The Regulation of p53 Synthesis

Regulace syntézy p53

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Summary
The regulation of p53 expression levels is critical in controlling p53 activity in normal and damaged cells. This is well illustrated by the E3 ubiquitin ligase MDM2 that targets p53 for proteasomal degradation under normal conditions and is essential for controlling p53 activity during development. MDM2 is over-expressed in human cancers and together with some other E3 ligases that have also been implicated in controlling p53 stability, which emphasises the importance of post-translational regulation of p53 expression. At the level of synthesis, *TP53* mRNA levels do not change in response to stresses and it is instead its rate of translation initiation that provides the mechanism of choice for expression control. More recent work has shown that *TP53* mRNA plays an important role in mediating the cellular regulation of p53 activity. We will discuss the regulation of p53 synthesis and its implications for controlling p53 activity under normal conditions and during different types of stress response.

Key words
p53 synthesis – stress response – IRES – proteinbiosynthetis – physiological stress – RNA-binding proteins

Souhrn
Regulace exprese proteinu p53 je kritická pro kontrolu jeho aktivity v normálních i poškozených buňkách. Velmi dobře je popsána úloha E3 ubikvitin ligázy MDM2, která je za normálních podmínek zodpovědná za degradaci p53 v proteazomu a je esenciální při kontrole aktivity p53 během vývoje organizmu. Nadměrná exprese MDM2 spolu s některými dalšími E3 ligázami podílejícími se rovněž na regulaci stability p53 byla prokázána u řady lidských nádorů, což jen podtrhuje význam posttranslační regulace hladiny proteinu p53. Za stresových podmínek se hladina *TP53* na úrovni mRNA zásadně nemění, naopak vše nasvědčuje tomu, že syntéza proteinu p53 je řízena především na úrovni iniciace translace, což představuje významný mechanizmus zodpovědný za kontrolu exprese p53. Na druhou stranu současné práce ukazují, že i *TP53* mRNA hraje důležitou roli při regulaci aktivity proteinu p53 v buňce. Proto jsme se v této práci zaměřili a diskutujeme mechanizmy zodpovědné za kontrolu syntézy proteinu p53 a jejich úlohu při regulaci p53 aktivity za normálních podmínek a při různých typech stresu.

Klíčová slova
syntéza p53 – odpověď na stres – IRES – proteosyntéza – fyziologický stres – RNA-vazebné proteiny

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Background

The tumour-suppressor protein p53 integrates a diverse range of cellular stress signals, such as those that arise from DNA damage, nucleotide deprivation, hypoxia or mitotic-spindle defects, to regulate factors and genes that mediate transient cell biological effects including cell-cycle arrest, repair and metabolic changes or irreversible apoptosis and senescence. The importance of p53 in tumour suppression is illustrated by the fact that almost half of all human cancers have mutation within the *TP53* gene leading to loss of p53 activity. In addition, cancer cells have many other types of defects in the ability to activate or respond to p53 [1]. Overall, it is apparent that most, if not all, cancers are defective in the normal p53 response. Manipulation of the p53 pathway, either by increasing p53 expression in tumour cells that harbour wild type p53 or by activating p53 downstream pathways in cells carrying a defective p53, are major goals of the pharmaceutical industry in the search for novel anti-cancer therapies.

As a sequence specific transcription regulator, p53 causes the induction of a large number of gene products that together induce different types of cell biological effects that best respond to the type and intensity of the causative damage or stress. Some of the more "classic" genes include $p21^{WAF/CIP1}$, Gadd45 and 14-3-3 for cell cycle regulation and Bax, Noxa and PUMA for regulation of apoptosis. One of the outstanding questions in p53 cell biology is how the cells can distinguish between different types of stress so that activation of p53 induces the correct cell biological response. This is likely to be a complicated mechanism involving parallel pathways but the answers should help us to elucidate some fundamental questions regarding p53 and pave the way for more specific therapeutic intervention within the p53 pathway. Here we will focus on the mechanisms that control *TP53* mRNA translation.

Stress-dependent Regulation of mRNA Translation

It has been estimated from analyses of mRNAs loaded with ribosomes, so called translational profiling, that somewhere between 10 and 15% of mRNA translation is stress responsive [2]. This indicates, perhaps not surprisingly, that regulated protein synthesis plays an important role in the cellular response to changes in the environment and a number of stresses, including nutrient deprivation [3], temperature shock [4], hibernation [5], oxygen shock (hypoxia or oxidative stress) [6], DNA damage [7] and viral infection [8] result in a global reprogramming of protein synthesis. There are several ways in which translation can be modulated by stress signalling pathways. The initiation of translation is in the vast majority initiated around the cap structure located in the 5'UTR of the mRNA. This recruits eIF4E, which binds eIF4G and eIF4A and in turn recruits the eIF3 complex and leads to the recruitment of the pre-initiation complex, which scans the mRNA for the initiation codon whereupon the 60S subunit is recruited and translation starts. The main regulatory step in translation is *via* initiation, although there are examples of regulated elongation. Several of the steps in the cascade that lead up to the final recruitment of the 60S subunit to the pre-initiation complex are subject to regulation. For example, the eIF4BP1/2 factors compete for 4G-4E in a phosphorylation dependent manner that is a target for different pathways, including mTOR, UV stress, or hypoxia [9]. Another common target for translation suppression is eIF2α (Fig. 1). Activation of PKR by double stranded RNAs, most importantly in response to viral infection, leads to a general suppression of cap-dependent translation [10]. However, some mRNAs such as the interferons, GCN4, or CEBP, that are required during the stress response, have evolved mechanisms in which phosphorylation of eIF2α instead promotes synthesis. mRNAs that are required to produce proteins that are needed for the repair process evade the general translation repression. In the case of yeast transcription factor GCN4 the mRNA carries four short untranslated open reading frames (uORFs) that under low levels of nutrient help to overcome the eIF2α phoshorylation mediated repression of global translation by post-termination 40S subunits to reinitiate at the authentic GCN4 start codon [11]. Another condition in which cells benefit from a broad reduction in synthesis of new proteins is during the unfolded protein response, which occurs after stress to the endoplasmic reticulum during hypoxia or nutrient starvation. In this situation the cells aim to restore the balance between newly synthesised and mature proteins by upregulation of chaperones and the degradation of unfolded proteins (ERAD pathway) as well as subdued protein synthesis *via* phosphorylation of eIF2α by the ER stress-associated kinase PERK. Other members of this kinase family are GCN2 which is activated in response to low levels of amino acids. Another interesting 3' untranslated RNA element for regulation of cap-dependent translation is the IFN-γ-induced GAIT complex which includes EPRS, NSAP1, ribosomal pro-

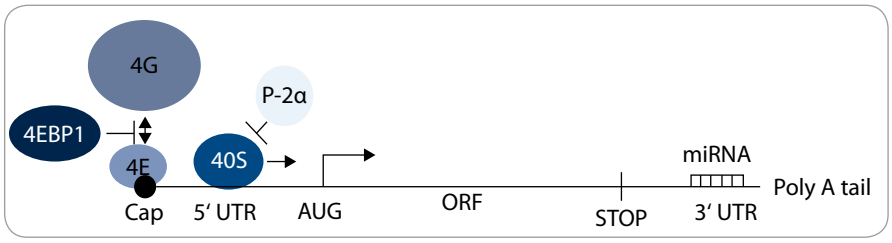

Fig. 1. Several stress pathways modify protein synthesis. Phosphorylation of eIF4EBP1/2 by kinases such as mTOR regulates the interaction between eIF4E (4E) and eIF4G (4G) and thereby suppresses cap-dependent translation in general. Viral infection or nutrient deprivation leads to general inhibition of protein synthesis *via* PKR and PERK kinases, respectively, that phosphorylate the eIF2α initiation factor. Eukaryotic mRNAs that produce factors needed for the stress response have evolved mechanisms to circumvent these types of general translation inhibition. Specific mRNA translation control is mediated by the interaction with miRNA (negative) or by the use of Internal Ribosome Entry Sites (IRES) (positive) (not indicated).

tein L13a and GAPDH which binds the RNA and eIF4G simultaneously inhibiting eIF3 and thereby inhibiting translation initiation [12]. A notable exception is VEGF-A, which evades IFN-γ-induced translation suppression by GAIT during hypoxia by the stabilisation of hnRNP L that promotes an RNA conformer that permits high level translation [13].

Some eukaryotic mRNAs also harbour structured RNA sequences in their 5′UTRs that have the capacity to promote cap-independent translation initiation by direct recruitment of the 40S subunit to the mRNA, thus bypassing cap-dependent mechanisms. This offers another level of regulation of translation initiation that is mRNA specific and it is interesting that such Internal Ribosome Entry Sites (IRES) are often located within mRNAs that are involved in controlling growth, stress response and apoptosis [14]. These structures are unique for each mRNA and still relatively little is known about how cells mediate specific control of each IRESs in response to various stress signalling pathways.

Another mechanism that offers more in terms of specific regulation of mRNA translation comes from RNA hybridisation. More recent years have seen a rapid increase in the numbers of small non-coding RNAs and there are today over 1,000 described and the number is likely to increase further. The expression of microRNAs is regulated by most known cellular pathways and have a broad use in controlling stress induced protein expression [15]. The miRNAs are formed and processed in the nucleus but interact with target mRNA in the cytoplasm to suppress synthesis and/or to target the mRNAs for degradation. Other forms of non-coding RNAs, some of which can exceed 1,000 nts are also starting to become known as regulators of gene expression.

Thus, the presence of regulatory element in the mRNAs can affect translation initiation efficiency *via* a number of mechanisms but also control the expression of isoforms.

Stress-dependent Regulation of p53 Synthesis

One common feature of p53 activation by different pathways is an increase in protein expression levels. This together with post-transcriptional modifications and interactions with co-factors promotes the specificity of p53 complexes required to differentiate its activity towards certain cell biological effects. The increase in p53 levels requires suppression of pathways that control its turnover rate, most notably ubiquitin-dependent degradation mediated by the E3 ligase MDM2 (HDM2 in human). *TP53* mRNA levels do not change in response to cell stress and a number of studies have shown that p53 expression is regulated at the level of translation [16]. This regulation is mediated *via* different regions of the *TP53* mRNA and *via* different mechanisms. Both 5′ and 3′ untranslated regions (UTRs) of the *TP53* mRNA were shown to modulate translation independently [17] and together [18], as well as sequences within the coding region [19]. The coding region of the *TP53* mRNA located downstream of the initiation codon of the full length p53 regulates the synthesis of an alternative translation product termed (p53/47 or ΔNp53) (Fig. 2). Two independent reports [20,21] showed that this N-terminal truncated isoform of p53 lacks the N-terminal transactivation domain and the MDM2 binding site and thus has different activity and stability as compared to the full length form. Unlike the alternative splicing or differential promoter usage which controls the expression of isoforms of the p53 family members p63 and p73, the p53/47 isoform is generated by translation initiation from an in-frame initiation codon at position 40 *via* the activity of an IRES located within the 5′ of the p53 coding sequence [22]. This IRES is activated by the PERK kinase in response to the endoplasmic reticulum (ER) stress and has important implications for p53 activity. The full length p53 induces G1 arrest without affecting the G2 phase of the cell cycle, while p53/47 has no effect on G1 but induces G2/M arrest [23]. The full length p53 causes G1 arrest by inducing the expression of the $p21^{CDKN1a}$ which targets cyclin-E/CDK2 activity, while p53/47 controls expression of 14-3-3σ that instead prevents cyclin-B-dependent activity. In addition to the IRES controlling p53/47 expression, structures in the 5′UTR of p53 also have the capacity to control cap-independent p53 translation. Together, these two IRESs show distinct cell cycle phase-dependent activity, with the IRES for full-length p53 being active at the G2-M transition and the IRES for p53/47 show highest activity at the G1-S transition [22]. These observations suggest that alternative translation initiation of the *TP53* mRNA plays an important role in controlling the p53-dependent gene expression and cell cycle progression. The presence of two independent IRESs within the *TP53* mRNA, differentially regulating protein expression, has also been reported in the VEGF mRNA [24]. Also, IRESs present within coding sequences and mediating the translation of truncated versions of the proteins, have been reported in the $p58^{PITSLRE}$ [25] and the oestrogen receptor [26] mRNAs.

As of today, little is known about the cellular factors that control *TP53* mRNA translation but several proteins have been linked with the *TP53* mRNA. It is reported that p53 negatively regulates its own translation by direct binding of the p53 protein to its 5′UTR, although the precise mechanism of this translational inhibition has not been elucidated [27]. One possibility is that it might induce conformational alteration to the *TP53* mRNA which can suppress translation by a mechanism similar to FGF2 mRNA [28]. Also hnRNP1 (PTB) and hnRNPC1/C2 are known to interact with the 120 nt coding region of *TP53* mRNA and enhance the *TP53* IRES mRNA translation [29,30]. In line with these observations, hnRNP Q has more recently been reported to bind the 5′ region of *TP53* mRNA and regulate its translation [31]. Furthermore ribosomal protein L26 (RPL26) and Nucleolin bind to the 5′UTR of p53 after DNA damage and control p53 translation [32].

The internal *TP53* IRES is required for endoplasmic reticulum-induced cell cycle arrest but it also plays an important role in controlling p53 activity in response to DNA damage. The first evidence that the cellular pathways that mediate the response to irradiation influence p53 synthesis came from treatment of ML-1 cells that in presence of proteasomal inhibitor cycloheximide

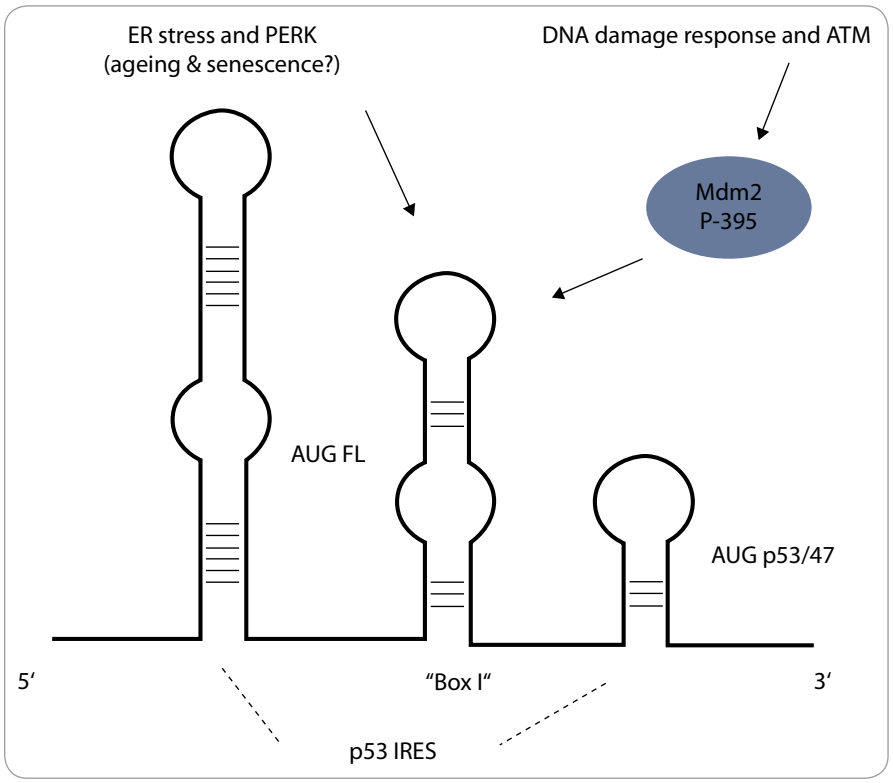

Fig. 2. The *TP53* mRNA plays an important role in regulating p53 activity in response to different stress pathways. Stress to the endoplasmic reticulum leads to a G2 arrest that helps the cell to restore the balance between newly synthesised and mature proteins. This requires the induction of the alternative translated p53/47 isoform *via* the PERK kinase. DNA damage activates the ATM kinase which recruits Mdm2 to the *TP53* mRNA and stimulates p53 synthesis and suppresses p53 degradation.

failed to increase p53 levels [33]. Supporting this model, several groups using metabolic labelling with ^{35}S-methionine and subsequent immunoprecipitation of p53 protein, showed that newly synthesised p53 accumulates quickly in the cell following DNA damage caused by IR [27,32,34], short ultraviolet (UVC) light irradiation [35], or etoposide [36]. It is now established that in response to genotoxic stress, p53 accumulates in the cell as a result of decreased p53 degradation by the E3 ubiquitin ligase MDM2 and by increased *TP53* mRNA translation. The mechanisms that control p53 synthesis in response to genotoxic stress include the activation of the ATM (Ataxia Telangiectasia Mutated) kinase. ATM phosphorylates, either directly or *via* activation of other kinases, residues on the N-terminus of p53 and the C-terminus of MDM2 at Ser 395 adjacent to the RING domain that harbours its E3 ligase activity. These modifications are important for p53 stabilisation and activation. The phosphorylation on p53 at residues 15, 18 and 20 correspond to p53 activity but it was less clear how phosphorylation an MDM2 C-terminus mediates p53 stability. Later work showed that phosphorylation of MDM2 at Ser 395 promotes the binding of the *TP53* mRNA to the C-terminus of MDM2 [37]. This interaction is required for the stabilisation of p53 following ATM activation and is mediated by an increase in the rate of p53 synthesis as well as a decrease in MDM2-dependent degradation of p53. It is interesting that the region of the *TP53* mRNA that binds to the RING domain of MDM2 also encodes the amino acids that interact with the N-terminus of p53 and controls its rate of degradation. Thus, from the same region of the *TP53* gene, two motifs have evolved for the control of p53's rate of synthesis and degradation by controlling the activity of MDM2. This IRES also regulates p53/47 expression in response to ER stress and it is interesting that the same RNA sequence of p53 plays a critical role in the p53 response *via* two different signalling pathways mediated *via* different factors and with different biological responses (Fig. 2).

Several ribosomal proteins are associated with MDM2 (L5, L11, L23, L26, S7, S3, 5S rRNA) which have an inhibitory interaction for ubiquitin ligase activity to stabilise p53 [38–40] and it will be interesting to identify the role of RNPs in *TP53* mRNA translation in the cell cycle and under genotoxic stress conditions. A similar, but negative role, of mRNP complex was shown to regulate translation of the drosophila FMR1 gene that encodes an RNA binding protein which binds the ribosomal proteins L5 and L11 along with 5S rRNA represses the translation of an mRNA encoding the microtubule-associated protein Futsch [41].

As MDM2 binds the internal *TP53* IRES to control the rate of p53 synthesis, it classifies as an IRES transacting factor (ITAF). The complex nature of regulation of cellular IRES-mediated translation under different pathophysiological conditions suggests that ITAFs are responsible for sensing changes in cellular metabolism and influence IRES activity. ITAFs are well known for their nuclear cytoplasmic translocation and generally belong to the group of nuclear ribonucleoproteins (hnRNP A1, C1/C2, I, E1/E2, K, Q and L). The most common explanations offered for how ITAFs work is that they (1) can act as a RNA chaperone modulating the three dimensional structure of the IRES to attain the correct structure for the 40S ribosome to land; (2) can build or abolish bridges between mRNA and ribosome along with canonical initiation factors; or (3) can take the place of the canonical initiation factors building bridges between the mRNA and ribosome [42–45]. On the basis of MDM2 binding to *TP53* mRNA that harbours IRES, it is tempting to speculate that it acts on p53 translation *via* one or more of these possible mechanisms to stimulate p53 synthesis following DNA damage. Although the precise mechanism of how MDM2 binding to *TP53* mRNA following ATM activation leads to a stimulation in p53 syn-

thesis remains unclear, we predict that the mRNP complex of the *TP53* IRES is built during transcription, post-transcriptional processing and export.

TP53 mRNA and MDM2 Interaction in the Nucleolus

The oncogene-induced protein, $p14^{ARF}$, binds to MDM2 and leads to p53 activation. This interaction retains MDM2 in the nucleolus and has together with the binding to ribosomal factors been hypothesised to play an important role in sensing dysfunctions in ribosomal biogenesis and trigger the p53 pathway. Given the canonical roles of the nucleolus in rRNA transcription, pre-rRNA processing and nascent ribosome subunit assembly thereby positively controlling cell growth, it is evident that maturation of other non-ribosomal RNA also occurs in the nucleolus [46,47]. In addition, it has been shown that irradiation and genotoxic stress targets MDM2 to the nucleolar compartment, implicating that different stress pathway act to retain MDM2 in this structure. Recent work indicates that the mechanism for oncogene-induced and genotoxic-induced nucleolar localisation is mediated *via* different pathways. In the latter it was shown that ATM-phosphorylation on residue 395 played a critical role as well as the binding to the *TP53* mRNA but seems not to involve $p14^{ARF}$. This accumulation of MDM2 in a *TP53* mRNA-dependent fashion is associated with the SUMOylation of MDM2, indicating that the structural modification of MDM2 at serine 395 that opens up the RNA binding pocket of MDM2 also stimulates its interaction with SUMO-regulating E3 ligases [37]. It is not known if the accumulation of MDM2 in the nucleoli following DNA damage plays a role in regulating p53 synthesis but it is interesting to note that PML (promyelocytic leukaemia protein) which binds MDM2 has also been shown to interact with ribosomal protein L11 and as this factor also interacts with MDM2. Thus it is possible that they together play a role in targeting MDM2 to the nucleolus and perhaps also in regulating *TP53* mRNA translation [48]. The structural changes in MDM2 associated with the modification of serine 395 and the consequent *TP53* mRNA interaction is supported by previous work showing that nucleotide binding to the RING domain of MDM2 upon localisation to the nucleolus causes a conformational change in the MDM2 RING domain [49]. In a similar way, earlier studies on ATP binding on *Escherichia coli* DnaA protein revealed a conformational change upon ATP binding that facilitates its interaction with DnaB [50] and further ATP binding also induces RecA filament assembly on single-stranded DNA [51].

Uncoupling of MDM2's RNA dependent nucleolar localisation and its E3 activity using mutational analysis has been experimentally challenging [52,53]. However, our earlier results on the E3 dead mutant MDM2 (C464A) that has partially lost its capacity to stimulate translation and RNA binding indicates that E3 activity is not required for the stimulation of translation.

Perspectives

mRNA translation control of p53 in response to stress signalling pathways has been an area of research overshadowed by studies on the regulation of p53 turnover rate. More recent works imply that the mechanisms of regulating p53 steady state levels are in fact a coordinated affair involving synthesis and degradation. In response to DNA damage, MDM2 switches from degrading p53 to promote its synthesis. Whether other stress pathways also stimulate MDM2-mediated synthesis of p53 is not known. p53 itself is not the only protein in this pathway that requires tight regulation of expression. It is easy to forget that MDM2 binds other factors involved in cancer related processes and one could envision that the synthesis of MDM2, and other factors in the p53 pathway, will also be subject to control of synthesis and degradation. There is, for that matter, no reason why the concept of co-regulated synthesis and degradation would not be used more widely in regulatory pathways outside p53. Another question is the regulation of the p53 family members, p63 and p73. To this date, the regulation of synthesis of these factors has not yet received much attention.

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