifs in p53 related to protein acetylation, p300 was subjected to peptide selection from a phage-peptide library. The identification of a second flexible p300-binding motif within the PPR of p53 has been shown to be required for acetylation and p53 binding to promoter sites (118). These observations provide a mechanism to explain how p300/CBP binding to p53 increases protein stability and transcriptional activity. The p53 BR has been shown to be acetylated by p300/CBP at Lys372, 373, 381, and 382 (119–121). P/CAF, a histone acetyltransferase (HAT) associated with p300/CBP, has also been shown to acetylate Lys320 within the second PPR (119). Acetylation at position 373 in p53 by p300/CBP leads to cell apoptosis, whereas acetylation at 320 by P/CAF leads to cell-cycle arrest (122). In colorectal cancer (CRC), ArhGAP30, a Rho GTPase-activating protein, is a crucial regulator of p53 acetylation and activity. This protein binds to the C-terminus of

p53 and facilitates the p300-mediated acetylation of p53 at Lys382. A low expression of ArhGAP30 is correlated with poor survival in CRC patients, showing that ArhGAP30 is a potential marker of CRC (123). This finding shows how p53 acetylation works in a fine-tuned mechanism that affects the gene-expression patterns and cell fate in normal physiology and cancer development.

The BR of p53 binds to non-specific sequences in DNA (124–126) and also regulates the sequence-specific binding of the core (127, 128). Using analytical ultracentrifugation, an inverse relationship was observed between the number of acetyl groups attached to the C-terminus of p53 and its ability to bind DNA (129). More recently, electron microscopy and single-molecule experiments have provided a basis to understanding how full-length p53 scans and recognizes specific DNA-responsive elements. The synergistic model of scanning and DNA recognition is based on the ability of the C-terminus to rapidly translocate as the core domain hops along DNA with transient associations for rapid scanning (130–132).

The transfer of acetyl groups to the ϵ -amino group of lysine residues in histones represents one of the best characterized post-translational modifications for chromatin regulation. Acetyl groups are also transferred to non-histone proteins, as shown above for p53, and modulate protein function by changing stability, cellular localization, and protein–nucleotide/protein–protein interactions. The acetylation status is maintained by the opposing activities of HATs and histone deacetylases (HDACs) in a controlled manner. In the case of p53, HDAC-1, -2, and -3 are all capable of downregulating p53 function, showing that the deacetylation of p53 is part of the mechanism that controls the physiological activity of p53 (133). However, AMP-activated protein kinase (AMPK) phosphorylates and inhibits the p53 deacetylase SIRT1, promoting p53 acetylation and apoptosis in hepatocellular carcinoma (134). This finding shows that HDACs are also involved in cancer development. In CML cells with Bcr–Abl-independent imatinib resistance, the resistance mechanism includes the aberrant acetylation of p53 and other proteins due to the upregulation of HDACs and the downregulation of HATs, indicating HDACs as targets for imatinib-resistant leukemia cells (135). In contrast, the pathogenic protein AML1/ETO that is involved in t(8;21) acute myeloid leukemia has been shown to be proteasome-degraded when treated with panobinostat, a HDAC inhibitor, and does not require functional p53 or the activation of conventional apoptotic signaling in a mouse model (136).

The Effect of p53 Mutations and Aggregation on its Functions and Cancer Development

The evaluation of 3,281 samples of 12 different tumor types revealed 127 mutated genes in different signaling and enzymatic processes, and the TP53 gene was detected as the most frequently mutated gene (42% of samples) (25). The current database of TP53 mutants (<http://p53.fr>) reveals 45,000 somatic mutations, most of which provide advantages to a specific cell clone in its microenvironment, increasing its survival or reproduction. These driver mutations normally trigger clonal expansions and tumorigenic development (137).

Factors influencing the TP53 mutational frequency in tumors may be classified by their high heterogeneity and the different cancer subtypes, the stage of cancer development, and ambient factors, such as viral and bacterial infection. Depending on the cancer type, TP53 mutations can range from <5%, as in cervical carcinoma, to 90% in ovarian carcinoma. For instance, a molecular search in breast carcinoma revealed four major subtypes of TP53 mutations with variable frequency, ranging from 12% in luminal A and 30% in luminal B to 72% in HER2-E and 80% in basal-like (138, 139). Considering the stage of development, a low frequency of TP53 mutations was reported for primary prostate tumors (between 10 and 20%) in contrast to metastatic tumors (up to 50%) (140). In CML disease, the occurrence of TP53 mutation is more frequent in the blastic phase (141, 142). Concerning exogenous factors, most human viruses impair p53 activity. In cervical cancer, the human papillomavirus E6 protein targets p53 for degradation (143). Bacterial infection has recently been shown to trigger the p53 pathway and to activate p53 isoforms (144). Moreover, the p53 R249S variant is often observed in liver cancer as being associated with aflatoxin B1 food contamination (145).

Mutations affecting amino acid sequences (i.e., missense mutations) are commonly observed in the TP53 gene. In the case of TP53, monoallelic alterations occur within six hot-spot sites in the DBD (R175, G245, R248, R249, R273, and R282). Even though missense mutations are predominant in tumors, other genetic alterations have already been described in TP53 (146), as observed in osteosarcoma with a high frequency of TP53 gene deletion (147, 148). Furthermore, TP53 germline mutations cause a rare autosomal-dominant cancer predisposition called Li-Fraumeni syndrome (149, 150). Most predisposed subjects present a variety of tumor types and carry a specific p53 germline mutation with approximately 90–95% penetrance (151). Similarly, dominant-negative mutations in TP53 are involved in tumor growth and development in glioblastoma (152). It is clear that we are still far from fully comprehending the complex behavior of TP53 mutations and signatures in human cancer and its effects on the p53 protein network, even considering that TP53 expresses eight differentially spliced mRNAs and is translated into 12 isoforms.

In fact, there is no unanimous resolution for the following question: in what circumstances can p53 act as a tumor suppressor or oncogene? The transcriptional activity of p53 may range from total inactivation to an activity greater than that of the wt form (153). The high frequency of p53 mutations in several tumors and the fact that p53^{-/-} mice display marked early onset and cancer predisposition depict p53 as an important tumor suppressor (94, 154). In contrast, mouse models have shown that to work as a tumor suppressor, p53 does not respond to acute DNA damage but to the oncogene-induced expression of the p19^{ARF} tumor suppressor, which activates p53 via the sequestration and inhibition of Mdm2 (155, 156). In addition, mice deficient for p21, Puma, and Noxa are not able to trigger p53-mediated apoptosis, G1/S cell-cycle arrest, and senescence but remain free of tumor development for at least 500 days compared with p53-null mice. This finding suggests that the induction of apoptosis, cell-cycle arrest, and senescence is dispensable for the p53-mediated suppression of tumor development and that genomic stability or metabolic adaptation are more important for p53 suppressor

activity (157). Surprisingly, mice bearing three lysine-to-arginine substitutions at p53 acetylation sites (K117R, K161R, and K162R) retain the ability to regulate energy metabolism and reactive oxygen species production compared with p53-null mice, showing that metabolism regulation and antioxidant function are crucial events for the suppression of early onset tumorigenesis (158). Knock-in mice expressing an allelic series of p53 TAD mutations have revealed that the transactivation of p53 is essential for tumor suppressor activity but is associated with a small set of novel p53 target genes (159).

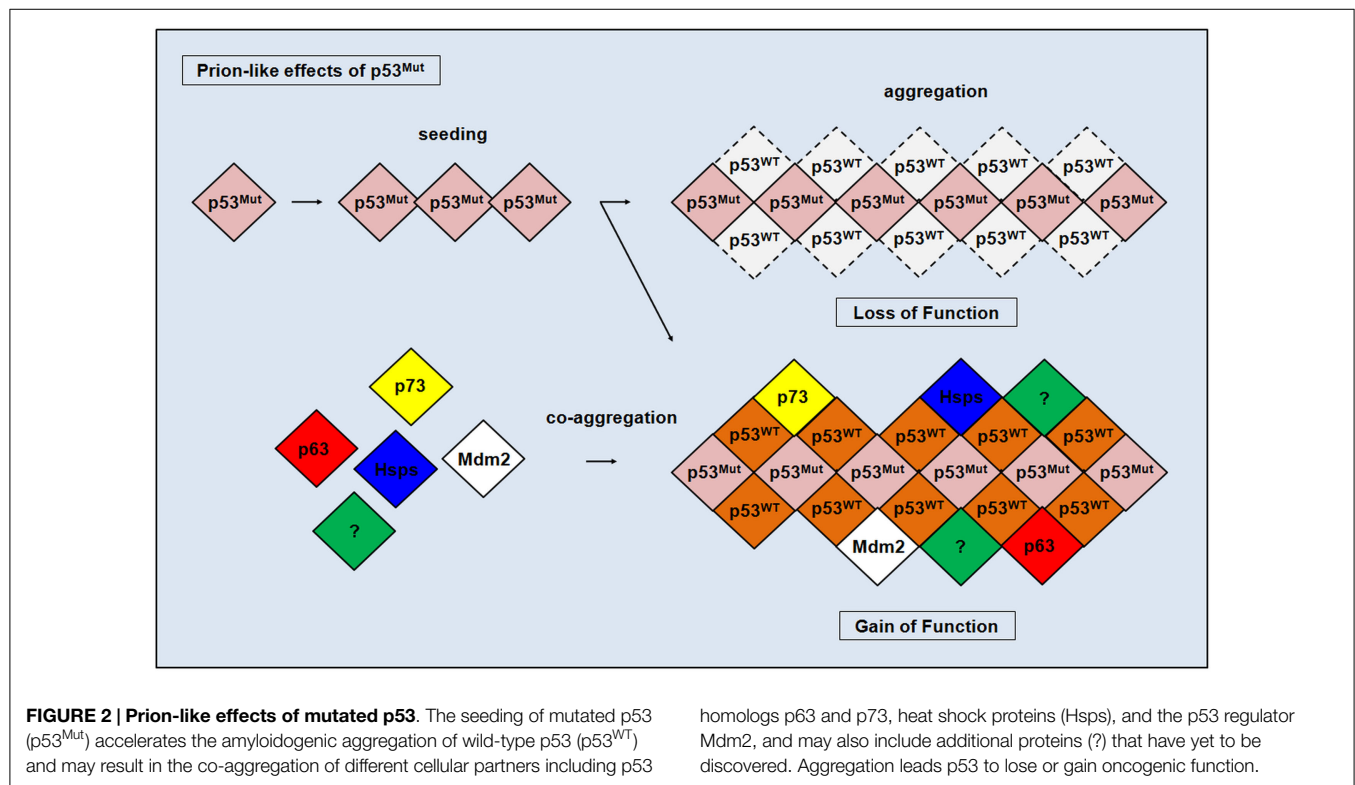
p53 mutations may lead to different effects: (i) mutant p53 may lack the activity of wt p53 (loss-of-function – LoF) (ii) mutant p53 may acquire oncogenic activity without disturbing wt p53, (iii) mutant p53 may inhibit wt p53 through a dominant-negative effect and trigger oncogenic functions and (iv) mutant p53 may inhibit wt p53 protein through a dominant-negative effect and reduce activity. The acquisition of oncogenic activity by mutated p53 was first revealed in experiments using the transfection of mutant p53 into TP53-null cells, which revealed the formation of tumors in mice (160–162). In addition to the gain of function, mutated p53 may operate through a dominant-negative mechanism, with the formation of hetero-oligomers between the mutant protein and wt p53 (163, 164). A possible dominant-negative effect may also be observed with mutated p53 and its ancestral p63 and p73 paralogs. In contrast, mass spectrometry experiments have revealed that p63 and p73 homotetramers are able to form mixed tetramers after 30 min of incubation. Conversely, neither p53 and p73 nor p53 and p63 homotetramers were able to exchange components after a 24-h incubation, showing a divergent evolution of the oligomerization domain within the p53 family (165). Furthermore, a gain-of-function phenotype of mutated p53 has been shown by the co-aggregation of p63 and p73 (166), showing that the creation of dominant-negative p53 through hetero-oligomerization is not exclusive. It is likely that dominant-negative mechanisms occur via high-level oligomeric states, in which aggregated mutant p53 sequesters wt p53 into mixed oligomers (22, 23). Several oncogenic functions of mutated p53 have been characterized, and for more in-depth information, please refer to specialized literature (167, 168). Of note, the c-Abl kinase also forms homo- and hetero-oligomers with its binding partner Abi-1 in a kinase-dependent manner (169). In the chimeric Bcr–Abl protein, the oligomerization domain of Bcr has been revealed to be crucial for the transforming function of this aberrant protein (170), but no relationship has been demonstrated in terms of the involvement of higher oligomeric states in leukemogenesis. Few works have linked protein aggregation to cancer development. Another important tumor suppressor that is inactivated in several types of cancer, the retinoblastoma (Rb) protein shares low stability and oligomerization with p53 (171). In addition, the polymerization of the splicing factor proline and glutamine (SFPQ) was shown to be essential for the cellular functions of this tumor suppressor (172) linking aggregation not only to the pathological aspects of cancer but also to functional roles of cellular nucleic acid metabolism.

The thermodynamics of wt and mutant p53 (R175H, C242S, R248Q, R249S, and R273H) were explored at the end of the 1990s through biophysical techniques and revealed irreversible

denaturation and aggregation under certain conditions for the wt and studied mutants (173). In 2003, our group designed a study to examine the aggregation of the p53 core domain (174–176). More recently, we explored whether the wt and the p53 hot-spot mutant R248Q aggregates like an amyloid under physiological conditions and whether mutant p53 can seed the aggregation of wt p53 (22). Using a cohort of structural and cellular approaches, we established the amyloid nature of wt and mutant p53 aggregation. We showed that a seed of amyloid oligomers formed from the p53 hot-spot mutant R248Q accelerated the aggregation of wt p53 into an inactive conformation (22). We showed that a prion-like behavior of p53 would explain the dominant-negative and gain-of-function effects of mutant p53 (Figure 2). Recently, Fersht showed that different p53 mutants aggregate through a complex order mechanism and that co-aggregation can occur with wt p53 and p63 or p73 (177, 178). To address the role of aggregated p53 and the prion-like effect in triggering cancer development and progression, we observed a greater extent of mutant p53 co-aggregation with amyloid oligomers in breast cancer MDA-MB-231 cells compared with wt p53 cells (MCF7) (22). In line with our observations, similar results were observed in the biopsies of breast cancer patients carrying specific p53 mutations (R175H, H193L, I195L, Y234C, G245S, and R248Q) (29) and in the skin tissue of six patients with basal cell carcinoma (179). Furthermore, prostate cancer tissues revealed high levels of p53 immunostaining within aggregates containing mutant and wt p53 (180). Finally, cancer stem cells from a unique population of high-grade serous ovarian carcinoma (HGSOC) revealed that p53 aggregation is associated with its inactivation and platinum resistance. During differentiation to their chemosensitive progeny,

these cells lost their tumor-initiating capacity and p53 aggregates (181). Moreover, using two-dimensional gel electrophoresis and mass spectrometry, these authors discovered that aggregated p53 works uniquely by interacting with proteins involved in cancer cell survival and tumor progression (181). Altogether, based on this recent *ex vivo* evidence, the involvement of p53 aggregation in cancer appears to be undisputed. Although a prion-like mechanism would explain the dominant-negative and gain-of-function effects during p53 aggregation, several questions are still awaiting exploration to define it as an etiological factor for cancer pathogenesis, invasiveness and metastasis (182).

Prions are transmissible polypeptide particles that undergo a conformational change from their cellular form (PrP^C) to a β -sheet rich form (PrP^{Sc}). This conformational modification is associated with the transmissible characteristics and pathogenesis of several diseases, including bovine spongiform encephalopathy and Creutzfeldt–Jakob disease in humans (183, 184). A genuine prion should be transmissible *in cell* and *in vivo*. Features linking p53 to prions are the conformational conversion of p53 during tumorigenesis (wt p53 to mutated p53) and the ability of mutant p53 to sequester wt p53 into amyloid species. Another interesting shared feature of p53 and PrP is their nucleic acid binding ability (23, 185–187). Although there are common characteristics between p53 and prions, no transmissible mechanisms for p53 have yet been demonstrated (182). Controversially, recent works have independently shown mechanisms of p53 secretion and uptake by cells (188–190), yielding the possibility that p53 is a transmissible particle. The Kirsten-Ras (K-Ras) oncogene protein has been shown to participate in p53 suppression by inducing Snail, and the depletion of Snail is able to induce p53



in K-Ras-mutated cancer cells but not in wt cancer cells (191, 192). Therefore, a direct correlation between K-Ras-mediated p53 suppression and tumorigenesis has been established in lung and pancreatic cancers, which have a higher frequency of K-Ras mutations (193). p53 suppression in response to the K-Ras oncogene occurs through a Snail-dependent mechanism in which p53 is secreted from cells and then taken up by K-Ras-mutated cells in caveolin-1-mediated endocytosis (189). Moreover, full-length p53 aggregates have been shown to penetrate HeLa and NIH3T3 cells via macropinocytosis and induce the aggregation of intracellular p53 (190). Although mouse models are still lacking, these surprising *in cell* transmissibility mechanisms of p53 have prompted new discussions about our proposed prion-like mechanism and have instigated ongoing experiments by our group and others to demonstrate the involvement of p53 aggregates in cancer pathogenesis and progression.

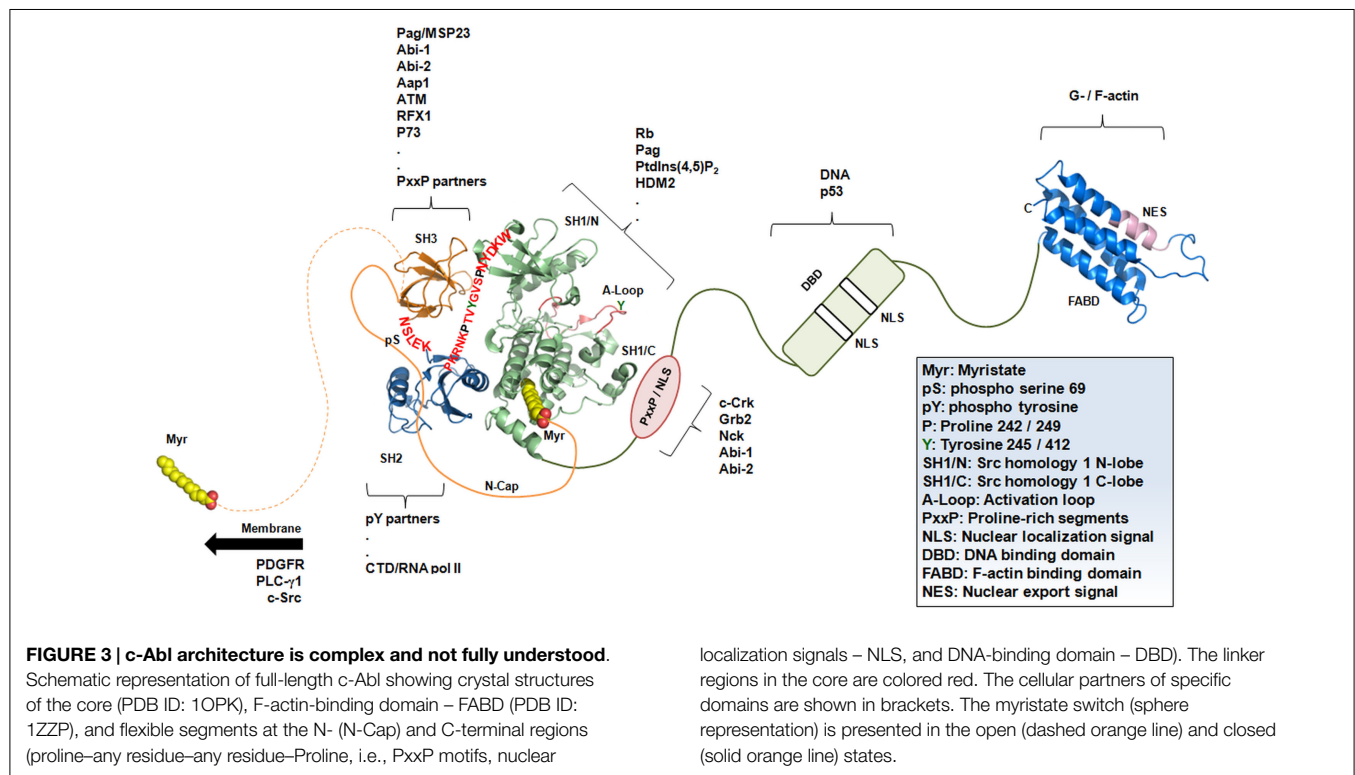
p53 aggregation and gain-of-function effects can be minimized by small molecule intervention. For instance, CDB3 is able to rescue the conformation of unstable p53 mutants (194), allowing an increase in the protein half-life to reach the nucleus and act as a tumor suppressor. In the case of PRIMA-1, its conversion to compounds that form adducts with thiols in mutant p53 is sufficient to induce apoptosis in tumor cells (195). Furthermore, resveratrol has been shown to inhibit carcinogenesis through the induction of p53-dependent cell death. Indeed, the transient transfection of wt p53 allowed H1299 cells to become more sensitive to the pro-apoptotic effects of resveratrol (196). Efforts to increase health quality are based on treatment, diagnosis and prevention. The existence of a direct correlation between the prion-like effect of aggregated p53 and tumorigenesis remains an open question, but we believe that it may provide the basis

for new therapeutic interventions, early diagnosis, and cancer prevention.

Impact of Unfolded c-Abl Core Segments on its Functions and Activity Regulation

The proper phosphorylation control of specific serine, threonine, or tyrosine residues is a fundamental step in cellular growth, survival, and death and is achieved through the tight regulation of protein kinases. As shown previously, tumor suppressors have the opposite effects. Under several types of stress, their fundamental role is to maintain genome integrity, and this is achieved by activating or repressing the transcription of specific genes to undergo biological processes, including the induction of apoptosis and growth arrest. Because kinase activity is involved in gene expression, metabolic pathways, cell growth and differentiation, membrane transport, and apoptosis, their activity must be tightly regulated. There are 518 human kinase sequences (1.7% of the entire genome), and the tyrosine kinase family is the largest with 90 members. This group of kinases is divided into 58 receptor tyrosine kinases (RTKs) and 32 non-receptor tyrosine kinases (nRTKs) (197).

c-Abl belongs to the nRTK group and has a modular and complex architecture flanked by unfolded segments (**Figure 3**). The proper dynamics of these segments play an important role during kinase activation/inhibition and cellular localization. The c-Abl core is composed of in-tandem Src homology (SH) 3 and SH2 domains that negatively regulate SH1 kinase activity. The SH3–SH2–SH1 domains are flanked by disordered regions at the most N-terminal region of the SH3 domain, which comprises the N-Cap region (residues 1–83), the short linker (residues



139–143: NSLEK) connecting the SH3–SH2 domains, and a longer linker between the SH2–SH1 domains (residues 237–254: PKRKNKPTVYGVSPNYDKW).

Pivotal studies have clarified the participation of SH3–SH2 domains in activity regulation and oncogenic signaling of c-Abl (198–200). The presence of the SH3 domain has been shown to inhibit the transforming activity of c-Abl *in vivo* but has no effect on the *in vitro* activity, suggesting that cellular inhibitors may act in kinase inhibition (200). Not only mutations or depletions within the c-Abl SH3 domain but also the phosphorylation of a specific SH3 Tyr residue (Tyr89) are able to disrupt its negative regulatory effect (201). In the 1990s, the consensus SH3-binding site was identified as a proline-rich segment (202). In the case of c-Abl, an intramolecular SH3-domain interaction is able to regulate c-Abl activity through SH3 binding to the longer linker connecting SH2 to SH1 (203, 204). Although cellular partners have been shown to inhibit c-Abl activity (205), including retinoblastoma (Rb) protein (206), Abl-interactor proteins 1 and 2 (207, 208), ABL-associated protein-1 (209), the 23-kD macrophage stress protein MSP23 or PAG (210), F-actin (211), and BRCA1 protein (212), Superti-Furga's group provided a basis for understanding the auto-inhibition mechanism of the c-Abl protein through the N-terminal Cap. These researchers concluded that an SH3 domain-dependent cellular inhibitor is dispensable (213). Furthermore, two consecutive crystallographic studies conducted by Superti-Furga and Kuriyan's group extended the understanding of c-Abl auto-inhibition. These studies revealed that during inhibition, a myristoyl/phosphotyrosine switch regulates the docking and accessibility of the SH2 domain to the C-lobe of the kinase domain (KD) and that c-Abl can be activated by phosphotyrosine ligands through binding to a specific motif in the SH2 domain (FLVR motif) (214, 215).

A second crystal structure of the c-Abl core revealed more about the participation of the N-Cap segment for c-Abl inhibition. The phosphorylation of Ser69 in the N-Cap mediates interactions with the shorter linker connecting SH3 and SH2 (NSLEK region), showing that this disordered N-terminal region not only participates in presenting the myristoyl group to the kinase but also may work as a stabilizer (216). More recently, our group proposed a cascade-like mechanism for c-Abl inhibition. In this mechanism, N-Cap–SH2 interactions monitored by chemical shift perturbation analysis would provide guidance for myristate binding in the C-terminal pocket of SH1 (217). Although no unanimous data exist concerning the real c-Abl inhibitory mechanism (cellular inhibitors or auto-inhibition), functional and structural characteristics make an autoinhibitory mechanism more favored and accepted. In contrast, in a perspective paper published in *Nature Cell Biology*, Wang proposed a speculative model of c-Abl co-inhibition that accommodates both inhibitory mechanisms (218). It is likely that both mechanisms may influence c-Abl inhibition depending on the cellular context.

In c-Src, a prominent family of kinases composed of nine members (c-Src, Yes, Fyn, Hck, Lck, Lyn, Blk, Fgr, and Yrk), a phosphotyrosine residue in the C-terminal region of the kinase (pY527) interacts with the SH2 domain (219–221) and is considered a surrogate mechanism of the N-Cap-myristoyl group. Controversially, a c-Src crystal structure revealed a similar pocket

for myristate binding (222), and more recently, membrane binding and myristoylation have been shown to regulate stability and kinase activity (223). In the case of c-Abl, transiently transfected wt and variant forms lacking myristoyl have been detected by immunoblots in crude membrane-enriched fractions (215), showing that membrane targeting may be a multifactorial event. One possible surrogate for myristate, as an inhibitor molecule, is phosphatidylinositol-4,5-bisphosphate [PtdIns (4,5)P₂], the product of PLC- γ 1 (224). In the case of c-Src, myristoylation is assisted by a polybasic cluster of amino acids that tightly bind the kinase to membrane regions (225, 226). These regulatory differences between c-Src and c-Abl kinase members reveal the complexity and plasticity of tyrosine-related kinases (227).

Small-angle X-ray scattering studies of an activated c-Abl form, in which the N-Cap region is depleted and two additional mutations are introduced (P242E/P249E), revealed an elongated shape consistent with the SH2 domain sitting at the top of the SH1 N-lobe (216). This SH2-kinase intramolecular interaction has been shown to be necessary for Bcr–Abl catalytic activity and was validated as an allosteric target for therapeutic intervention because its disruption completely abolished leukemia formation in mice (228). Altogether, these data suggest that, upon complete activation, the N-Cap-myristoyl tether would detach from the SH1 C-terminal pocket. However, a theoretical model proposed that the equilibrium fraction of c-Abl in which myristoyl is unlatched is only ~0.5% (229). An intermolecular autophosphorylation of Tyr412, located at the activation loop, followed by the phosphorylation of Tyr245, which is located in the longer linker between the SH2–SH1 domains, also participates in enzyme activation (230). To understand the possible role of a detached N-Cap-myristoyl tether inside the cell, our group asked whether myristoylated c-Abl would bind to the cellular membrane. Although c-Abl was initially shown to be present in pseudopodia protrusions, after stimulation with hepatocyte growth factor (HGF) in thyroid cancer cells (231), we demonstrated that the N-Cap-myristoyl tether may play a role in protein inhibition and also may direct the c-Abl protein to anchor in the membrane as an additional mechanism to stabilize this disordered segment, which may also be linked to early apoptotic signaling (217). Although we proposed a link to apoptosis during transport to the membrane, the membrane pool of c-Abl is also linked to cytoskeletal reorganization (232, 233), cell migration, and neurite outgrowth. For example, c-Abl activation downstream to the platelet-derived growth factor receptor (PDGFR) has been shown to require functional phospholipase C- γ 1 (PLC- γ 1), creating a bidirectional link between PLC- γ 1 and c-Abl in the membrane ruffling signaling pathway (224).

The Impact of Unfolded C-Terminal Segments of c-Abl on its Nuclear Functions

c-Abl kinase is not exclusive in the broad spectrum of biological functions triggered by its activity because neonatal lethality was observed in c-Abl^{-/-} mice and in mice carrying a truncated form of the c-Abl in which the C-terminal region was depleted (234, 235). The C-terminal region of c-Abl is composed of more than 600 residues with several flexible and disordered segments containing three nuclear localization signals (NLSs) (236, 237), an

NES (238) with multiple proline-rich motifs and multiple protein-binding sites; a DBD (239) and a structured F-actin-binding domain (FABD) (Figure 3). In addition to the FABD (240), structural information from this C-terminal region is still lacking probably because of its increased flexibility and the presence of non-crystallizable segments. Indeed, the DBD of c-Abl does not bind DNA with a high degree of sequence specificity (241), and

a computational prediction of its potential secondary structure revealed that it is mainly formed of coil conformations (242).

The presence of NLS and NES confers to the protein the ability to shuttle between these two cellular compartments depending on the cellular requirements (Figure 4). The nuclear c-Abl pool is involved in the control of cell-cycle-dependent and DNA damage-induced gene expression. The main regulator of

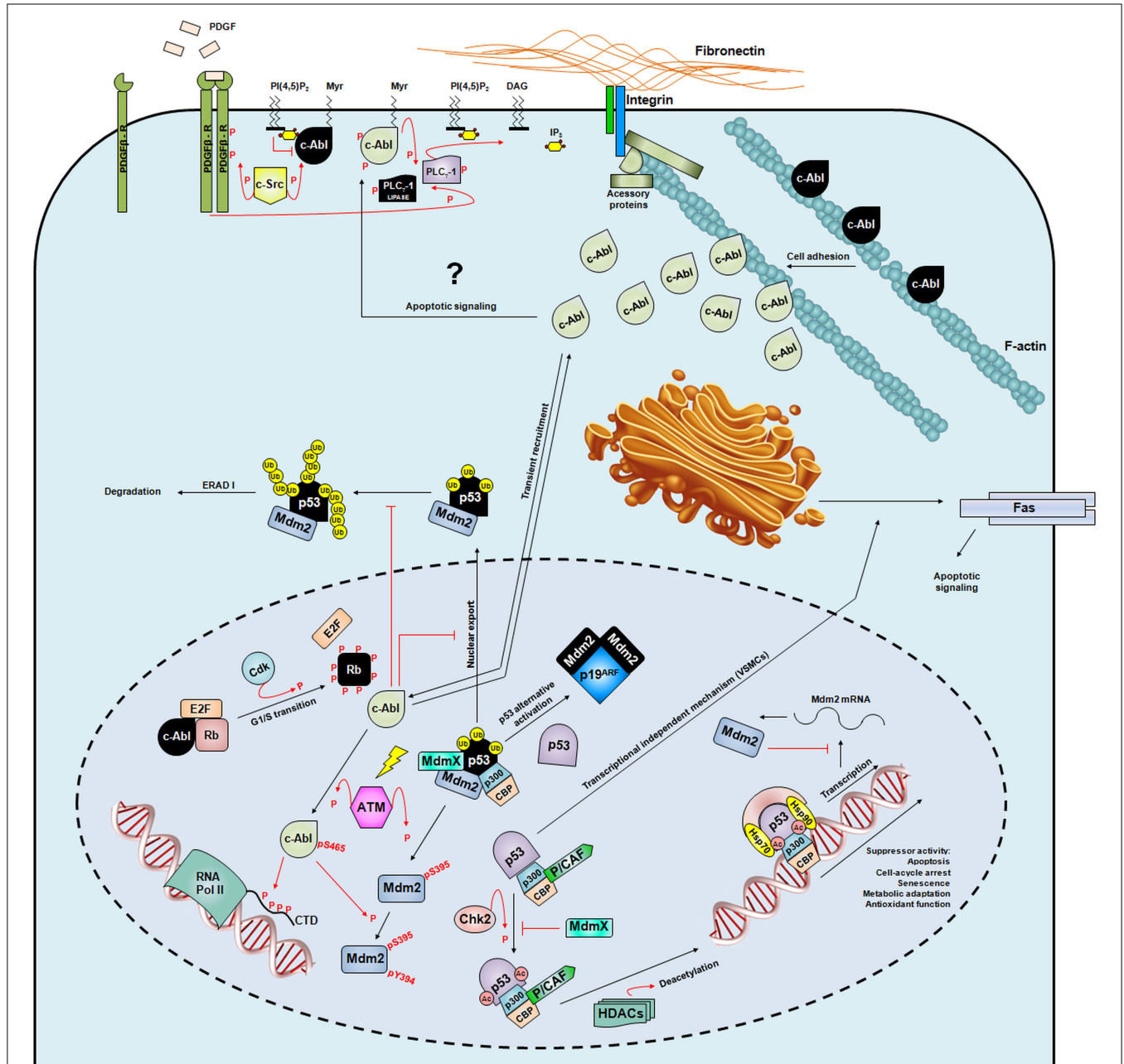


FIGURE 4 | Intracellular signaling of c-Abl. A schematic representation of the broad spectrum of c-Abl signaling pathways in different sub-cellular compartments. The nuclei are shown by dashed lines. Inactivated c-Abl, Rb, p53, Mdm2, and PLC-1 proteins are colored black to distinguish them from activated forms (represented in different colors). "P" in red means phosphorylation, "Ub" in yellow ubiquitination,

"Ac" in light red acetylation, and the yellow ray is genotoxic stress. Red and black arrows represent signaling through phosphorylation and promoting activity, respectively, and red lines with a crossbar indicate signaling inhibition. Molecules anchored to the membrane are: PI(4,5)P₂ – phosphatidylinositol-4,5-bisphosphate, Myr – myristate, and DAG – diacylglycerol.

nuclear c-Abl is the retinoblastoma (Rb) tumor suppressor, which binds through its C-terminal tail to the c-Abl ATP-binding lobe (206). In resting G1 cells, Rb is found unphosphorylated, and this is the active state for its growth-inhibitory activity. Upon G1/S transition, Rb becomes hyperphosphorylated by cdk/cyclin kinases (243) and releases the Rb/c-Abl complex, allowing c-Abl to become activated. The assembly of Rb-mediated complexes, such as the ternary complex of E2F/Rb/c-Abl, has been shown to be important for cell-cycle arrest (244). Moreover, the cytostatic effect of c-Abl (245) has been shown to be dependent not only on Rb but also on p53 (237) (**Figure 4**). However, Sawyer's group observed that growth suppression triggered by c-Abl requires p53 but not Rb (246).

The 52 tandem heptapeptide repeats (YSPTSPS) in the carboxyl-terminal domain (CTD) of RNA polymerase II are extensively phosphorylated on serine, threonine, and tyrosine residues as well as by O-linked glycosylation (247). The RNA pol II CTD tail has been shown to be a specific phosphorylation target for nuclear activated c-Abl but not c-Src (248). CTD phosphorylation by c-Abl requires an active c-Abl SH2 domain that binds to partially phosphorylated CTD and increases processivity (249), but a second c-Abl CTD-interacting domain at the c-Abl C-terminal region is also required (250).

Nuclear c-Abl is activated through phosphorylation on Ser465 by ataxia telangiectasia-mutated (ATM) kinase after ionizing radiation (251, 252), which also phosphorylates the major p53-negative regulator Hdm2 on Ser395 (253, 254) (**Figure 4**). Activated c-Abl phosphorylates Hdm2 on Tyr394 (255), and through this phosphate-exchanging mechanism, Hdm2 impairs its inhibitory activity toward p53, which becomes active in the nucleus (256, 257). Although c-Abl and p53 are both activated upon genotoxin exposure (258), it appears that only p53 is considered a universal sensor of genotoxic stress (259). Depending on the extent of DNA damage, p53 signaling can induce the transcription of genes involved in at least three processes that govern cell fate: cell-cycle arrest, cellular senescence, and apoptosis (260).

The Impact of Unfolded c-Abl Segments on its Cytoplasmic Functions and Cellular Localization

We have recently shown that the N-Cap and SH3 domains of c-Abl acquire microsecond–millisecond motions upon N-Cap association with the SH2-L and that the N-Cap-myristoyl tether likely triggers the protein to anchor to the membrane in a function- and stability-dependent mechanism (217). Although membrane anchoring is linked to early apoptotic signaling, the membrane pool of c-Abl is also involved in biological processes, such as membrane ruffling, mitogenesis, and chemotaxis. Upon PDGF stimulation, PDGFRs dimerize and recruit c-Src and PLC- γ 1 to activate membrane-bound c-Abl (261). Moreover, c-Abl and the Abl-related gene (Arg) have been shown to form an inducible complex with PDGFR and change phosphoryl groups (262). After activation, c-Abl binds to PLC- γ 1 and inhibits its lipase activity through phosphorylation (224, 261).

The cytosolic pool of c-Abl is able to bind the F-actin network (263), and upon binding, its kinase activity is abolished (211) (**Figure 4**). A proline-rich region in the C-terminus of c-Abl has also been shown to sequester G-actin and bundle F-actin filaments *in vitro* (263), a mechanism that has also been observed for Arg (264). The detachment of cells from the extracellular matrix (ECM) does not influence the kinase activity of the nuclear and cytoplasmic c-Abl pools, but during cell adhesion to fibronectin, c-Abl becomes activated, and the nuclear pool is transiently recruited to the cytosol. These results suggest that c-Abl can induce integrin signaling to the nucleus to coordinate adhesion and cell-cycle signals (265). During the cell cycle, the cdc2 Ser/Thr kinase is required for G1/S and G2/M transitions and is responsible for the hyperphosphorylation of the c-Abl C-terminal region, which may participate in cell-cycle regulation (239). Altogether, the one-gene-one-function paradigm is beyond the c-Abl biological roles. Because of its intrinsic complexity and flexibility, more than 20 years of research have been required to begin deciphering its physiological functions, and it is unclear how many more years will be needed to completely understand this enigmatic and hard-working kinase.

The Involvement of c-Abl in Leukemia and Solid Tumors

Chronic myeloid leukemia is a biphasic disease with a chronic phase ranging from 3 to 4 years that is characterized by a massive expansion of granulocytic cells. This step is followed by a blast phase in which cell differentiation is blocked, leading to extramedullary infiltrates of immature myeloid or lymphoid cells (blasts) in the peripheral blood, liver, spleen, or lymph nodes. Beyond the clinical features of hematopoietic malignancies, such as CML, the c-Abl oncoprotein is also involved in malignant solid tumors of the breast, lung, colon, and kidney (266–269). In these tumors, the role of c-Abl activation is not linked to Bcr–Abl translocation but is associated with gene amplification, protein overexpression, oncogenic tyrosine kinases, chemokine receptors, oxidative stress, and negative regulatory protein inhibition (270, 271).

The discovery of the oncogenic effect of the c-Abl protein originates from early studies of the Moloney leukemia virus (MLV), which triggers a thymic-independent lymphatic neoplasm upon inoculation in mice (272, 273). The discovery of a small, abnormal chromosome named the Philadelphia chromosome (Ph) was the first consistent chromosomal abnormality associated with a specific type of leukemia (274). In 1973, this shortened chromosome was characterized as the reciprocal translocation t(9;22) through quinacrine fluorescence and Giemsa staining (275). The Ph chromosome is found in the myeloid, erythroid, megakaryocytic, and B lymphoid lineages, indicating that it represents a stem cell-proliferative disorder.

At the molecular level, Ph translocation creates a hybrid gene known as *bcr-abl* that encodes three main protein isoforms (p190^{Bcr-Abl}, p210^{Bcr-Abl}, or p230^{Bcr-Abl}) with different leukemic phenotypes (69). Genomic breaks occur within the break cluster region (*bcr*) gene at three different regions classified as minor-bcr, major-bcr, and micro-bcr (276). In the *c-abl* gene, a non-random

break also occurs within the first intron (277), and this break removes the residues corresponding to the N-Cap-myristoyl tether. As a result, the chimeric Bcr–Abl loses the pivotal N-Cap-myristoyl inhibitory mechanism and becomes highly active. The *bcr–abl* fusion is considered a hallmark of CML pathogenesis. Because the anchoring of *c-Abl* to the membrane through the N-Cap-myristate tether is linked to pro-apoptotic signaling (217) and Bcr–Abl is exclusively cytosolic (278), we believe that this “missing link” in the Bcr–Abl chimera may help explain the apoptotic resistance phenotype of Bcr–Abl-positive cells. Through the activation of the transcription factor STAT5, Bcr–Abl specifically increases the expression of the antiapoptotic proteins Bcl-2 and Bcl-X (279–281). Additionally, Bcr–Abl has been shown to prevent mitochondrial cytochrome *c* release through the inhibition of Bad by phosphatidylinositol 3-kinase/Akt-dependent signaling (282). Moreover, the mechanism explaining the anti-apoptotic mechanism of Bcr–Abl has been shown to occur downstream of mitochondrial cytochrome *c* release, preventing the binding of Apaf-1 to caspase 9 (283). The involvement of a membrane-bound *c-Abl* in apoptotic signaling requires further exploration.

c-Abl Mutations are a Consequence of a Drug-Resistance Phenotype

Different from p53, which contains mutations that are associated with dominant-negative, loss-of-function and gain-of-function mechanisms in cancer, *c-Abl* mutations are a consequence of a drug-resistance phenotype. The front-line and well-accepted therapy for early diagnosed chronic CML patients is 2-phenylaminopyrimidine or imatinib mesylate (284, 285), which has also been shown to be effective for blast crisis (286). Although effective, continuous treatment with this ATP-competitive Bcr–Abl inhibitor leads to patient relapse in the majority of cases due to KD-acquired mutations. However, this resistance mechanism is not exclusive (287–289). Indeed, imatinib resistance has been shown to occur without amplification or mutations in Bcr–Abl (290). Moreover, the overexpression of multidrug-resistance genes may also participate in imatinib resistance (291, 292). The exploration of a drug-resistance signature using microarrays has revealed the upregulation of apoptosis-related genes and the downregulation of genes involved in energy metabolism in K562 sublines established with different dosages of imatinib (293). Imatinib insensitivity is not only related to a drug-resistance phenotype. Ph-positive stem cells (CD34+) from patients with chronic phase CML are equally resistant to the drug (294) and exhibit enhanced MAP kinase activity after treatment (295). Moreover, microarrays comparing CML CD34+ cells with normal CD34+ cells have revealed Bcr–Abl-induced functional alterations, such as increased cell-cycle and proteasome activity (296). Altogether, these imatinib-insensitive stem cells likely explain the maintenance of a minimal residual disease phenotype and relapse.

Almost a year after Druker’s work, which showed drug efficacy, a series of patient follow-up studies evidenced a high frequency of point mutations within the KD ATP-binding region or within the P-loop associated with poor prognosis (297–299).

Controversially, several imatinib-resistant KD mutants have been shown to remain sensitive to this drug (300). Crystallographic studies of the wt and the T315I hot-spot mutant have aided the understanding of the contacts of imatinib within the *c-Abl* KD (301, 302) and the mechanisms underlying resistance (303) but even the second-generation (nilotinib and dasatinib) and several third-generation ATP-competitive kinase inhibitors succumb to overcome T315I gain-of-function effects (304–306). The involvement of the myristate pocket in kinase inhibition gives rise to the targeting of Bcr–Abl through allosteric mechanisms. Although some imatinib-resistant Bcr–Abl mutants are sensitive to the myristate-like inhibitor GNF-2, T315I is not (307). However, the association of nilotinib and GNF-5, a close derivative of GNF-2, was able to prolong the survival of mice in a Bcr–Abl T315I xenograft model (308). The recent idea of targeting Bcr–Abl is based on allosteric inhibitors targeting the myristate pocket, the Bcr N-terminal coiled-coil oligomerization domain (309) or the SH2–KD interaction (228). Understanding how Bcr–Abl works in a dynamic and synergistic manner will provide the missing clues to increase the repertoire of targetable segments for drug design.

Future Directions

The growing number of proteins with in-tandem structured and unstructured regions will challenge the next generation of researchers to uncover the mechanisms underlying their intramolecular dynamic synergism. Several of these unstructured segments located at the N- or C-termini or between structured regions were previously not thought to participate in protein activity, regulation, cancer initiation, and abrogation. This scenario is currently changing due to a combination of 10 years of efforts in cellular and molecular signaling and the use of atomic and sub-atomic methods, such as small-angle X-ray scattering (310) and NMR. It is now possible to reconstruct models of partially structured domains and flexible linkers at sub-atomic resolution, as shown for the quaternary structures of full-length p53 in a DNA-bound complex (311, 312), and also to estimate the frequency of the conformational distribution for an ensemble of totally unstructured segments. These methods are complementary to atomic resolution methods that are at the limit of providing information from disordered regions. More than 30% of eukaryotic genomes encode unfolded regions with more than 30 residues, and this number increases to 80% in cancer-associated proteins. The challenge for the next era will be to reveal the hierarchical interactome of these very flexible proteins and to understand how they act synergistically to promote homeostasis and tumorigenesis.

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