Inter-Molecular Dynamics in Gene Expression

Department of
Molecular Genetics

Yosef Shaul

Nina Reuven, Julia Adler, Dan Levy, Dorit Cohen, Peter Tsvetkov, Yaarit Adamovich, Vicky Meltser, Dana Rabinovich

☎ 972 8 934 2320
FAX 972 8 934 4108
@ yosef.shaul@weizmann.ac.il
www.weizmann.ac.il/molgen/members/shaul.html

Introduction

Our main goal is uncovering the principles of molecular dynamics in living cells. Our general approach is to follow cell response to physical forces (radiation), chemicals (drugs) and biological agents (viruses). The rationale is that certain biochemical pathways are dormant in the unchallenged cells. Each of these insults activates different molecular processes that we are monitoring and investigating. Apart from understanding the processes themselves, knowing the molecular basis of their evolvement (history) and memorization (future) is equally important.

The proteins that provide the machinery for growth and maintenance of the living cell turn over by complex processes of synthesis and degradation. It is believed that proteins fold into defined 3D structures, however recently it has become acknowledged that many proteins contain disordered regions, and some are even completely disordered under physiological conditions. These natively unfolded or intrinsically disordered proteins (IDPs) are involved in many cellular processes, including transcription regulation and signal transduction. We found some new principles in proteasomal degradation of IDPs.

The term IDPs does not adequately describe the functional significance of this category of proteins. The use of the term IDP may also give the impression that these proteins are in some way 'inferior' to structured proteins. This is completely wrong, given the fact that such proteins are mainly associated with higher organisms and functions. We think the term '4D proteins' would better describe these proteins, simply based on the fact that their structures are not fixed, as is generally the case for '3D proteins', but rather defined by time and space. IDPs, therefore, are more highly ordered than the 3D proteins and the term 4D proteins would more accurately define this group of proteins.

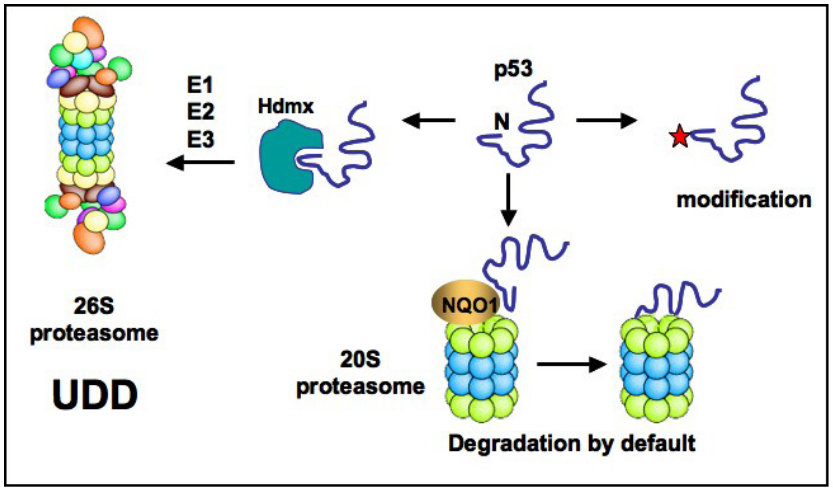

Fig. 1 *p53 degradation by default. P53, as a protein with IDP regions, is prone to degradation by the 20S proteasome in a ubiquitin-independent manner. Three mechanisms of protection are described; modification, NQO1 association, and interaction with a partner (such as Hdmx). Ubiquitin-dependent degradation (UDD) would then be required to degrade stabilized p53.*

Protein structure and degradation

Protein degradation is an essential step in gene expression by regulating the level of end product. The degradation of the majority of cellular proteins is mediated by the proteasomes. Ubiquitin-dependent proteasomal protein degradation is executed by a number of enzymes that interact to modify the substrates prior to their engagement with the 26S proteasomes. The 26S proteasome is made of two complexes, the 20S and the 19S. The role of 19S is to unfold the proteins to gain entry into the 20S particle, where the protein is cleaved into short peptides. Thus, some of the functions of the 19S complex are expected to be dispensable for degradation of IDPs (4D proteins). Indeed, in cell free systems at least some of the IDPs are digested by 20S particles in the absence of the 19S in a ubiquitin-independent manner. In fact, we have demonstrated that susceptibility to the 20S proteasome is a hallmark of IDPs and can be used for their operational definition. An example is the tumor suppressor p53, which is a short-lived protein that accumulates following exposure to different types of stress and induces cell cycle arrest or apoptosis. Both the p53 N-terminal trans-activation domain and the C-terminal regulatory domain were identified as unstructured regions. We found that the N-terminus region facilitates and serves as a signal for degradation by the core 20S proteasomes.

In vivo degradation of IDPs; the principle of "degradation by default"

We also addressed the important question of whether IDPs are present as such within the living cell and undergo degradation via the 20S proteasome. A growing number of proteins bearing disordered regions are reported to undergo ubiquitin-independent degradation. Degradation of p53 has been intensively studied and indeed p53 has become a hallmark for ubiquitin-dependent 26S proteasomal degradation. Several specific E3 ubiquitin ligases were reported to bind and poly-ubiquitinate p53, marking it for degradation by the 26S proteasomes. We found that p53 is also susceptible to degradation in a ubiquitin-independent

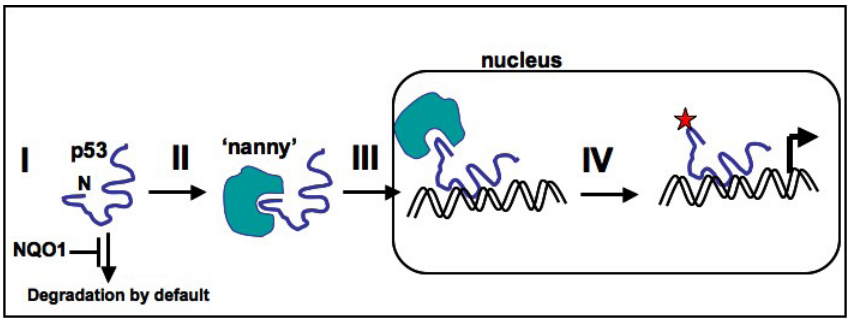

Fig 2. *The nanny model. The newly synthesized p53 (step I) interacts with a partner (step II) that plays a role of nanny, namely protects p53 from degradation by default and ensures p53 safely reaching the site of action (step III). Under this condition p53 as a transcription factor is under repression. The nanny partner therefore prepares p53 but does not license it to function. The last step (IV) is activation of p53 by modification.*

manner that is mediated via the core 20S proteasomes. Experiments both in vitro and in vivo suggest that in contrast to degradation of p53 by the 26S proteasomes, degradation by the 20S proteasomes does not require interaction with E3 ubiqiuitin ligases or any tagging such as poly-ubiquitination. Degradation of p53 by the 20S proteasomes is the result of inherent features of the p53 protein and therefore we term it degradation "by default". p73, a p53 family member, was also found to undergo degradation "by default" via the 20S proteasomes in the cells (Fig 1).

We further showed that the process of degradation "by default" is regulated in order to allow proper protein accumulation. Two different mechanisms were discovered to protect the proteins from degradation "by default". In one, large protein complexes support protein stability by protecting from 20S proteasomal degradation. The second mechanism is executed by NADH quinone oxidoreductase-1 (NQO1), a ubiquitous enzyme that utilizes NADH to catalyze the reduction of various quinones. We found that NQO1 plays a role of 20S proteasome "gatekeeper", regulating the degradation of certain substrates. Purification of the 20S proteasomes from mouse livers and human red blood cells showed that NQO1 is physically associated with the 20S proteasomes but not with the 26S proteasomes. NQO1 also binds a subset of short-lived proteins including p53, and protects them from 20S proteasomal degradation. Binding of NQO1 to these proteins is augmented in the presence of NADH and inhibited by dicoumarol, an inhibitor of NQO1, which competes with NADH. Inhibition of NQO1 by dicoumarol or NQO1 knockdown induces ubiquitin-independent degradation of these proteins. In vitro degradation assays further confirmed that NQO1 together with NADH selectively protect p53 from 20S proteasomal degradation and dicoumarol reduces the protection. The binding of NQO1 to the 20S proteasomes and the ability of NQO1 to bind and protect a subset of short-lived proteins from 20S proteasomal degradation suggest that NQO1 is a novel important regulator of degradation "by default" (Fig 1).

Steps in maturation of IDPs; the nanny model

Given the susceptibility of IDPs to degradation by default we have to assume that biosynthesis of IDPs has to be uniquely protected, processed and matured. To investigate IDP maturation we used p53 as a model. The unstructured N-terminus of p53 contains the transcription activation (TA) domains and the biding site for Mdm2/Hdmx (residues 1-42), and the proline rich area and the second TA domain (residues 40-92). We found that the N-terminal disordered region of p53 is critical for p53 degradation by the 20S proteasome in vitro and in vivo (degradation by default). We have further demonstrated that artificial and natural N-terminus binding proteins are highly effective in rescuing p53 from degradation by default.

Hdmx is a paralog of Mdm2, and although it shares high sequence and structural similarity with Mdm2, it lacks E3 ligase activity and thus cannot sensitize p53 to degradation. There is some evidence that Hdmx can stabilize p53 on the protein level, either by binding p53 directly to compete with Mdm2 for interaction with p53 or by binding to Mdm2 to compromise Mdm2 E3 ligase activity. However, despite the ability of Hdmx to stabilize p53, Hdmx still acts as a repressor of p53,

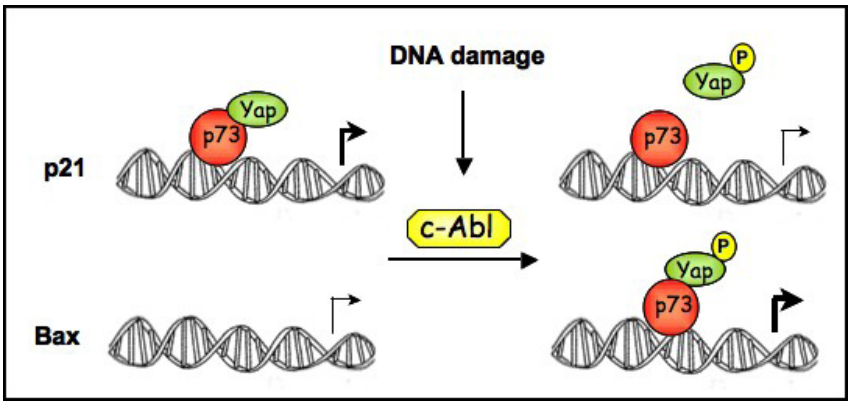

Fig. 3 *The life and death switching. In response to DNA damage cell growth is either arrested by the activation of the p21 promoter or cells undergo apoptosis by the activation of proapoptotic genes such as Bax. Both promoters are activated by p73 and the question is how the decision is made regarding differential activation of the target genes. c-Abl that is activated under these conditions modifies the transcription coactivator Yap. The modified Yap dissociates from p21 and jumps to the Bax promoter.*

since, like Mdm2, the binding of Hdmx to the N-terminus of p53 prevents its transcription activation. We found that Hdmx plays a novel role of protecting p53 from degradation by default. Thus Hdmx has a conflicting activity; protecting p53 from proteasomal degradation on one hand, and inhibiting its activity on the other hand. For p53 to function, some instructive signals have to ensue that dissociate Hdmx from p53, such as those that give rise to N-terminus p53 phosphorylation. At this stage p53 becomes associated with other macromolecules such as DNA and other transcription activators and coactivators. We define the role of Hdmx as "nanny", namely it ensures p53 production but does not provide the license to act (Fig 2).

The biosynthesis of IDPs requires the presence of a nanny partner. This "nanny" role of Hdmx to protect p53 at its infancy stage when it is prone to degradation by default may be more general. Indeed we have found that in the context of the AP-1 transcription factor c-Jun plays the role of nanny for c-Fos. c-Fos undergoes degradation by default, but it can be protected by NQO1 resulting in accumulation in the cytoplasm. In the presence of c-Jun, the NQO1-c-Fos complex dissociates to form c-Jun-c-Fos complex that translocates to the nucleus. c-Jun, therefore, protects c-Fos from degradation by default and induces nuclear localization.

DNA damage signaling

We study the molecular basis of the cellular response to DNA damage. We have characterized a novel signaling-pathway that is initiated by double strand breaks (DSB) and triggers activation of the non-receptor tyrosine kinase c-Abl. This kinase in turn tyrosine phosphorylates p73, a member of the p53 tumor suppressor family. The modified p73 accumulates by translocation to the nuclear matrix. In addition, Yap1, a transcription co-activator, interacts with p73 through the p73 PPPY motif, and increases p73 transactivation of apoptotic genes. The ubiquitin E3 ligase Itch, like Yap1, interacts with p73. Given the fact that both Itch and Yap1 bind p73 via the PPPY motif we hypothesized that Yap1 may also function to stabilize p73 by displacing Itch binding to p73. We found that the interaction of Yap1 and p73 is necessary for p73 stabilization. Yap1 competes with Itch for binding to p73, and prevents Itch-mediated ubiquitination of p73. Accumulated p73-Yap1 complex activates the downstream apoptotic genes to elicit apoptosis, unless the damage has been repaired or the DNA fragments are excluded. Thus, Yap1 a transcription coactivator not only potentiates the transcription activation of its target activator (p73) but also regulates its level.

We also detected a pre-apoptotic phase, so far overlooked, whereby c-Abl inactivates the repair machinery and induces micronuclei (MNi) formation to exclude the un-repaired DNA fragments, possibly to eliminate extensive DNA rearrangements. The process of MNi formation, in particular the role of c-Abl in this process, is under investigation.

A mechanism in promoter switching; a death and life decision

The signaling pathway has to reach a decision-making point. This rather intuitive notion demands to be understood at the molecular level by identifying the decision makers. For example, activation of p73 by c-Abl in response to DNA damage induces apoptosis. However, given the fact that the cell growth-arrest genes are potential targets of p73 as well, the question of how the desired gene is selected becomes very important. Initially it had been proposed that p73 is selectively recruited to the proapoptotic genes provided p300 is functional to acetylate p73. Others suggested that Yap1 is required for the selective activation in addition to functional PML. However, the signal that capacitates Yap1 to select the desired genes remained an enigma. We have at least in part solved the underlying mechanism of decision-making.

We found that Yap1 is a direct substrate of c-Abl and in response to DNA damage it becomes phosphorylated at position Y357. Tyrosine phosphorylated Yap1 is a more stable protein, displays much higher affinity to p73 and selectively coactivates the p73 proapoptotic target genes. Furthermore, we found evidence for Yap1 to be a decision maker that switches between p73 proapoptotic and p73 growth arrest target genes based on its phosphorylation state. Thus, our data provide evidence for an important mechanism for c-Abl activation in response to DNA damage insults whereby modification of a transcription coactivator plays a role of decision-making between life and death (Fig 3).

The molecular basis of virus-host cell interactions; HBV as a metabolic gene

Hepatitis B virus (HBV) is a widespread human specific infectious agent that provides an excellent model to investigate virus-host cell interaction. HBV enhancers and promoters are activated by a number of transcription factors that are mainly expressed in liver cells such as HNF3 and nuclear receptors (i.e. HNF4α, RXRα and PPARα). We found that a short-term fast that turns on the gluconeogenic program robustly induces HBV gene expression in a mouse model. This induction is completely reversible by re-feeding, and depends on Pgc1α, a fasting-induced co-activator of gluconeogenic genes that serves also as a co-activator of HBV. We conclude that HBV transcription is tightly regulated by changes in the body's nutritional state through the metabolic regulator Pgc1α (Fig 4).

Based on its enhancer composition, which links nutritional signals that control hepatic glucose and fat metabolism in the liver to HBV gene expression and replication, it appears that the virus has adopted a regulatory system that is unique to the major hepatic metabolic genes. We hypothesize that by mimicking the expression of key genes implicated in glucose homeostasis, HBV sophisticatedly exploits the host resources to ensure its persistence. This unique virus-host interaction,

mediated by metabolic events in the liver, may provide new mechanisms for previously unexplained clinical phenomena, such as the observed diversity in disease severity between different geographical areas that differ in nutritional habits. Furthermore, the dependency of HBV on nutritional signals sets the stage for viral manipulation by controlling food intake, and opens additional avenues towards food or nutritional therapy as an effective anti-HBV weapon.

HBV and cancer

HBV is highly associated with hepatocellularcarcinoma (HCC) incidence. The HBx protein of HBV has been suggested to be the putative oncogene. Using the two-hybrid system we identified HBXAP as an HBx interacting protein. HBXAP encodes a nuclear protein with an apparent molecular mass of 215 kD which contains several distinct structural motifs including the PHD domain. Recent studies have indicated that HBXAP plays a role in transcriptional regulation and chromatin remodeling. HBXAP has been shown to function as a histone chaperone in the nuclei while its binding partner, hSNF2H, possesses nucleosome-dependent ATPase activity. The HBXAP/hSNF2H complex (or RSF complex) mediates nucleosome deposition and generates regularly spaced nucleosome arrays. At the cellular level, RSF participates in chromatin remodeling by mobilizing nucleosomes in response to a variety of growth modifying signals and environmental cues. Such nucleosome remodeling is essential for transcriptional activation or repression, DNA replication and cell cycle progression. Recently, a growing body of evidence has accumulated to support a novel role of chromatin remodeling in cancer. Interestingly HBXAP gene amplification has been found in many tumors. Dr. Shih, our collaborator from John Hopkins, has found that over expression of HBXAP is critical in certain ovarian cancers that have reached the aggressive stage. HBXAP appears to be a new oncogene.

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