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Short Article

Oxidative Stress Diverts tRNA Synthetase to Nucleus for Protection against DNA Damage

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SUMMARY

Tyrosyl-tRNA synthetase (TyrRS) is known for its essential aminoacylation function in protein synthesis. Here we report a function for TyrRS in DNA damage protection. We found that oxidative stress, which often downregulates protein synthesis, induces TyrRS to rapidly translocate from the cytosol to the nucleus. We also found that angiogenin mediates or potentiates this stress-induced translocalization. The nuclear-localized TyrRS activates transcription factor E2F1 to upregulate the expression of DNA damage repair genes such as BRCA1 and RAD51. The activation is achieved through direct interaction of TyrRS with TRIM28 to sequester this vertebrate-specific epigenetic repressor and its associated HDAC1 from deacetylating and suppressing E2F1. Remarkably, overexpression of TyrRS strongly protects against UV-induced DNA double-strand breaks in zebrafish, whereas restricting TyrRS nuclear entry completely abolishes the protection. Therefore, oxidative stress triggers an essential cytoplasmic enzyme used for protein synthesis to translocate to the nucleus to protect against DNA damage.

INTRODUCTION

TyrRS is an essential component of the translation machinery in the cytoplasm. As a member of the aminoacyl-tRNA synthetase family, TyrRS catalyzes a two-step reaction to link tyrosine to the 3′-end of its cognate tRNA to generate Tyr-tRNA^{Tyr} as a substrate for the ribosome for protein synthesis (Carter, 1993; Giegé, 2006). In addition to the evolutionarily conserved catalytic core (mini-TyrRS), TyrRS in segmented organisms (from insects to humans) contains a C-terminal domain (C-domain) that is dispensable for aminoacylation (Liu et al., 2002; Wakasugi and Schimmel, 1999). TyrRS was the first tRNA synthetase identified as having extracellular cytokine-like functions, which are activated when the C-domain is separated from mini-TyrRS (Wakasugi and Schimmel, 1999). Mutations in TyrRS are causally associated with Charcot-Marie-Tooth disease (CMT), the most common inherited peripheral neuropathy affecting both motor and sensory neurons (Jordanova et al., 2006). Intriguingly, loss of aminoacylation activity in TyrRS is not required for causing the disease (Storkebaum et al., 2009), suggesting that TyrRS could be linked to CMT through functions other than aminoacylation.

In a recent study (Fu et al., 2012), we discovered the nuclear localization of human TyrRS, and found that its nuclear localization signal (NLS) sequence (^{242}KKKLKK^{247}) was evolved from a tRNA anticodon-binding hexapeptide. The sequence is located in a key position for tRNA binding so that the NLS is blocked when TyrRS is bound with its cognate tRNA. Indeed, knocking down the expression of tRNA^{Tyr} robustly enhances the nuclear import of TyrRS, suggesting that the nuclear transport of TyrRS is coordinated with the demands of protein synthesis in the cytoplasm (Fu et al., 2012). However, the function of nuclear-localized TyrRS is unknown.

It is well established that the demand of global protein synthesis decreases under stress conditions, where the translation machinery is diverted to preferentially promote synthesis of stress-response genes (Holcik and Sonenberg, 2005). One of the mechanisms to downregulate global translation is via stress-induced tRNA cleavage by the activation of angiogenin, a tRNA-specific ribonuclease that cleaves tRNA at the anticodon loop (Li and Hu, 2012; Thompson and Parker, 2009). Angiogenin suppresses protein synthesis not by depleting the tRNA pool but rather by generating 5′ tRNA halves (5′-tiRNAs) that cooperate with translational silencer YB-1 to inhibit translation initiation (Ivanov et al., 2011).

Here we report that oxidative stress stimulates the nuclear transport of TyrRS and the stimulation appears to involve angiogenin. We also show that nuclear-localized TyrRS functions to protect against DNA damage in mammalian cells and in zebrafish. The protection is provided through TyrRS sequestering TRIM28 and HDAC1 from repressing transcription factor E2F1, thus promoting the expression of DNA damage repair genes.

RESULTS

Angiogenin and Oxidative Stress Enhance TyrRS Nuclear Localization

The NLS of TyrRS is normally blocked by the anticodon loop of its cognate tRNA (Fu et al., 2012), which is the predominant

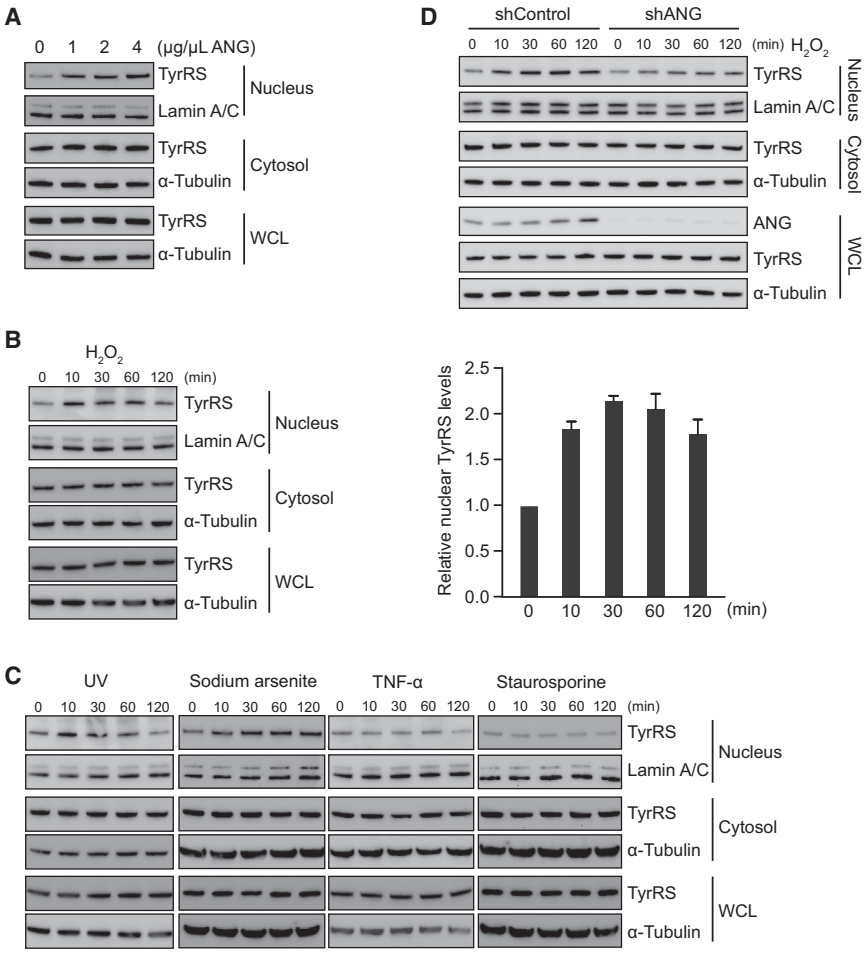

Figure 1. Angiogenin and Oxidative Stress Enhance TyrRS Nuclear Localization

(A) Angiogenin (ANG) enhances TyrRS nuclear localization in HeLa cells. WCL indicates whole cell lysate. Lamin A/C and α-tubulin served as nuclear and cytoplasmic markers, respectively.

(B) H_2O_2 stimulates TyrRS entering the nucleus of HEK293T cells. The quantified result (right) represents average ± SEM (n = 2).

(C) UV and sodium arsenite, but not TNF-α and staurosporine, treatments stimulate TyrRS entering the nucleus of HEK293T cells.

(D) Knockdown of endogenous angiogenin in HEK293T cells decreases the amount of TyrRS that enters the nucleus in response to H_2O_2 treatment.

See also Figure S1.

site for angiogenin cleavage (Li and Hu, 2012; Thompson and Parker, 2009). This consideration prompted us to investigate if angiogenin can regulate TyrRS nuclear localization. Although $tRNA^{Tyr}$ appears to be insensitive to angiogenin cleavage under stress (Fu et al., 2009; Hanada et al., 2013; Saikia et al., 2012), we found that adding angiogenin into the culture medium of HeLa cells dramatically enhances the nuclear import of TyrRS in a concentration-dependent manner (Figure 1A). Angiogenin specifically stimulates the nuclear localization of TyrRS (Figure S1A available online), but not SerRS (seryl-tRNA synthetase) whose NLS was not found to be involved in tRNA binding (Xu et al., 2012). Consistent with previous reports (Fu et al., 2009; Hanada et al., 2013; Saikia et al., 2012), cellular $tRNA^{Tyr}$ appears to be intact during the angiogenin treatment (Figure S1B). However, angiogenin does have the capacity to cleave $tRNA^{Tyr}$ transcript in vitro (Figure S1C). The cleavage happens even in the presence of TyrRS, and the cleaved $tRNA^{Tyr}$ fragments can no longer bind to TyrRS (Figure S1C).

Because various stress conditions activate angiogenin (Li and Hu, 2012; Pizzo et al., 2013), we investigated how stress affects TyrRS nuclear localization. HEK293T cells were exposed to five unique stress conditions: UV (oxidative stress), H_2O_2 (oxidative stress), sodium arsenite (oxidative stress), tumor necrosis factor alpha (TNF-α; inflammatory stress), and staurosporine (apoptotic stress). We found that TNF-α and staurosporine did not have an obvious effect on TyrRS relocalization, whereas UV, H_2O_2, and sodium arsenite treatments induced TyrRS nuclear translocation within 10 min, suggesting that nuclear import of TyrRS is a specific response to oxidative stress (Figures 1B and 1C). The enhanced nuclear import lasted from 30 min to more than 2 hr depending on the specific treatment (Figures 1B and 1C). We also subjected HeLa cells, two human osteosarcoma cell lines (Saos2 and U2OS), and a mouse motorneuron MN-1 cell line to H_2O_2 treatment. The treatment consistently induced TyrRS nuclear translocation in all cell types tested (Figure S1D), suggesting that TyrRS has a ubiquitous role in the cell nucleus in response to oxidative stress.

To test if angiogenin is involved in the oxidative stress-induced nuclear translocation of TyrRS, we knocked down the expression of angiogenin in HEK293T cells (Figure 1D). The knockdown decreased the amount of TyrRS that enters the nucleus in response to H_2O_2 treatment, suggesting that angiogenin does play a role in stimulating the nuclear translocation of TyrRS under oxidative stress (Figure 1D). Possibly, regardless of whether or not cleavage happens, angiogenin can compete with TyrRS for binding to $tRNA^{Tyr}$ and thereby remove the tRNA from blocking the NLS of TyrRS.

Nuclear TyrRS Promotes the Expression of DNA Damage Repair Genes

To investigate the nuclear function of TyrRS, we used the SmartChip Real-Time PCR System (WaferGen Biosystems) to compare the transcriptional profile of TyrRS-overexpressed HEK293T cells with that of control cells transfected with an empty vector. (The overexpression method was used because it enhances TyrRS nuclear localization; Fu et al., 2012.) Among the valid readouts from 718 genes, the expression of 59 genes was significantly upregulated (Figure S2A), whereas expression

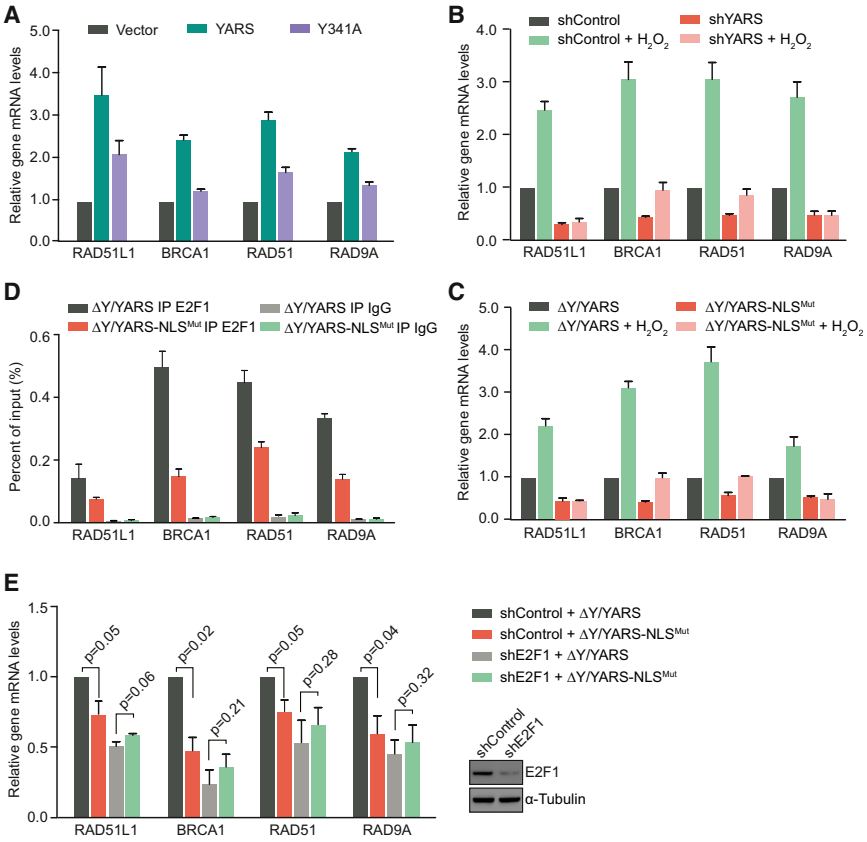

Figure 2. Nuclear TyrRS Promotes Expression of DNA Damage Response Genes by Activating E2F1

(A) Overexpressing TyrRS in HEK293T cells upregulates DNA damage response genes and a mutant form of TyrRS (Y341A) is less active.

(B) Knockdown of endogenous TyrRS in HEK293T cells decreases the expression of DNA damage response genes. H_2O_2 significantly stimulated the expression of DNA damage response genes in control cells that express normal amount of TyrRS. The response to H_2O_2 was significantly reduced or completely diminished in TyrRS-knockdown cells.

(C) Restricting TyrRS nuclear localization in HEK293T cells decreased the expression of DNA damage response genes and inhibited the response to H_2O_2.

(D) Nuclear TyrRS promotes E2F1 binding to its target DNA repair genes in HEK293T cells. DNA binding is detected with ChIP-qPCR assays with antibodies against E2F1 or IgG (control).

(E) The upregulation of DNA damage response genes by TyrRS depends on E2F1. Real-time PCR and E2F1 knockdown are used to detect the effect of E2F1 on transcription levels of DNA damage response genes in HeLa cells.

See also Figure S2. All graphs represent average ± SEM (n = 3).

of nine genes (TERT, CSF1, CD68, GPX1, EPDR1, SLC5A4, ID2, CYP2C19, and TRAF5) was significantly downregulated as a result of TyrRS overexpression. (Up- or downregulations are considered "significant" if the change in expression level was more than 30% in TyrRS-overexpressed cells relative to control cells.) Interestingly, among the 59 upregulated genes, 19 were noted to be involved in DNA damage response and cell cycle regulation (highlighted in red in Figure S2A). For example, the top two genes on the list—RAD51L1 and BRCA1—function in sensing and repair DNA damage, respectively (Sigurdsson et al., 2001; Wu et al., 2010). To validate the transcription profile result, we performed in-house, real-time PCR on RAD51L1, BRCA1, and two other DNA damage response genes on the list (RAD51 and RAD9A). The result clearly confirmed the specific upregulation of these genes in TyrRS-overexpressed HEK293T cells (Figure 2A). A similar result was also obtained using HeLa cells (Figure S2B).

To rule out the possibility that the DNA damage response is an artifact of TyrRS overexpression, we knocked down the expression of endogenous TyrRS in HEK293T cells by a short hairpin RNA targeting the 3′ UTR region of its mRNA (shYARS; Figure S2C). The knockdown significantly decreased the expression level of RAD51L1, BRCA1, RAD51, and RAD9A (Figure 2B), confirming that TyrRS promotes the expression of DNA damage response genes. H_2O_2 treatment significantly stimulated the expression of these DNA damage response genes in control cells that express a normal amount of TyrRS (Figure 2B). In contrast, in TyrRS-knockdown cells, the response to H_2O_2 treatment was significantly reduced or abolished (Figure 2B), indicating that TyrRS plays a key role in mediating the oxidative stress response to promote the expression of DNA damage response genes.

Considering that a simple knockdown of TyrRS may affect global transcription through a general effect on translation, and to confirm that TyrRS is acting from the nucleus to promote the DNA damage response, we devised a strategy to specifically exclude TyrRS from the nucleus without affecting its cytoplasmic role in aminoacylation. Particularly, we compensated the TyrRS-knockdown cells with a mutant form of TyrRS (^{242}NNKLNK^{247}) that has a normal activity for aminoacylation but displays a severe deficiency in nuclear import (Fu et al., 2012). This NLS-mutated TyrRS gene was expressed in HEK293T cells in conjunction with the shYARS to maintain a normal level of TyrRS in the cytoplasm while knocking down the expression of endogenous TyrRS that can enter the nucleus (ΔY/YARS-NLS^{Mut}) (Figure S2C). As a control, a wild-type (WT) TyrRS gene was expressed with the shYARS to give a normal level of TyrRS in both the cytoplasm and the nucleus (ΔY/YARS) (Figure S2C). Importantly, the exogenously expressed WT TyrRS was able to respond to H_2O_2 treatment to enter into the nucleus (Figure S2C). Thus, we have successfully created a system to specifically investigate the nuclear function of TyrRS. Using this system, we found that excluding TyrRS from the nucleus significantly decreased the expression level of RAD51L1, BRCA1, RAD51, and RAD9A in HEK293T cells (Figure 2C), confirming that it is the nuclear-localized TyrRS that promotes the expression of these genes. Consistent with the result in Figure 2B, H_2O_2 treatment significantly promoted

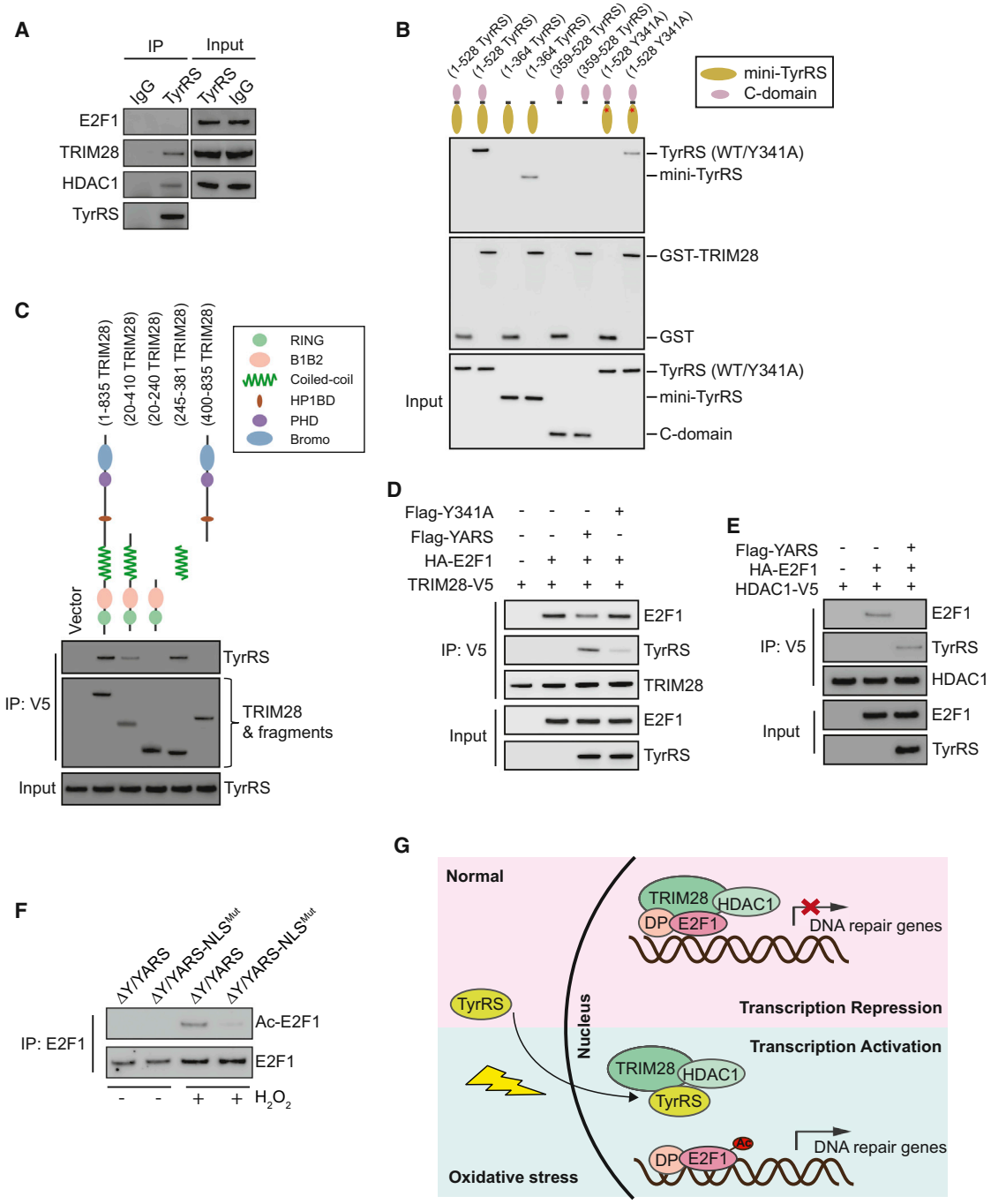

Figure 3. TyrRS Interacts with TRIM28 and Blocks HDAC1 Activity

(A) Endogenous interaction of TyrRS with TRIM28 and HDAC1, but not E2F1, was detected with coIP in HeLa cells.

(B) Domain mapping using GST pull-down experiments reveals that both mini-TyrRS and C-domain of TyrRS contribute to the interaction with TRIM28. The Y341A mutation, designed to alter the conformation between mini-TyrRS and C-domain, weakens the TyrRS-TRIM28 interaction.

(C) Domain mapping reveals that the coiled-coil domain of TRIM28 is responsible for the TyrRS interaction. V5-tagged TRIM28 deletion mutants were cotransfected with Flag-tagged TyrRS into HEK293T cells for coIP with anti-V5 antibodies. TyrRS was detected with anti-Flag antibodies.

(D) WT, but not Y341A, TyrRS inhibits the interaction between E2F1 and TRIM28. V5-tagged TRIM28, HA-tagged E2F1, and Flag-tagged WT or Y341A TyrRS were cotransfected into HEK293T cells for coIP using anti-V5 antibodies. E2F1 and TyrRS proteins were detected by anti-HA and anti-Flag antibodies, respectively.

(E) TyrRS ablates the interaction between E2F1 and HDAC1. V5-tagged HDAC1, HA-tagged E2F1, and Flag-tagged TyrRS were cotransfected into Saos2 cells for coIP using anti-V5 antibodies.

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the expression of DNA damage response genes in control cells, however, in cells with deficient TyrRS nuclear translocation, the effect of H_2O_2 was dramatically reduced or completely abolished (Figure 2C).

TyrRS Promotes DNA Damage Response Genes' Expression through E2F1 Activation

We hypothesized that TyrRS promotes the expression of DNA damage response genes by regulating a transcription factor. E2F1 was a likely candidate because it is known to regulate cell-cycle progression and DNA repair genes. Interestingly, analysis of the published data from the Encyclopedia of DNA Elements (ENCODE) project, which used chromatin immunoprecipitation (ChIP) sequencing analysis to identify transcription factor binding sites on the human genome, revealed that all 19 of the significantly upregulated genes related to DNA damage repair or cell cycle regulation have an E2F1 binding site. Supporting a role for E2F1 in mediating the response, of the 59 genes that are significantly upregulated by TyrRS (Figure S2A), 90% were noted to have E2F1 binding sites. Among all transcriptional factors included in ENCODE, E2F1 is the most frequently bound transcription factor to the regulatory elements of these 59 genes.

To confirm the E2F1 binding and to assess the impact of nuclear TyrRS, we performed a ChIP assay in HEK293T cells expressing WT or nuclear import-deficient TyrRS. We were able to identify a consensus DNA binding sequence of E2F1 (TTTCC/GCGC) in the transcriptional regulatory region of RAD51L1, BRCA1, RAD51, and RAD9A genes (Figure S2D). E2F1 binding was consistently detected for these genes (Figure 2D). Remarkably, the E2F1 binding to all 4 genes is significantly reduced when TyrRS is excluded from the nucleus (Figure 2D). This result not only supports the hypothesis that nuclear TyrRS regulates gene expression through E2F1, but also suggests that the potential mechanism of the TyrRS regulation involves promoting the DNA interaction of E2F1 (see below).

Because other members of the E2F family share the consensus DNA binding sequence of E2F1, we tested another family member E2F6 to see if the binding to the DNA damage response genes is specific to E2F1. As shown in Figure S2E, E2F6 shows weak binding to BRCA1 and virtually no binding to RAD51L1, RAD51, or RAD9A genes. Furthermore, excluding TyrRS from the nucleus enhances E2F6 binding to the BRCA1 gene, opposite to the effect on E2F1. We postulate that this phenomenon may result from the competition between E2F1 and E2F6 for binding to the same target site on BRCA1. Regardless, the promoting effect of TyrRS appears to be specific for E2F1.

We also tested if TyrRS itself can bind to these genes to regulate their expression directly. The result from our ChIP analysis is negative (Figure S2E), suggesting that the nuclear TyrRS regulation on the expression of DNA damage response genes is unlikely to be driven by direct DNA binding.

Finally, we knocked down the expression of endogenous E2F1 in HeLa cells expressing WT or nuclear import-deficient TyrRS. In either case, the mRNA levels of RAD51L1, BRCA1, RAD51, and RAD9A were decreased when E2F1 is knocked down (Figure 2E), consistent with the expected role of E2F1 in promoting the expression of DNA damage response genes. Interestingly, much of the promoting effect of E2F1 is lost when TyrRS is excluded from the nucleus (Figure 2E), indicating that E2F1 is largely dependent on TyrRS nuclear localization to mediate the expression of these DNA damage response genes. On the other hand, when E2F1 is knocked down, nuclear TyrRS slightly inhibits rather than promotes the expression of these DNA damage response genes (Figure 2E), suggesting that the promoting effect of nuclear TyrRS completely depends on E2F1.

TyrRS Interacts with TRIM28 and HDAC1 in the Nucleus

To understand how TyrRS enhances the transcriptional activity of E2F1, we tested whether TyrRS can interact with E2F1. We performed a coimmunoprecipitation (coIP) experiment using TyrRS antibodies to pull down E2F1; however, no interaction was detected (Figure 3A). This result is consistent with our result from the ChIP analysis showing the lack of TyrRS binding to the E2F1-bound DNA damage response genes (Figure S2E), suggesting the effect of TyrRS on E2F1 is through an indirect mechanism.

To search for interaction partners of TyrRS in the nucleus that can potentially influence E2F1, we performed an interactome study (Figure S3A). Among a number of nuclear proteins identified as potential interaction partners of TyrRS by mass spectrometry analysis, TRIM28 (also known as KAP1) and HDAC1 were on the list (Figure S3B). Intriguingly, TRIM28 has been reported to interact with E2F1 to repress its transcriptional activity through HDAC1-dependent deacetylation (Wang et al., 2007; Figure 3G, upper panel).

We confirmed the interaction between the endogenous TyrRS and TRIM28 with coIP (Figure 3A). Using purified, GST-tagged recombinant TRIM28 protein, but not GST protein alone, we were able to pull down purified His-tagged TyrRS (Figure 3B), suggesting a direct interaction between TyrRS and TRIM28. The interaction between the endogenous TyrRS and HDAC1 was also confirmed with coIP (Figure 3A); however, direct interaction between TyrRS and HDAC1 was not detected (data not shown), suggesting that the TyrRS-HDAC1 interaction is likely mediated through TRIM28.

To further understand the TyrRS-TRIM28 interaction, we tested the interaction with mini-TyrRS and the C-domain. GST-TRIM28 was able to pull down mini-TyrRS (res. M1-I364), but not the C-domain (res. E359-S528), but the interaction with mini-TyrRS is much weaker than with the full-length TyrRS (Figure 3B). These results suggest that, while the N-terminal mini-TyrRS is more important than the C-domain, both contribute to the TRIM28 interaction and specific structural features of the

(F) Nuclear TyrRS promotes E2F1 acetylation in Saos2 cells. Saos2 cells were transfected with ΔY/YARS or ΔY/YARS-NLS^{Mut} construct, selected by puromycin for 10 days, and treated with H_2O_2 for 1 hr when indicated. The acetylated E2F1 was detected by IP of the endogenous E2F1, followed by western blot analysis against acetylated-lysine residues.
(G) Schematic illustration of how TyrRS activates E2F1 transcription under oxidative stress.
See also Figure S3.

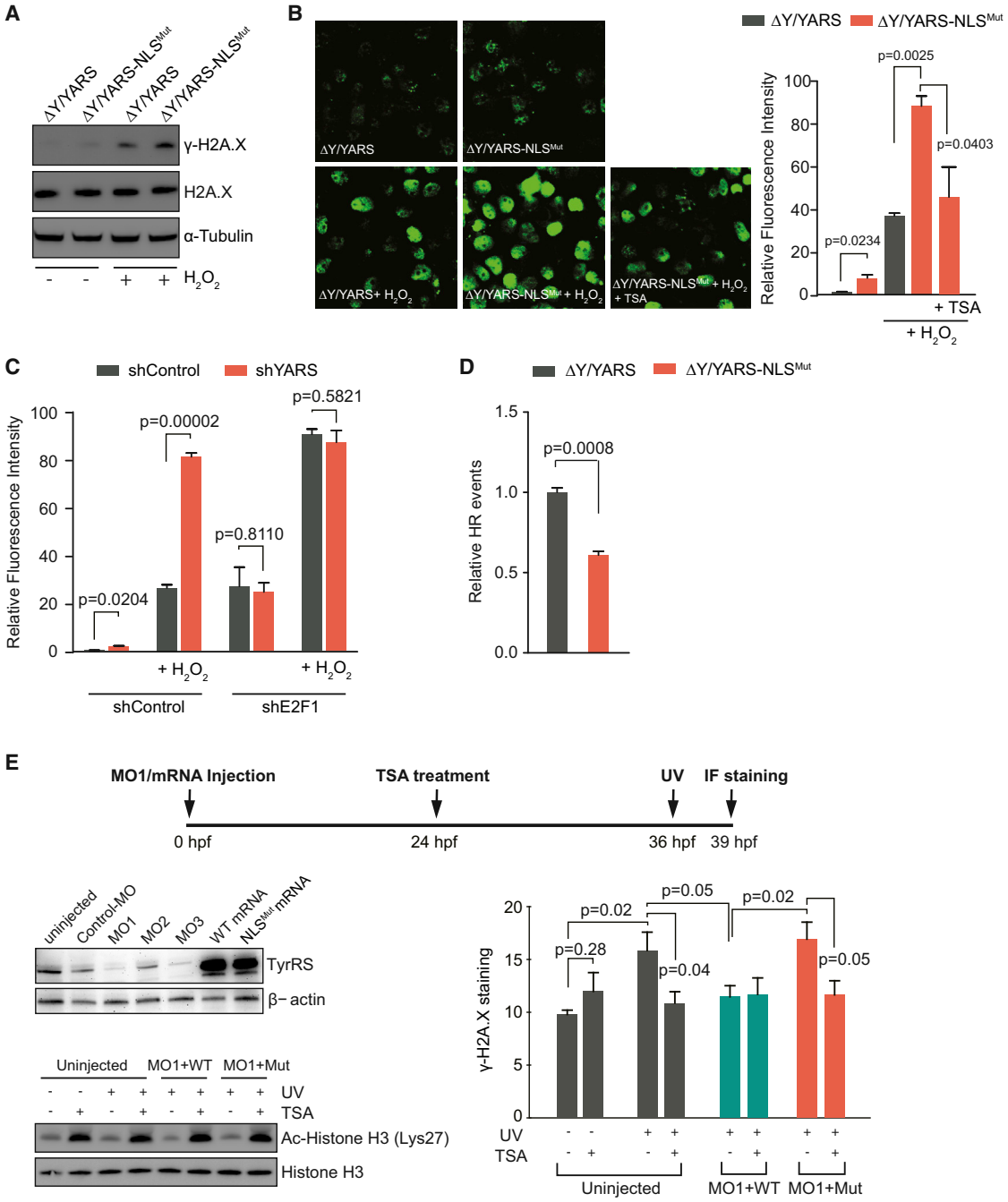

Figure 4. Nuclear TyrRS Protects Cells and Organism from DNA Damage

(A) Nuclear TyrRS protects HEK293T cells from H_2O_2 induced DNA damage. The levels of γ-H2A.X in ΔY/YARS or ΔY/YARS-NLS^{Mut} transfected cells, with or without H_2O_2 treatment (1 hr), were detected with western blot. Total H2A.X and α-tubulin served as loading controls.

(B) Nuclear TyrRS protects against DNA damage in HeLa cells. Cells were treated with H_2O_2 for 1 hr and immunofluorescently stained for γ-H2A.X, or pretreated with TSA (200 nM) for 12 hr before the H_2O_2 treatment. Images and fluorescence intensities were analyzed and quantified using ImageJ. The bar graph represents average ± SEM (n = 3).

(C) The DNA damage protection effect of TyrRS depends on E2F1. HeLa cells with or without E2F1 knockdown were transfected with shControl or shYARS constructs. After 1 hr H_2O_2 treatment, the cells were immunofluorescently stained for γ-H2A.X. Fluorescence intensities were analyzed and quantified using ImageJ. The bar graph represents average ± SEM (n = 3).

(D) Nuclear TyrRS promotes DNA repair by HR. U2OS cells carrying EGFP-HR substrate were transfected with ΔY/YARS or ΔY/YARS-NLS^{Mut} and I-SceI constructs. Transfected cells were assayed for EGFP-positive events with fluorescence-activated cell sorting analysis. Graph represents average ± SEM (n = 3).

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intact protein is important for molecular association. Consistent with this result, a point mutation on the full-length protein (Y341A), which was designed to cause a conformational change between mini-TyrRS and the C-domain (Yang et al., 2007), significantly weakens the TRIM28 interaction (Figure 3B). These results were also confirmed with coIP experiments (Figure S3C).

TyrRS Sequesters TRIM28 and HDAC1 to Promote E2F1 Acetylation and Activation

TRIM28 is a member of the tripartite motif family, which includes three zinc-binding domains—a RING, a B-box type1 (B1), and a B-box type 2 (B2)—and a coiled-coil domain at the N terminus, a central HP1 binding domain, and a plant homeodomain and a bromodomain at the C terminus (Figure 3C). In the role of TRIM28 to suppress the transcriptional activity of E2F1, the coiled-coil domain is used to interact with E2F1, whereas the plant homeodomain and bromodomain recruit HDAC1 to reverse E2F1 acetylation (Wang et al., 2007). To gain insight on how TyrRS may interact with TRIM28 to affect the transcriptional activity of E2F1, we mapped the TyrRS binding site on TRIM28. The result clearly revealed that the coiled-coil domain of TRIM28 was necessary and sufficient for TyrRS binding (Figure 3C). Therefore, the E2F1 binding site on TRIM28 is also responsible for TyrRS interaction, suggestive of a potential competition between E2F1 and TyrRS for TRIM28 binding. Indeed, overexpression of TyrRS weakened the E2F1-TRIM28 interaction (Figure 3D). The Y341A TyrRS, which can enter into nucleus as efficiently as WT TyrRS (Figure S3D) but has diminished interaction with TRIM28 (Figures 3B and S3C), showed no obvious effect in blocking the interaction between E2F1 and TRIM28 (Figure 3D). Consistently, Y341A TyrRS was less active than WT TyrRS in promoting the expression of DNA damage response genes (Figure 2A), indicating that the transcriptional activation effect of TyrRS is specifically mediated through its interaction with TRIM28.

Because TRIM28 recruits HDAC1 to E2F1, inhibition of the E2F1-TRIM28 interaction by TyrRS would affect E2F1's access to HDAC1. Because the retinoblastoma protein is also known to repress E2F1 transcriptional activity by recruiting HDAC1 in a similar but independent fashion of TRIM28 (Magnaghi-Jaulin et al., 1998), we used the retinoblastoma protein-negative cell line Saos2 to test the effect of TyrRS on E2F1-HDAC1 interaction. As shown in Figure 3E, the E2F1-HDAC1 interaction is ablated by TyrRS overexpression in Saos2 cells.

We further tested whether nuclear TyrRS could affect the acetylation level of E2F1 in Saos2 cells. Although the level of acetylated E2F1 in Saos2 cells was below the detection limit (Figure 3F, lanes 1 and 2), we detected the acetylated E2F1 by treating the cell with H_2O_2, which enhances TyrRS nuclear localization (Figure S1D). Consistent with the role of nuclear TyrRS in blocking the access of HDAC1 to E2F1, the acetylation level of E2F1 was significantly reduced as TyrRS was excluded from the nucleus (Figure 3F, lanes 3 and 4). Importantly, acetylation of E2F1 enhances its transcriptional activity by increasing its DNA binding affinity (Martínez-Balbás et al., 2000). Therefore, the ability of TyrRS to promote E2F1 acetylation correlates well with our earlier result from ChIP analysis showing that nuclear TyrRS enhances the DNA interaction of E2F1 (Figure 2D).

Nuclear TyrRS Protects Cells from DNA Damage

Having demonstrated that nuclear-localized TyrRS promotes the expression of select DNA damage repair genes, we investigated the potential for TyrRS to protect cells from DNA damage. First, we compared the level of DNA damage in HEK293T cells expressing WT versus nuclear import-deficient TyrRS following H_2O_2 treatment. For this purpose, phosphorylated histone H2A.X at S139 (γ-H2A.X) was used as a biomarker for DNA double-strand breaks. Without H_2O_2 treatment, the level of γ-H2A.X was below the detection limit (Figure 4A). However, with H_2O_2 treatment, γ-H2A.X could be detected and the level of γ-H2A.X in ΔY/YARS-NLS^{Mut} cells was higher than that in ΔY/YARS cells (Figure 4A), suggesting that nuclear TyrRS has a protective effect on DNA damage.

To verify this result in a different cell type, we performed immunofluorescence staining of γ-H2A.X in HeLa cells expressing WT or nuclear import-deficient TyrRS. Although H_2O_2 treatment induced DNA damage (as indicated by the γ-H2A.X foci) in both group of cells, the level of induction in cells expressing nuclear import-deficient TyrRS was at least 2-fold higher than that in cells expressing WT TyrRS (Figure 4B), confirming that nuclear TyrRS protects cell from DNA damage.

To further confirm that the protective role of TyrRS against DNA damage is not an artifact from the exogenously expressed protein, we monitored DNA damage in TyrRS-knockdown HeLa cells and obtained essentially the same result (Figures S4A and 4C). Interestingly, when we knocked down E2F1 expression in this experiment, TyrRS can no longer protect against DNA damage (Figures S4A and 4C). This observation, in combination with the result of Figure 2E, clearly suggests that the protective role of TyrRS against DNA damage is specifically mediated through E2F1 and its transcriptional activity on DNA damage response genes.

As the TyrRS-upregulated genes RAD51L1, BRCA1, and RAD51 are involved in homologous recombination (HR) during the DNA damage repair process (Bindra et al., 2004; Feng and Zhang, 2012; Johnson et al., 2011), we examined whether nuclear TyrRS has an effect on HR. We used an EGFP-based HR assay as previously described (Wang et al., 2012), in which the EGFP expression cassette is disrupted by an 18-bp I-SceI cleavage site insertion that contains two in-frame stop codons. Expression of I-SceI endonuclease generates DNA double-strand breaks at the I-SceI cleavage site, which is repaired by HR using an internal

(E) Protective effect of TyrRS against DNA damage in zebrafish. The knockdown efficiency of synthesized antisense morpholinos (MOs) against the endogenous zebrafish TyrRS and the expression levels of the injected human TyrRS mRNAs were detected with western blot. β-actin served as a loading control. The embryos of zebrafish were injected with MO-1 and synthesized WT or NLS^{Mut} TyrRS mRNAs, followed by TSA and UV treatments. Immunofluorescence staining against γ-H2A.X was used to detect DNA damage. The activity of TSA was confirmed by detecting acetylated histone H3 (Lys27) levels in the fish samples. Total histone H3 served as the loading control. The γ-H2A.X foci in each embryo were calculated and analyzed using ImageJ. Graph represents average ± SEM (n = 4 to ~8). See also Figure S4.

EGFP donor fragment as a template to restore EGFP expression. Excluding TyrRS from the nucleus significantly reduced EGFP expression (Figures 4D and S4B), confirming the effect of nuclear TyrRS in promoting DNA repair by HR.

Nuclear TyrRS Protects Zebrafish from DNA Damage

Finally, we explored the potential protective effect of TyrRS against DNA damage in vivo using zebrafish as a model organism. A similar strategy to the one utilized in vitro was implemented in vivo to specifically exclude TyrRS from the nucleus without affecting protein synthesis in the cytoplasm. To suppress endogenous (nuclear localization competent) TyrRS in zebrafish, we designed three antisense morpholinos (MOs) and found MO1 to be most effective (Figure 4E). MO1 was then coinjected with WT or NLS^{Mut} TyrRS mRNA into the fish embryos. For uninjected fish, UV radiation significantly increased DNA damage as detected by γ-H2A.X staining ($p = 0.02$). In comparison, fish injected with MO1/WT TyrRS mRNA exhibits much lower level of DNA damage after the UV treatment ($p = 0.05$) (Figure 4E), supporting that TyrRS is strongly protective again DNA damage in vivo. The strong effect correlates with the high level of TyrRS expression from the injected mRNA; however, overexpression of NLS-mutated TyrRS had no protective effect at all (Figure 4E), confirming that TyrRS-mediated protection depends on its nuclear localization.

HDAC Inhibitor Mimics the Role of TyrRS in DNA Damage Protection

We have shown that through TRIM28 interaction, nuclear-localized TyrRS can functionally block HDAC1 from inactivating E2F1 (Figure 3G, lower panel). We investigated whether an HDAC1 inhibitor could phenocopy nuclear TyrRS with regard to DNA damage protection. Trichostatin A (TSA), a selective inhibitor of the class I and II HDACs (including HDAC1), was used to treat HeLa cells expressing nuclear import-deficient TyrRS. Interestingly, under H_2O_2 treatment, TSA could compensate for the lack of nuclear TyrRS to protect the cells against DNA damage (Figure 4B). The effect of TSA was also tested in the zebrafish system (Figure 4E). The TSA treatment alone appears to enhance DNA damage, although the effect is not statistically significant. However, under UV stress, TSA treatment is significantly protective again DNA damage ($p = 0.04$). Interestingly, no additional protection is provided by the TSA treatment in fish that overexpress TyrRS, and the loss of protection by restricting TyrRS nuclear entry can be completely compensated by the TSA treatment ($p = 0.05$) (Figure 4E). Therefore, it appears that the role of nuclear TyrRS is functionally equivalent to an HDAC inhibitor in providing DNA damage protection.

DISCUSSION

Fifteen years after cytokine-like activities were discovered for extracellular TyrRS (Wakasugi and Schimmel, 1999), this study reveals a function for nuclear TyrRS in DNA damage protection. As illustrated in Figure 3G, under oxidative stress, TyrRS translocates to the nucleus and directly interacts with TRIM28. The interaction sequesters the TRIM28/HDAC1 repressor complex, activates transcription factor E2F1, and promotes the expression of DNA response and repair genes such as BRCA1 and RAD51. The enhanced expression of DNA repair genes as a result of TyrRS nuclear translocation is presumably protective. Indeed, restricting TyrRS nuclear entry in cells undergoing oxidative stress causes elevated levels of DNA double-strand breaks. Furthermore, overexpression of TyrRS is strongly protective against DNA damage in zebrafish, suggesting a potential therapeutic value of TyrRS in disease states hallmarked by DNA damage.

It is intriguing to note that the protective effect of nuclear TyrRS can be chemically mimicked by the use of the HDAC inhibitor TSA (Figures 4B and 4E). The effect of HDAC inhibitors on DNA damage is controversial (Conti et al., 2010; Kachhap et al., 2010). HDAC inhibitors have been shown to have both radiation-sensitizing and radiation-protective effects (Brown et al., 2008; Groselj et al., 2013; Miller et al., 2011). The radiation-sensitization effect can be explained by the negative impact of an HDAC inhibitor on genome stability, which exposes DNA to DNA-damaging agents (Eot-Houllier et al., 2009). However, chromatin structure relaxation is also important in allowing the access of DNA repair proteins to the sites of DNA damage (Murr et al., 2006). Our results are consistent with a radiation-protective effect of HDAC inhibitors and raise the possibility that the protective effect of an HDAC inhibitor may be derived through a functional mimicry of nuclear TyrRS and its related pathways.

Our interactome study showed that nuclear TyrRS may interact with other proteins involved in DNA damage response, for example, DNA-PK and PARP1 (Figure S3B), suggesting that the protective role of nuclear TyrRS against DNA damage may result from more than E2F1 activation. Furthermore, TRIM28 is a universal transcriptional corepressor that not only represses E2F1, but also other transcription factors involved in DNA damage response, such as ERBB4, STAT3, and p53 (Gilmore-Hebert et al., 2010; Iyengar and Farnham, 2011; Tsuruma et al., 2008; Wang et al., 2005). TRIM28 is also directly involved in the DNA damage response as the substrate of ATM. Following phosphorylation at Ser824 by ATM, TRIM28 mediates chromatin relaxation by an unknown mechanism to facilitate the repair of DNA double-strand breaks (Ziv et al., 2006). We did examine and find no difference in the phosphorylation level of TRIM28 at Ser824 in ΔY/YARS versus ΔY/YARS-NLS^{Mut} HEK293T cells after H_2O_2 treatment (data not shown). This is consistent with the observations that, in E2F1 knockdown cells, the protective effect of TyrRS is completely abolished (Figure S4A) and nuclear TyrRS can no longer promote the expression of DNA damage response genes (Figure 2D), indicating that the protective effect of TyrRS against DNA damage is predominantly regulated through the E2F1 signaling pathway.

Oxidative stress plays a major role in various neurodegenerative diseases (Federico et al., 2012; Klein and Ackerman, 2003). Interestingly, many players in this DNA-damage response pathway have been linked to neurodegeneration. As mentioned, causal mutations in TyrRS are associated with CMT and the mechanisms are unlikely to relate to loss of aminoacylation (Jordanova et al., 2006; Storkebaum et al., 2009). Angiogenin, which promotes TyrRS nuclear localization, is a susceptibility gene for amyotrophic lateral sclerosis (ALS) as well as for Parkinson disease (van Es et al., 2011). Loss-of-function mutations

of angiogenin are found in patients with ALS (Greenway et al., 2006; Skorupa et al., 2012; Thiyagarajan et al., 2012) and consistently, angiogenin treatment is neuroprotective in mice models of ALS (Subramanian et al., 2008). E2F1 is another example. Overactivation of E2F1 can cause aberrant cell-cycle progression and specific neuronal cell death in Parkinson disease (Höglinger et al., 2007) and in inherited deafness associated with mitochondria stress (Raimundo et al., 2012). On one hand, the neuroprotective role of angiogenin and our observation that angiogenin enhances nuclear translocation of TyrRS suggest that the nuclear role of TyrRS may be neuroprotective but disrupted by CMT-causing mutations; on the other hand, the susceptibility of E2F1 activation to neurodegeneration raises the possibility that CMT-causing TyrRS mutants may be neurodegenerative through overactivation of E2F1. In any case, the nuclear function of TyrRS revealed in this study provides insights for understanding how mutations in TyrRS are causally linked to CMT.

Aminoacyl-tRNA synthetases are among the most abundant proteins in cells. The abundance was presumed to result from a large cellular demand for protein synthesis. However, we observe here that a severe knockdown of TyrRS in human cells did not generate obvious toxicity, at least within the time of our experiments (2–3 days). This and other observations (Seburn et al., 2006) support a concept that the levels of cellular tRNA synthetases are in large excess for what is needed to simply support protein synthesis. This overabundance as well as the unique ability of TyrRS, provided by the dual functional NLS, to coordinate nuclear localization with the demand of protein synthesis may be some of the key reasons for the tRNA synthetase to act in a stress response.

EXPERIMENTAL PROCEDURES

Detection of tRNA^{Tyr} Cleavage

In vitro transcribed tRNA^{Tyr} was radiolabeled and incubated with recombinant TyrRS and angiogenin. tRNA cleavage was detected by electrophoretic mobility shift assay (EMSA). To detect tRNA cleavage in vivo, total RNA of angiogenin treated cells was extracted by using Trizol (Invitrogen) and subjected to Northern blot analysis.

Cellular Localization of TyrRS

Cells treated with angiogenin, UV (50 J/m^2), H_2O_2 (200 μM), sodium arsenite (500 μM), TNF-α (100 ng/ml), or staurosporine (1 μM) were subjected to nuclear-cytoplasmic fractionation and western blot analysis or inmunofluorescence staining to analyze the nuclear translocalization of TyrRS.

Identification of TyrRS Interaction Partners in Nucleus

The nuclear fraction of TyrRS-overexpressed HeLa cells was subjected to IP and subsequently mass spectrometry analysis at the Scripps core facility. The interactions of interest were further confirmed with coIP and GST-pull down assays.

Detection of DNA Damage in Cells and Zebrafish

Western blot analysis and immunofluorescence staining of γ-H2A.X were used to detect DNA damage levels in HEK293T or HeLa cells. Homologous recombination assay were carried out in U2OS cells. For the zebrafish experiment, synthetic mRNAs (1 ng/embryo) and MOs (4 to ~5 ng/embryo) were injected into the yolks of one- to two-cell stage embryos. After UV (720 J/m^2) treatment, in the absence or presence of TSA (1 μM), the fish embryos were subjected to immunofluorescence staining of γ-H2A.X. All the experiments involving zebrafish were conducted according to the guidelines established by the Institutional Animal Care and Use Committee (IACUC) at The Scripps Research Institute, IACUC approval number 09-0009.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.molcel.2014.09.006.

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