The Paradox of p53: What, How, and Why?

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Unlike the rather stereotypic image by which it was portrayed until not too many years ago, p53 is now increasingly emerging as a multifaceted transcription factor that can sometimes exert opposing effects on biological processes. This includes pro-survival activities that seem to contradict p53’s canonical proapoptotic features, as well as opposing effects on cell migration, metabolism, and differentiation. Such antagonistic bifunctionality (balancing both positive and negative signals) bestows p53 with an ideal attribute to govern homeostasis. The molecular mechanisms underpinning the paradoxical activities of p53 may be related to a protein conformational spectrum (from canonical wild-type to “pseudomutant”), diversity of DNA response elements, and/or higher-order chromatin configuration. Altogether, this functional flexibility positions p53 as a transcriptional “super hub” that dictates cell homeostasis, and ultimately cell fate, by governing a hierarchy of other functional hubs.

Deciphering the mechanisms by which p53 determines which hubs to engage, and how one might modulate the preferences of p53, remains a major challenge for both basic science and translational cancer medicine.

Thirty-five years ago, the arena of cancer biology was introduced to a new putative oncogene, p53 (Kress et al. 1979; Lane and Crawford 1979; Linzer and Levine 1979; Melero et al. 1979; Smith et al. 1979). However, one decade later, p53 was reincarnated as an ultimate tumor suppressor (Wolf and Rotter 1984; Baker et al. 1989; Eliyahu et al. 1989; Finlay et al. 1989; Oren 1992; Berns 1994), widely hailed for its ability to drive the apoptotic demise of cancer cells (Yonish-Rouach et al. 1991). Now, rebounding trends increasingly turn our attention to the fact that, at least in some biological contexts, p53 can actually unequivocally support cell survival, even if the beneficiary cell happens to be cancerous (Vousden and Prives 2009). Does this mean that p53 should now, once again, be considered as an oncogene? Most probably not. Nevertheless, this tells us that we have to part with the old stereotypic image of p53 as a simple-minded tumor suppressor and come up with more sophisticated understanding of what exactly p53 does and for what purposes.

Some of the stereotypic perception of p53 is historically based, originating from the initially disappointing observation that p53 is seemingly dispensable for normal development (Donehower et al. 1992). Gratifyingly, p53-null mice were found to be more resilient to radiation-induced apoptosis (Clarke et al. 1993; Lowe et al. 1993). Consequently, p53 was studied for many years primarily in response to DNA damage or other acute “organism-threatening” conditions. This might have masked other, more...
“mundane” attributes of p53. However, the current dogma is gradually shifting toward the concept that p53 may have the choice of regulating a variety of cellular processes either positively or negatively, thereby actively maintaining homeostasis under less dramatic but much more frequently encountered conditions. Thus, the celebrated “guardian of the genome” (Lane 1992) is evolving into the “guardian of homeostasis.”

To realize its homeostatic agenda, p53 may paradoxically regulate the expression of genes exerting opposing effects on the same cellular process. In that regard, p53 can be envisioned as possessing a dynamic “sliding scale” of functions, ranging from canonical tumor suppressor attributes (e.g., pro-death, anti-cell migration, and quenching of reactive oxygen species [ROS]) to diametrically opposite activities that are typically associated with oncogenes (e.g., pro-survival, pro-migration, and induction of ROS). The term “antagonistic bifunctionality,” describing paradoxical activities of a single molecule that enable more effective responses to changing conditions and thereby contribute to biological robustness, has been coined previously in studies of biological circuits (Hart and Alon 2013). It now appears that p53 may also belong to the growing family of regulatory molecules that qualify for this title. Yet, many questions still remain: “What” paradoxical cell processes does p53 regulate? How do the mechanics of paradoxical transactivation operate? And, why has evolution ended up relying seemingly on only one molecule for such crucial bidirectional choices? In fact, when viewed through these glasses, much of the literature is testament to the notion that p53 sometimes implements binary and paradoxical cell processes.

WHAT DO WE MEAN BY “PARADOXICAL CELL PROCESSES”?

Let us start off with apoptosis, as an example. p53 is a direct transactivator of a hoard of pro-apoptotic genes including BAX, the BH3-only genes PUMA and NOXA, the death receptors CD95, DR4, and DR5, and many more (Riley et al. 2008). However, proteins encoded by several p53 target genes actually can tone down or even inhibit apoptosis. The canonical p21 protein is perhaps the best-documented example (Polyak et al. 1996; Janicke et al. 2008), but the list also includes others such as 14-3-3σ, Plk2, Btg2, Btg3, and DDR (Rouault et al. 1996; Hermeking et al. 1997; Shimizu-Yoshida et al. 2001; Burns et al. 2003; Ongusaha et al. 2003; Ou et al. 2007). Although some of those genes serve primarily to elicit cell-cycle arrest and thereby indirectly spare the cells from death (Jones et al. 2005), others have overt pro-survival activities. Even in the former case, although cell-cycle arrest and apoptosis are superficially teleologically equivalent as effectors of tumor suppression, they differ greatly in that proliferation arrest may spare also damaged cells from a death sentence, potentially sowing the dormant seeds of cancer.

Another setting in which p53 may generate opposing outcomes is cell migration and wound healing. These programs underpin vital processes in multicellular organisms, ranging from embryonic development to aging. Cell migration can also be inappropriately activated during tumor progression, in which it serves as a key step in metastatic spread (Mego et al. 2010). Thus, it is perhaps no surprise that p53 “takes an interest” in these processes. Indeed, numerous studies support the notion that p53 inhibits cell migration, tissue renewal, and wound healing (Tyner et al. 2002; Schoppy et al. 2010; Muller et al. 2011). For instance, p53 represses epithelial-to-mesenchymal transition (EMT), an essential process in wound healing as well as a major driver of malignant progression in many types of cancer (Wang et al. 2009) by various mechanisms, including the transactivation of microRNAs of the miR-200 family, which target the EMT transcription factors ZEB1 and ZEB2 (Howe et al. 2011; Kim et al. 2011). Notably, p53 pathway hyperactivation accelerates tissue deterioration and promotes the appearance of age-associated pathologies in mice (Tyner et al. 2002; Maier et al. 2004). This premature aging, potentially explainable by augmented attrition of adult stem cells (SCs), argues that sustained p53 engagement can present a significant barrier to tissue renewal.
Paradoxically, p53 was also reported to facilitate tissue renewal and EMT in some settings (Kane and Greenhalgh 2000; Sablina et al. 2003; Nakade et al. 2004). For example, loss of p53 was shown to lead to severe defects in hair follicle regeneration and accelerated deterioration of the intestinal epithelium in mice with mosaic deletion of Atr (Ruzankina et al. 2009). Similarly, in a Pten;K-ras mouse model, p53 showed a positive role in ovarian cancer cell migration, as well as in survival (Mullany et al. 2012). This role for p53 in promoting cell migration, which appears to be at odds with its canonical perception, further highlights its antagonistic bifunctionality.

Homeostatic regulation of metabolism is emerging as a major function of p53. As such, p53 engages in intricate cross talk with master regulators of metabolism, such as mTOR and AMPK (AMP-activated protein kinase). Similar to apoptosis and migration, the metabolic impact of p53 is also highly context-dependent and may lead to confounding conclusions if one takes the facts at face value. For example, during energetic stress due to nutrient deprivation, AMPK can phosphorylate and activate p53 (Imamura et al. 2001; Jones et al. 2005). However, in high-glucose conditions, metformin-activated AMPK actually inhibits p53 (Nelson et al. 2012). The complexity does not end here, but extends also to the cross talk between p53 and mTOR. p53 restrains mTOR by transcriptionally activating the expression of an assortment of proteins, including the exemplary TOR inhibitor TSC2 (Feng et al. 2005). In some instances, p53-dependent mTOR inhibition is AMPK-dependent (Budanov et al. 2004; Feng et al. 2005). In turn, p53 can be inhibited by mTOR (Mun-gamuri et al. 2006), which makes good sense intuitively. However, under different conditions, p53 is actually up-regulated, rather than inhibited, by mTOR (Lee et al. 2007). Together, this complex regulatory network between mTOR, AMPK, and p53 modulates numerous aspects of cellular metabolism, including ROS, autophagy, and lipid metabolism (detailed in the following paragraphs).

Energy metabolism is intricately linked to production and neutralization of ROS (Sabhawal and Schumacker 2014). p53 positively regulates the expression of antioxidant proteins, such as sestrins (Budanov et al. 2004), aldehyde dehydrogenase 4 (ALDH4) (Yoon et al. 2004), and TP53INP1 (Cano et al. 2009). Concurrently, p53 represses the expression of pro-oxidant genes such as nitric oxide synthase (NOS2) (Ambs et al. 1998) and cyclooxygenase 2 (COX2) (Subbaramaiah et al. 1999). Yet, on the other hand, p53 can augment cellular ROS by activating genes such as PIG3/TP53IP3, proline oxidase (PIG6/POX), and ferredoxin reductase (FDXR) (Polyak et al. 1997; Liu and Chen 2002; Rivera and Maxwell 2005) and inhibiting G6PDH, malic enzymes, and manganese superoxide dismutase (Zhao et al. 2005; Jiang et al. 2011, 2013). The generally accepted rationale behind these seemingly conflicting effects of p53 is that mild ROS induces a p53-dependent growth arrest and antioxidant response, whereas excessive ROS might be conducive to p53-dependent apoptosis (Kruiswijk et al. 2015). That being said, the question still remains as to the “design logic” of p53 governing the paradoxical pivot between these two opposing processes.

Autophagy is a lysosome-dependent catabolic pathway that recycles building blocks derived from long-lived proteins or damaged organelles. Like p53, autophagy can also exert opposing effects on cell fate. While serving as a survival mechanism under conditions of relatively mild or transient nutrient deprivation, allowing the cell to optimize the use of its limited resources, autophagy can also lead, in extreme cases, to cell death. At first glance, autophagy appears to be a tumor suppressive mechanism, because mice heterozygous for the autophagy gene Beclin1 are tumor-prone (Qu et al. 2003; Yue et al. 2003) and Beclin 1 deletions are associated with 40%–75% of sporadic human breast, ovarian, and prostate cancers (Aita et al. 1999). However, autophagy can also benefit cancer through its ability to protect tumor cells against metabolic stress, hypoxia, and antineoplastic therapies (Rouschop and Wouters 2009). Not surprisingly, p53 can both promote and inhibit autophagy (Morselli et al. 2009; Mairui et al. 2010; Maddocks and Vousden 2011). This may be dependent on cell-type-specific subcel-
lular distribution of p53 (Tasdemir et al. 2008), as well as on the cellular metabolic baseline, which differs greatly between normal and cancerous cells. Indeed, nutrient (amino acid) starvation has opposing effects on autophagic flux in p53-depleted mouse embryonic fibroblasts and in human colon carcinoma cells (Scherz-Shouval et al. 2010).

Furthermore, the cellular metabolic baseline itself may be influenced by p53. This is exemplified by the impact of p53 on lipogenic status. Thus, p53 regulates genes involved in lipid transport in the liver (Goldstein and Rotter 2012), and it also facilitates transport of fatty acids to the mitochondria to undergo catabolism (Zaugg et al. 2001). Moreover, under glucose starvation p53 induces LIPIN1, a key modulator of the fatty acid metabolism transcriptional regulators PGC1-α, PPARα, and SREBP (Finck et al. 2006; Assaï et al. 2011). p53 can also directly repress the expression of SREBP1c and two of its lipogenic target genes, fatty acid synthase (FASN) and ATP citrate lyase (ACLY) (Yahagi et al. 2003). Importantly, many of those transcriptional effects are exerted by p53 also under basal conditions, in the absence of notable stress, thereby enabling p53 to fine-tune the lipid metabolic landscape of the pertinent cells and tissues.

Given that p53 keeps a tight rein on lipogenesis, it may not be surprising that it has been implicated in diseases related to lipid metabolism, such as type 2 diabetes, obesity, and hepatic steatosis (fatty liver disease) (Yahagi et al. 2003; Minamino et al. 2009; Liu et al. 2013). p53 is induced in the livers of mice suffering from hepatic steatosis (Yahagi et al. 2003, 2004) and chronic alcohol consumption (Derdak et al. 2011) and in adipocytes of obese mice (Yahagi et al. 2003). In these models, attenuation of p53 activity reduces disease by suppressing fat accumulation and liver damage (Derdak et al. 2013) and improving insulin sensitivity (Minamino et al. 2009). However, other studies suggest that p53 plays a protective role against the development of obesity, diabetes, and liver steatosis. When fed a high-fat diet, mice lacking p53 accumulate excessive hepatic lipids and body mass (Wang et al. 2013b). Moreover, the inability to properly activate p53 has been shown to increase metabolic stress. For instance, mice bearing an ATM-phosphorylation-resistant form of p53 develop insulin resistance and glucose intolerance (Armata et al. 2010). Complementary to this notion, “super-p53” mice, which express an extra copy of the p53 gene, benefit from superior glucose tolerance (Franck et al. 2012). These opposing effects of p53 deficiency on metabolic pathologies illustrate once more the bipolar nature of p53 and caution against a stereotypic description of the relationship between p53 and metabolism. Yet, they reinforce again the notion that p53 is a key regulator of metabolic homeostasis, not only at the cellular but also at the organismal level.

Another area of recent interest is the impact of p53 on stem cell differentiation. Here again, p53 facilitates some differentiation programs, while inhibiting others. Of note, several early studies have documented decreases in p53 protein and RNA levels during mouse embryonic stem cell (mESC) differentiation and mouse embryonic development in vivo (Chandrasekaran et al. 1981; Rogel et al. 1985; Sabapathy et al. 1997; Lin et al. 2005), which is thought to be coupled with decreased p53 transcriptional activity (Lin et al. 2005), cytoplasmic localization (Grandela et al. 2007; Qin et al. 2007; Han et al. 2008; Solozobova et al. 2009) and p53 conformational alterations (Sabapathy et al. 1997). Moreover, activation of p53 in mESCs counteracts differentiation by inducing various components of the Wnt signaling pathway (Lee et al. 2010). In apparent contradiction to the above, several in vitro and in vivo models have shown that reexpression of p53 in p53-null undifferentiated mESCs drives them toward a more differentiated state (Sabapathy et al. 1997; Komarov et al. 1999; Lee et al. 2005). One possible mechanism that may account for a positive effect of p53 on differentiation has to do with the impact of p53 on the expression of Nanog, a protein essential for embryonic stem cell self-renewal and maintenance of pluripotency (Silva et al. 2009); in mESCs, direct suppression of Nanog by p53 is sufficient to drive differentiation (Lin et al. 2005). A plausible explanation for the seemingly discrepant effects of p53 on mESC
differentiation is that differences in the signaling landscape of mESCs might modulate the ability of p53 to choose among different noncanonical transcription programs, with widely varying consequences for cell fate.

Adult SCs are necessary for normal tissue homoeostasis and are vital for regeneration after damage. Analogous to the situation in embryonic SCs, the proliferation, self-renewal, and differentiation status of adult SCs is also tightly controlled by p53. p53 exerts a positive influence on B-cell, neural, and myogenic differentiation (Shaulsky et al. 1991; Aloni-Grinstein et al. 1993; Montano 1997; Tamir and Bengal 1998; Hughes et al. 2000; Porrello et al. 2000; Cam et al. 2006; Zhang et al. 2006). Similarly, p53-null mammary glands from adult mice harbor increased numbers of undifferentiated SCs both in vivo (Jerry et al. 2000; Cicalese et al. 2009) and in vitro (Tao et al. 2011). One appealing mechanism for the p53-dependent maintenance of a limited pool of adult SCs is via the role of p53 in promoting asymmetric cell division (Cicalese et al. 2009); however, this is not the only way whereby p53 can encourage differentiation. Other mechanisms have been proposed to explain the positive effect of p53 on differentiation during the later stages of brown adipogenesis (Molchadsky et al. 2013) and in driving terminal differentiation of osteogenic cells (Radinsky et al. 1994).

In other settings, p53 actually appears to exert a negative effect on differentiation, resulting in the augmented differentiation of particular types of p53-null cells. This is exemplified by mesenchymal stem cells (MSCs). MSCs reside in the bone marrow and can differentiate into osteoblasts, adipocytes, and chondrocytes. When the balance of adipogenic or osteogenic factors is tipped, MSCs normally become committed toward a single lineage by activating lineage-specific transcription factors and repressing alternative lineage factors (Rosen and MacDougald 2006). Proper p53 function can be likened to a switchboard operator plugging-in environmental cues to drive and reinforce a suitable differentiation state. MSCs that lack p53 get their “wires crossed” and augment osteoblast differentiation markers Osterix and Runx2 (Lengner et al. 2006; Wang et al. 2006; Molchadsky et al. 2008; Rodriguez et al. 2009) concomitantly with inducing Pparg, a driver of adipocyte differentiation (Rodriguez et al. 2009). Thus, rather than simply serving as a positive regulator of differentiation, as one might expect from the well-documented inverse correlation between differentiation and cancer, p53 should be viewed also here as a moderator of differentiation homeostasis.

HOW DO THE MECHANICS OF DIVERSE TRANSSACTIVATION WORK?

Conceivably, “alternative lifestyle” changes underpinning the paradoxical effects of p53 might be related to differences in p53 protein conformation. Point mutations in the p53 DNA-binding domain elicit conformational and functional instability (Joerger and Fersht 2007), strengthening the notion that correct folding of p53 is vital for its “proper” canonical functions. Furthermore, p53 that is wild type (WT) by sequence is not automatically WT by nature, and actually needs to maintain its WT conformation by binding to a variety of molecular chaperones, including CCT (chaperonin-containing t-complex polypeptide 1) (Trinidad et al. 2013; Rivlin et al. 2014) and HSP70 (Walerich et al. 2009). Conformational maintenance of p53 has been associated with phosphorylation within the p53 amino-terminal domain (Wang and Chen 2003). Interestingly, transforming growth factor (TGF)-β, a cytokine intimately involved in cell migration, signals through “noncanonical” mutant p53 in a manner that depends on p53 phosphorylation on amino-terminal residues (Cordenonsi et al. 2007).

Moreover, it stands to reason that the extensively studied mutant p53 gain of function (GOF) (Oren and Rotter 2010; Muller and Vousden 2014) may be an exaggerated reflection of transcriptional activities that are normally explored also by WT-p53 under defined conditions. Embedded within this concept is the notion that, at least in some cases, the WT-p53 that is retained in a substantial percentage of tumors may be structurally and functionally altered in
a manner that converts it into a “pseudomutant” p53. In this way, such tumors may still reap the potential benefits of mutant p53 GOF even in the absence of TP53 gene mutations. Furthermore, some cancer-associated deregulated signaling pathways may force genetically WT-p53 to adopt “pseudomutant” properties, bypassing the selective pressure for TP53 mutations (Furth et al. 2015). However, when the signaling landscape of such cancer cells is profoundly altered (e.g., on exposure to acute stress), the canonical WT conformation of their p53 might be restored, reinstating a canonical p53 transcriptional program. This may explain why many gene expression studies using WT-p53-positive cancer cells have repeatedly revealed canonical target genes rather than “altered p53” targets. The experimental design typically compared cells treated with genotoxic agents to their nontreated counterparts, rather than focusing on the transcriptional effects of the endogenous WT-p53 under basal conditions.

An alternative and not necessarily contradicting notion is that target gene divergence is due not just to altered p53 protein states, but also to built-in differences in p53 response elements. Several studies have focused on this issue in relation to p53 binding to cell cycle versus apoptotic gene promoters. It has been suggested that different binding affinities of p53 to the regulatory DNA elements of proapoptotic versus cell-cycle inhibitory genes might be crucial (Weinberg et al. 2005). However, although there is a tendency for the promoters of many proapoptotic genes to bind p53 less avidly, there are some that do harbor high-affinity p53 binding sites (Szak et al. 2001). A complementary notion suggests that regulation of the efficiency of RNA polymerase II engagement and transcriptional commitment to p53 target genes transactivation, as dictated by core promoter architecture, plays an important role in regulating differential gene expression (Morachis et al. 2010). More recently, a mechanism that combines the complementary impacts of the p53 protein state and the structure of its cognate DNA response elements has been described (Timofeev et al. 2013). Specifically, it has been shown that binding of p53 to proapoptotic target genes and transcriptional activation of those genes, both in vitro and in vivo, relies on cooperative interactions between adjacent DNA-binding domains within the p53 tetramer. Hence, conditions that affect the strength of those cooperative interactions may modulate the transcriptional program executed by p53, providing a possible explanation for opposing outcomes under different conditions.

Higher-order chromatin architecture is also an important feature in transcriptional regulation. The preexisting three-dimensional chromatin landscape of a particular cellular state is thought to be a stable structure that may predispose to induction of distinct sets of target genes (Jin et al. 2013). Recently, it has been shown that enhancer regions distant from the actual p53 target genes interact intrachromosomally with those target genes to convey long-distance p53-dependent transcriptional regulation (Melo et al. 2013; Allen et al. 2014). In this scenario, a single distal enhancer may coordinately turn on the expression of multiple target genes that interact with that enhancer. It is therefore tempting to speculate that state-specific changes in the higher-order organization and accessibility of such distal p53-regulated enhancers may allow the cell to switch between different p53 transcriptional programs without altering the immediate context of each individual target gene.

The vast majority of studies addressing the transcriptional roles of p53 have focused on genes that are transactivated by p53. Nevertheless, a comparable number of genes are actually repressed by p53. For technical reasons, investigation of p53-mediated transactivation is easier and more straightforward than the study of p53-mediated transcriptional repression and consequently has been more rewarding publication-wise. Yet, p53-mediated transcriptional repression may be of equal importance and remains an underexplored area that may still hide many exciting surprises. Unlike transactivation, transcriptional repression by p53 is often (with some notable exceptions) not elicited through direct binding of p53 to recognizable p53-binding sequences within the DNA. Instead, it typ-
ically occurs via protein–protein interactions, which allows p53 to be recruited to specific genes by “piggybacking” on other transcription factors and DNA-binding proteins that recognize specific sequences within those genes. In doing so, p53 may obstruct pro-transcriptional interactions of its partners or recruit transcriptional corepressors such as Sin3a and histone deacetylases (Murphy et al. 1999; Ho and Benchimol 2003; Riley et al. 2008) to their binding sites. It is thus quite plausible that “altered” p53 interacts with a different repertoire of binding partners, which target it to a different panel of DNA response elements. This is likely to result in the repression of a different set of genes. The extent to which such “partner switching” contributes to the execution of “counterintuitive” programs by p53 is presently unknown and might provide interesting new insights.

For many years, p53 research has focused exclusively on the full-length (FL) p53, which has been characterized in great structural and functional detail. Nevertheless, there is growing recognition that alternative p53 isoforms (Bourdon et al. 2005), derived from the TP53 gene through the usage of multiple promoters, alternative translation initiation sites, and alternative splicing, possess a diverse range of biochemical and biological activities, including cancer-promoting activities (see Joruiz and Bourdon 2016). Changes in the relative abundance of p53 isoforms, which either augment or repress the transcriptional activity of coexpressed FL p53, have been implicated in cancer as well as in senescence and aging (Hafsi et al. 2013; Surget et al. 2013). As one example, the Δ133p53 isoform inhibits senescence in normal human fibroblasts, whereas the p53-β isoform promotes senescence (Fujita et al. 2009). In line with these observations, it has been proposed that increased Δ133p53 and decreased p53-β expression in colon carcinomas may reflect an escape from the senescence barrier during progression from adenoma to carcinoma (Fujita et al. 2009). Recently, a new isoform, p53Ψ, was shown to promote cell motility and invasion in a transcription-independent manner (Senturk et al. 2014), widening the “noncanonical” repertoire of p53. These multiple p53 isoforms, which increase greatly the level of complexity of p53-mediated transcription, might contribute significantly to the generation of discordant p53 programs.

As if matters were not complicated enough, p53 is only one member of an extended family of transcription factors, which includes also p63 and p73. Although superficially binding to the same consensus sites (Dotsch et al. 2010), each member of the family has its unique gene preferences, as illustrated also by the distinct phenotypes of p53, p63, and p73 knockout mice (Murray-Zmijewski et al. 2006).

Together, the combination of these many regulatory variables may have more impact than previously appreciated and may provide a logical mechanistic framework for the apparent antagonistic bifunctionality (and probably multifunctionality) of p53.

WHY RELY ON ONLY ONE MOLECULE FOR BIDIRECTIONAL PROCESSES?

Cells continuously adapt to changing conditions to maintain homeostasis by altering gene expression. By analogy to architectural blueprints, gene expression patterns can be envisaged as networks in which transcription factors and their target genes are pictured as nodes, connected to each other via hubs. This is thought to confer a hierarchical structure, whereby hubs play a central role in directing the cellular response to a given stimulus. Importantly, the number of extensively connected central hubs is far below that of hubs with few connections. The fact that most nodes make only a small number of significant connections renders a biological network more robust and less sensitive to noise (Shoval and Alon 2010). Another characteristic of transcriptional networks is that they facilitate efficient propagation and integration of signals by creating pathways in which very few “steps” are necessary to join any two nodes (Blais and Dynlacht 2005).

Within this framework, p53 might be viewed as a “super hub,” modulating the expression of numerous and varied secondary hubs and thus commanding a profound impact on...
cell fate (Vogelstein et al. 2000). The p53 iso-
forms and p53 family members, p73 and
p63, are all players in the same regulatory net-
work. So, indeed, p53 is connected to (almost)
everything. This network architecture, which
positions p53 as a critical "input sensor" that
safeguards against perturbations and equips
p53 with an exceptionally powerful toolbox to
tune its own activities, is highly advantage-
ous for maintaining homeostasis.

Of note, the concept of transcriptional hubs
is not at odds with the aforementioned molec-
ular mechanisms proposed for differential acti-
vation of distinct transcriptional programs by
p53. Altered states of WT-p53 with a "sliding
scale" of functions might be capable of differen-
tially engaging specific hubs, distinct p53-
binding DNA response elements might define
functional hubs, and higher-order chromatin
architecture might physically confine a subset
of functional hubs.

Opposing transcriptional outputs of p53
might be defined by the distinct number of
steps that signals need to travel to generate a
particular output. Moreover, in stress condi-
tions there may be a reordering of hub hierarchy,
bringing certain hubs more closely to p53 and
thus decreasing the number of necessary steps.
This setup most likely has implications for the
kinetics of differential gene transcription. Un-
der regular physiological conditions, associated
with manageable mild and transient stress, p53
may engage proximal and immediate hubs. Manageable stresses include, for example, repa-
ritable transient DNA damage or fluctuations in
oxygen or nutrient availability. These represent
a potential challenge to homeostasis, and p53
appropriately responds by inducing a transient
cell-cycle arrest (proliferation hubs). Alterna-
tively, p53 might induce antioxidant responses,
metabolic remodeling or promotion of catabo-
lim (metabolic hubs). These adaptive responses
allow cells to survive safely until the challenge
to homeostasis has been satisfactorily resolved.
In contrast, p53 might be situated more distantly
from apoptotic hubs, which might take longer
to fully engage. This is in line with the kinetics of
p53 transactivation in vivo. Although p53 can
bind promoters of both cell-cycle and pro-
apoptotic genes very rapidly, transcription of
proapoptotic targets is impeded for hours after
p53 binding, suggesting slow kinetics of engage-
ment of rate-limiting factors needed to trans-
activate those genes (Szak et al. 2001). This sug-
gests that resolution of the stress within an
acceptable time window, before all apoptotic
factors are fully in place, may dampen the pro-
dead signal and reinstate homeostasis without
risking the irreversible consequences of avoid-
able cell death.

Similar mechanisms of staggered kinetics
might be built also into other p53-regulated dis-
cordant processes. For instance, it is conceivable
that in wound healing p53 might initially engage
pro-migratory, pro-EMT hubs while orchestrat-
ing a subsequent wave of more distal anti-EMT
hubs to complete the resolution phase of the
wound healing process. This is in line with the
explicit need to precisely regulate the EMT re-
sponse temporally in normal cells, because
dampening of the delayed or “distant” hub
might be exploited by cancer to generate “a
wound that does not heal” (Dvorak 1986).

Protracted p53 activation, and perhaps the
resulting imbalance between immediate and de-
layed discordant transcriptional programs, ap-
pears to have a negative impact on homeostasis.
For instance, whereas basal levels of p53 facili-
tate the maintenance of glucose tolerance and
protect from metabolic disease, chronic activa-
tion of p53 (similar to that which occurs in
response to sustained metabolic stress due to
excess glucose or obesity) has been shown to
contribute to glucose intolerance (Hinault et
al. 2011). Likewise, signal-independent persis-
tent p53 activation can promote the aging pro-
cess (Tyner et al. 2002; Maier et al. 2004; Dumble
et al. 2007).

Another implication of the p53 “super hub"
conjecture is that cells lacking p53 may be par-
ticularly unable to tolerate perturbations to ho-
meostasis. This is exemplified by the observation
that individuals with Li–Fraumeni familial can-
cer syndrome (LFS), harboring a heterozygous
TP53 germine mutation, suffer from metabolic
disorders in addition to their high risk of devel-
oping cancer (Wang et al. 2013a). Indeed, per-
turbation of homeostasis is an inherent and
defining feature of cancer. The centrality of p53 for tumor suppression is dramatically illustrated by the fact that it is the most frequently mutated gene in human cancer (Petitjean et al. 2007). Thus, the disadvantage of “super hubs” is the hypersensitivity of the system to inactivation of the decisive indispensible hub, as occurs upon TP53 mutation. The fact that this potentially vulnerable network architecture has nevertheless persisted through evolution implies that the benefit of relying on a single bifunctional molecule must somehow outweigh the above disadvantage, with its associated increased cancer risk.

What other architectural benefits are built into the p53 module? Specifically, what functional benefit might be achieved by maintaining p53 as a single paradoxical component rather than splitting the two opposing functions into distinct components? In bacteria, maintaining enzymes with bifunctionality (e.g., enzymes that can act both as kinases and as phosphatases [Capra and Laub 2012]) has been shown to enable robustness within a regulatory circuit (Shinar et al. 2007). In other words, for bacteria, changes in the concentration of any of the components will not change the phosphorylation–dephosphorylation ratio (Hart and Alon 2013). Analogously, p53 bifunctionality might ensure that the balance between pro-survival and anti-survival outputs would remain robust despite minor perturbations within the circuit. This would allow accurate control of homeostasis in the face of naturally occurring fluctuations in upstream signals or in the concentrations of metabolites and oxygen.

An additional benefit of such network design might be exerted during more severe stress, when energy conservation, speed of response, and coherence of response are essential. Using a multifunctional single component is energy-efficient in times of genotoxic or excessive nutrient stress, averting the need to consume valuable resources that otherwise would be needed to synthesize new proteins. Reusing resources also ensures a more rapid response than synthesizing and assembling fresh transcriptional transactivators. Last, relying on a single molecule for two paradoxical programs has the dual benefit of inducing the desired output while simultaneously diverting stimulation from undesired transcriptional targets.

Interestingly, the bifunctionality of p53 seems to have gone through selective pressure in the course of evolution. Thus, p53 has co-evolved functions in maintaining genomic integrity in the face of genotoxic stress (Levine et al. 2011) together with its pro-survival activities (Rutkowski et al. 2011). As a further illustration, the p53 homologs in Caenorhabditis elegans and Drosophila dually regulate apoptosis and glucose metabolism (Mandal et al. 2005; Belyi et al. 2010).

Of note, evolutionary selection for bifunctionality is not limited to p53 and appears to be a common feature of many apoptosis-related molecules. For example, c-IAP1 is most widely known as an antiapoptotic factor; however, the cleaved carboxy-terminal domain of c-IAP1 actually has proapoptotic activity (Clem et al. 2001). Similarly, full-length BID has a pro-proliferation activity (Bai et al. 2005) and can serve as a DNA damage sensor to participate in protective cell-cycle arrest (Kamer et al. 2005; Zinkel et al. 2005), but the cleaved form of BID (t-BID) acts as a potent pro-death molecule (Gross et al. 1999).

More than 35 years after its discovery, and despite close to 80,000 pertinent scientific publications, the “paradox” of p53 is still far from being resolved. Can we develop the computational, technological, and biological tools to tackle this “super hub” challenge? Can we work together to overcome our current scientific biases and identify true patterns in the huge piles of data? Only the next 35 years will tell. But be ready for new surprises!

ACKNOWLEDGMENTS

The research performed in the authors’ laboratory is supported in part by a Center of Excellence Grant (1779/11) from the Israel Science Foundation, research grants from the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation, the Robert Bosch Stiftung, The Germany Israel Foundation (GIF), the Estate of John Hunter, and the Moross Integrated
Cancer Center. M.O. is incumbent of the Andre Lwoff Chair in Molecular Biology.

References

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by wild-type p53 utilizes histone deacetylases, mediated by interaction with mSin3a. 


The Paradox of p53: What, How, and Why?


## Subject Collection: The p53 Protein

<table>
<thead>
<tr>
<th>Topic</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeting the MDM2–p53 Protein–Protein Interaction for New Cancer Therapy: Progress and Challenges</td>
<td>Shaomeng Wang, Yujun Zhao, Angelo Aguilar, et al.</td>
</tr>
<tr>
<td>Structural Evolution and Dynamics of the p53 Proteins</td>
<td>Giovanni Chillemi, Sebastian Kehrloesser, Francesca Bernassola, et al.</td>
</tr>
<tr>
<td>Exploiting the p53 Pathway for Therapy</td>
<td>Chit Fang Cheok and David Philip Lane</td>
</tr>
<tr>
<td>The Regulation of Cellular Functions by the p53 Protein: Cellular Senescence</td>
<td>Crystal A. Tonnessen-Murray, Guillermina Lozano and James G. Jackson</td>
</tr>
<tr>
<td>The Transactivation Domains of the p53 Protein</td>
<td>Nitin Raj and Laura D. Attardi</td>
</tr>
<tr>
<td>The Evolution of the Ribosomal Protein–MDM2–p53 Pathway</td>
<td>Chad Deisenroth, Derek A. Franklin and Yanping Zhang</td>
</tr>
<tr>
<td>Somatic TP53 Mutations in the Era of Genome Sequencing</td>
<td>Pierre Hainaut and Gerd P. Pfeifer</td>
</tr>
<tr>
<td>Control of Cellular Aging, Tissue Function, and Cancer by p53 Downstream of Telomeres</td>
<td>Caitlin M. Roake and Steven E. Artandi</td>
</tr>
<tr>
<td>Inherited TP53 Mutations and the Li–Fraumeni Syndrome</td>
<td>Tanya Guha and David Malkin</td>
</tr>
<tr>
<td>TP53 Mutations in Hypodiploid Acute Lymphoblastic Leukemia</td>
<td>Evan Q. Comeaux and Charles G. Mullighan</td>
</tr>
<tr>
<td>Transcriptional Regulation by Wild-Type and Cancer-Related Mutant Forms of p53</td>
<td>Neil T. Pfister and Carol Prives</td>
</tr>
<tr>
<td>The Inherited p53 Mutation in the Brazilian Population</td>
<td>Maria Isabel Achatz and Gerard P. Zambetti</td>
</tr>
<tr>
<td>TP53 Mutations in Breast and Ovarian Cancer</td>
<td>Laxmi Silwal-Pandit, Anita Langerød and Anne-Lise Borresen-Dale</td>
</tr>
<tr>
<td>p53 and the Carcinogenicity of Chronic Inflammation</td>
<td>Andrei V. Gudkov and Elena A. Komarova</td>
</tr>
<tr>
<td>Oncogenic Mutant p53 Gain of Function Nourishes the Vicious Cycle of Tumor Development and Cancer Stem-Cell Formation</td>
<td>Yoav Shetzer, Alina Molchadsky and Varda Rotter</td>
</tr>
</tbody>
</table>

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