DDX3X and DDX3Y are redundant in protein synthesis

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Abstract

DDX3 is a DEAD-box RNA helicase that regulates translation and is encoded by the X- and Y-linked paralogs *DDX3X* and *DDX3Y*. While *DDX3X* is ubiquitously expressed in human tissues and essential for viability, *DDX3Y* is male-specific and shows lower and more variable expression than *DDX3X* in somatic tissues. Heterozygous genetic lesions in *DDX3X* mediate a class of developmental disorders called *DDX3X* syndrome, while loss of *DDX3Y* is implicated in male infertility. One possible explanation for female-bias in *DDX3X* syndrome is that *DDX3Y* encodes a polypeptide with different biochemical activity. In this study, we use ribosome profiling and *in vitro* translation to demonstrate that the X- and Y-linked paralogs of DDX3 play functionally redundant roles in translation. We find that transcripts that are sensitive to *DDX3X* depletion or mutation are rescued by complementation with *DDX3Y*. Our data indicate that *DDX3X* and *DDX3Y* proteins can functionally complement each other in human cells. *DDX3Y* is not expressed in a large fraction of the central nervous system. These findings suggest that expression differences, not differences in paralog-dependent protein synthesis, underlie the sex-bias of *DDX3X*-associated diseases.

Keywords: translational control, DEAD-box proteins, RNA, sex differences

Introduction

DDX3X is a ubiquitously expressed ATPase and RNA-helicase encoded by an essential gene on the X-chromosome, *DDX3X*. *DDX3X* escapes X-inactivation in a wide range of tissues (Cotton et al., 2015). Mutations in *DDX3X* are associated with numerous pathologies, including cancers like medulloblastoma (Floor et al., 2016; Kool et al., 2014; Oh et al., 2016), chronic lymphocytic leukemia (Ojha et al., 2015), squamous cell carcinoma (Stransky et al., 2011), Burkitt's lymphoma (Gong et al., 2020), and many others (Sharma and Jankowsky, 2014). Heterozygous missense or loss-of-function mutations in *DDX3X* are also implicated in intellectual disability and autism-spectrum disorders in females (Iossifov et al., 2014; Lennox et al., 2020; Ruzzo et al., 2019; Scala et al., 2019; Snijders Blok et al., 2015; Takata et al., 2018; Wang et al., 2018; Yuen et al., 2017), with the severity of phenotype correlating with the degree of reduction in *DDX3X* catalytic activity (Lennox et al., 2020).

DDX3X has a Y-chromosome paralog, *DDX3Y*, and the protein products of these two genes are more than 90% identical (Ditton et al., 2004). *DDX3Y* is located within the *Azoospermia Factor a (AZFa)* locus nested within the non-recombining region of the Y chromosome (Kotov et al., 2017). Expression of *DDX3Y* alone has been shown to rescue the specific infertility phenotype caused by deletion of *AZFa* (Ramathal et al., 2015), although these findings have been disputed in mice (Zhao et al., 2019). *DDX3Y* mRNA is broadly expressed across tissues, but activation of a testis-specific distal promoter produces a transcript isoform with a distinct 5' leader that also contributes to its translational control (Jaroszynski et al., 2011). Because of this, expression of *DDX3Y* was thought to be testis-specific, but later work suggested that *DDX3Y* is expressed more broadly in tissues across the human body (Uhlen et al., 2015)(data available from v19.proteinatlas.org). Furthermore, recent evidence suggests that depletion of *DDX3Y* results in neural differentiation defects, indicating wider tissue distribution of *DDX3Y* than previously assumed (Vakilian et al., 2015). Notably, *DDX3Y* expression is consistently lower than that of its X-linked paralog (Uhlen et al., 2015)(data available from v19.proteinatlas.org).

DDX3X and *DDX3Y* both encode for variants of DDX3, a DEAD-box RNA chaperone that facilitates translation initiation on mRNAs with structured 5' leaders (Calviello et al., 2020; Oh et al., 2016). We recently defined the set of genes that depend on DDX3X for their translation and proposed a model where this translation is facilitated by the resolution of these highly structured 5' leaders by 40S-associated DDX3X (Calviello et al.,

2020), consistent with prior work (Chen et al., 2018). However, the role of *DDX3Y* in translation and gene expression – and how similar or different its function is to *DDX3X* – remains incompletely understood.

Genetic diseases involving *DDX3X* such as *DDX3X* syndrome demonstrate severe sex bias (n=107, 104 females, 3 males). This cohort contains *de novo* genetic lesions in *DDX3X* that are exclusively heterozygous (or hemizygous in males) (Lennox et al., 2020). These observations have been attributed to the inability of the Y-linked *DDX3* paralog to functionally complement *DDX3X*, leading to embryonic lethality in males with inactivating mutations of *DDX3X* – whereas heterozygous females are viable, but suffer from symptoms of varying intensity. *Ddx3y* is unable to compensate for the loss of *Ddx3x* during embryonic development in mice (Chen et al., 2016) This is in contrast to the observation that mouse *Ddx3y* is able to compensate for the loss of *Ddx3x* during neural development, insulating male mice from ataxia and seizure phenotypes upon ablation of *Ddx3x*, but is unable to reverse the susceptibility to hindbrain malignancies conferred by *Ddx3x* loss (Patmore et al., 2020). Conversely, the loss of *DDX3Y* confers infertility in human males, despite the robust expression of *DDX3X* in germ-line tissues (Ramathal et al., 2015). In principle, sex bias in *DDX3X*-dependent disorders could arise from differential expression between *DDX3X* and *DDX3Y* across tissues, different activities of their gene products, dosage compensation by the wild-type locus, or other unknown mechanisms.

In this study, we tested the functions of *DDX3X* and *DDX3Y* proteins in translation through cell based and biochemical assays. We depleted endogenous *DDX3X* in male HCT 116 cells using an inducible degron system, complemented with either *DDX3X* or *DDX3Y* cDNAs, and measured translation and RNA abundance using ribosome profiling and RNA-seq. We find that the substitution of *DDX3X* with *DDX3Y* does not significantly affect translation or transcript levels. Using an in vitro reporter system, we demonstrate that genes that are robustly susceptible to depletion of *DDX3* do not show significant changes upon substitution of *DDX3X* with *DDX3Y*. Taken together with their distinct tissue-level expression patterns, our data suggests that the X- and Y-linked paralogs of *DDX3* are redundant in protein synthesis, implying tissue-specific variation of *DDX3Y* expression or another mechanism beyond translational control underlies the sex bias of *DDX3X* associated developmental disorders.

Materials and methods

DDX3 sequence alignment and phylogenetic tree

Reference protein sequences of human DDX3X and DDX3Y were obtained from RefSeq and alignment was performed using the MAFFT algorithm using the BLOSUM62 scoring matrix with default parameters (gap open penalty = 1.53; gap extension penalty = 0.123) (Kato et al., 2002). Protein sequences for mammalian DDX3 orthologs (both X and Y, where available and reliably annotated) were obtained using a combination of the NCBI Gene ortholog finder and PSI-blast (Altschul et al., 1997). Multiple sequence alignment was performed as described above, and a phylogenetic tree was constructed using the Interactive Tree of Life (iTOL) tool (v5.6.3) (Letunic and Bork, 2019).

Construction of DDX3X-degron cell line

HCT-116 cells expressing OsTIR1 were constructed as described in (Natsume et al., 2016). Briefly, a plasmid co-expressing gRNA for the AAVS1 safe-harbor locus and SpCas9 was co-transfected with plasmid encoding for OsTIR1 and homologous arms for the HDR at the AAVS1 locus. Selection was performed in 1 µg/ml of puromycin, colonies were picked and validated by PCR and western blot.

The HCT-116 OsTIR1 expressing cell line was transfected with 800 ng (total) of an equimolar mixture of pX330-based CRISPR/Cas plasmid with guide RNAs targeting the C-terminal end of DDX3X and 1200 ng of a short homology donor carrying the mAID degron tag with a G418 resistance cassette in a pUC19 backbone. Selection was performed in 700 µg/ml of G418, and single colonies picked and validated by PCR and western blot. Selection, picking, clonal expansion and validation were repeated twice (total of 3 rounds) to ensure a clonal cell line.

Induction of DDX3X degradation and transfection of DDX3X-FLAG or DDX3Y-FLAG

Partially codon-optimized DDX3X-FLAG and DDX3Y-FLAG were obtained from Twist Biosciences and cloned into an expression vector under the control of a CMV promoter. For ribosome profiling and *in vitro* translation lysates, expression plasmids (50 µg of DDX3X-FLAG and 75 µg of DDX3Y-FLAG per 15 cm plate, to equalize expression levels) were transfected into cells using a 3:1 ratio of lipofectamine 2000 to plasmid. 24 hours post-transfection, 500 µM Indole-3-acetic acid (IAA, Grainger, 31FY95) was added to the cells (in fresh media) from a 500 mM stock in DMSO. Protein levels were assayed by western blot.

Antibodies

Primary antibodies used in this study include rabbit polyclonal anti-DDX3 (1:5000 in 5% milk, custom made by Genemed Synthesis using peptide ENALGLDQQFAGLDLNSSDNQS) (Lee et al., 2008), anti-actin HRP (1:10000 in 5% milk, Santa

Cruz Biotechnology, sc-47778), anti-FLAG HRP (1:10000 in 5% milk, Sigma, A8592).

Ribosome profiling library construction

DDX3X-mAID tagged HCT-116 (one 15 cm dish at 80-90% confluency per replicate) cells expressing OsTIR1 were transfected with either DDX3X or DDX3Y. 24 hours post-transfection, media was changed and fresh media with 500 µM IAA was added to cells. Un-transfected cells were treated with either DMSO or IAA. 48 hours after auxin addition, cells were treated with 100 µg/ml cycloheximide (CHX) and harvested and lysed as described in (McGlinchey and Ingolia, 2017). Briefly, cells were washed with PBS containing 100 µg/ml CHX and lysed in ice-cold lysis buffer (20 mM TRIS-HCl pH 7.4, 150 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 100 µg/ml CHX, 1 % (v/v) Triton X-100, 25 U/ml TurboDNase (Ambion)). 240 µl lysate was treated with 6 µl RNase I (Ambion, 100 U/µl) for 45 minutes at RT with gentle agitation and further digestion halted by addition of SUPERase-In (Ambion). Illustra Microspin Columns S-400 HR (GE healthcare) were used to enrich for monosomes, and RNA was extracted from the flow-through using Direct-zol kit (Zymo Research). Gel slices of nucleic acids between 24-32 nts long were excised from a 15% urea-PAGE gel. Eluted RNA was treated with T4 PNK and preadenylated linker was ligated to the 3' end using T4 RNA Ligase 2 truncated KQ (NEB, M0373L). Linker-ligated footprints were reverse transcribed using Superscript III (Invitrogen) and gel-purified RT products circularized using CircLigase II (Lucigen, CL4115K). rRNA depletion was performed using biotinylated oligos as described in (Ingolia et al., 2012) and libraries constructed using a different reverse indexing primer for each sample.

RNA was extracted from 25 µl intact lysate (non-digested) using the Direct-zol kit (Zymo Research) and stranded total RNA libraries were prepared using the TruSeq Stranded Total RNA Human/Mouse/Rat kit (Illumina), following manufacturer's instructions.

Libraries were quantified and checked for quality using a Qubit fluorimeter and Bioanalyzer (Agilent) and sequenced on a HiSeq 4000 sequencing system (single end, 65 nt reads).

Pre-processing and alignment of NGS data

All NGS data was processed as previously described (Calviello et al., 2020). Briefly, adapter sequences were trimmed from the footprint reads, UMI sequences were removed and reads were collapsed. Reads aligned to rRNA or a collection of snoRNAs, tRNAs and miRNAs were discarded. The filtered reads were mapped to the hg38 version of the human genome using STAR (Dobin et al., 2013) and count matrices built as described previously (Calviello et al., 2020) using Ribo-seQC (Calviello et al., 2019). Only reads above 24 nucleotides long were included in the count. Read counts for all libraries are in Table S1.

Results

The DEAD-box RNA helicase DDX3 has two paralogs, one each on each of the sex chromosomes. The X-linked *DDX3X* gene is located on a non-pseudoautosomal region of the short arm of the X-chromosome (**Figure 1A**). *DDX3Y*, a paralog of *DDX3X*, is located on the long arm of the Y chromosome in the male-specific region (**Figure 1A**). The sex bias of DDX3X syndrome suggests that males with inactivating mutations in *DDX3X* suffer embryonic lethality, implying that human *DDX3Y* cannot functionally complement *DDX3X*. However, *DDX3X* and *DDX3Y* protein products show ~92% homology, with most of the differences in the N-terminal domain (**Figure 1B**), which is dispensable for RNA duplex unwinding (Floor et al., 2016). Nevertheless, a phylogenetic tree of available mammalian DDX3 protein sequences reveals significant homology between mammalian *DDX3Y*, and separation of the “DDX3Y clade” away from *DDX3X* (**Figure 1C**, **Figure S1**). In many cases, *DDX3Y* sequences of evolutionary distant mammals show greater homology than the paralogs within a given species (**Figure 1C**, **Figure S1**). This indicates both a high degree conservation of the DDX3 orthologs as well as a potential function for *DDX3Y* distinct from its X-linked paralog.

In order to dissect the similarities and differences between the functions of *DDX3X* and *DDX3Y*, we used a cell line we generated to rapidly and efficiently degrade endogenous DDX3 protein X in human male-derived colorectal cancer HCT 116 cells, which show a stable diploid karyotype (Natsume et al., 2016; Waldman et al., 1995). We tagged *DDX3X* with a 68 amino acid fragment of the Auxin Inducible Degron (AID) tag termed mini-AID (mAID) in parental cells expressing the auxin-responsive F-box protein, TIR1, from *Oryza sativa* (OsTIR1) (Natsume et al., 2016). Cells treated with the synthetic auxin, Indole-3-acetic acid (IAA) exhibit >90% reduction in *DDX3X* protein levels by 12 hours of treatment (Calviello et al., 2020) (**Figure 2A**). Using this system, we have previously shown that depletion of *DDX3X* (48-hour auxin treatment only) affects the translation of a subset of cellular messages (Calviello et al., 2020) (**Figure S2A**). Transcripts with increased *DDX3X* sensitivity have low basal translation efficiency and increased GC content and RNA structure within their 5' leader sequences (Calviello et al., 2020).

Transient transfections with either FLAG-tagged *DDX3X* or *DDX3Y* cDNAs followed by degradation of endogenous *DDX3X* protein (48-hour auxin treatment beginning 24 hours post-transfection) allowed for almost complete replacement of the endogenous DDX3 protein with exogenous *DDX3X* or *DDX3Y* (**Figure 2B-C**). We measured transcript levels and ribosome occupancy under

these experimental conditions using ribosome profiling and RNA-seq. Expression of *DDX3Y* shows little to no changes in ribosome occupancy across the entire transcriptome when compared to *DDX3X* (**Figure 2D**). Upregulated genes in *DDX3Y* vs *DDX3X* cells are related to the increased amount of *DDX3Y*-expressing plasmid that was required to be transfected to equalize protein levels (see Materials and Methods) (**Figure S2B-C**).

To increase statistical power to detect a difference between *DDX3X* and *DDX3Y*, we tested for differences between groups of transcripts. *DDX3X* sensitive transcripts show no change in their translation efficiency upon substitution of *DDX3X* with *DDX3Y* (**Figure 2E**; TE_{down} in depletion vs. control without transfection; Calviello et al., 2020). Additionally, *DDX3X*-sensitive transcripts show increased ribosome occupancy in 5' leaders compared to the CDS, consistent with previous observations (Calviello et al., 2020). However, *DDX3X*-sensitive transcripts show no change in the ratio of ribosomes in the 5' leader of the transcript to the coding sequence (5' riboskew) upon the expression of *DDX3Y* compared to *DDX3X* (**Figure 2F**). Furthermore, transcripts with the largest magnitude of raw changes in TE between *DDX3Y* and *DDX3X* expression (without considering statistical significance) show no propensity for increased 5' UTR GC content, a signature of DDX3 sensitivity, consistent with the marginal changes being due to a non-specific effect (**Figure S2D-E**). These observations suggest that the X- and Y-linked paralogs of DDX3 perform redundant functions in translation.

In order to further test the impact of *DDX3X* and *DDX3Y* on translation, we cloned a set of *DDX3*-sensitive 5' UTRs (defined in (Calviello et al., 2020)) upstream of a Renilla luciferase reporter and performed in vitro transcription, capping and 2'-O methylation to generate a set of reporter RNAs. We made translation extracts from HCT 116 cells either depleted of endogenous *DDX3X* or depleted and complemented by transient transfection of exogenous *DDX3X* and *DDX3Y* as described previously (**Figure 3A-B**; Calviello et al., 2020). As previously observed, 5' UTRs of *DDX3* sensitive mRNAs also confer DDX3 dependence to a luciferase reporter, while the control RNA with a simple 5' UTR remains unaffected (**Figure 3C**). Consistent with the ribosome profiling results, *DDX3* sensitive 5' UTRs show no change in reporter output when translated with extracts containing only *DDX3Y* compared to *DDX3X* (**Figure 3D**). This includes the ODC1 reporter, which is sensitive to every perturbation in *DDX3X* that we have tested, included depletion and inactivating mutants (Calviello et al., 2020); **Figure 3E**). These data further

reinforce the notion that DDX3X and DDX3Y are functionally redundant in regulation of translation.

The results above suggest that DDX3Y can rescue translational deficiencies caused by loss of DDX3X. To reconcile this with the observed sex-bias in DDX3X-dependent disorders, we examined FANTOM-CAGE expression data for the paralogs across multiple human tissues (Lizio et al., 2015). Our examination confirmed that as previously reported, expression of *DDX3Y* is prominent in the tissues of the male reproductive system (prostate, seminal vesicle, testes etc.; **Figure 4A, Figure S3**). Furthermore, *DDX3Y* expression is undetectable in several cell types in the brain, including the midbrain, hippocampus, thalamus and amygdala (**Figure 4A, Figure S3**). Taken together, we found that *DDX3X* and *DDX3Y* functioned similarly in translation and are differentially expressed at the RNA level between human tissues.

Discussion

The DEAD-box protein DDX3 is encoded by two paralogous genes in humans, *DDX3X* and *DDX3Y*, the protein products of which show 92% sequence identity (**Figure 1B**). Heterozygous inactivating mutations in *DDX3X* are linked to DDX3X syndrome, a neurodevelopmental delay and autism-spectrum disorder (Lennox et al., 2020). DDX3X syndrome shows a severe sex bias with >95% of known cases affecting females. This sex bias coupled with the observation that inactivating mutations are heterozygous in females (Lennox et al., 2020), suggests that *DDX3Y* is unable to compensate for the impairment of DDX3X function in brain despite recent observations that DDX3Y may also modulate neural development (Vakilian et al., 2015). In fact, the presumed causative *DDX3X* mutations in males diagnosed with DDX3X syndrome shows only very mild impairment of protein function, such that the heterozygous mother remains asymptomatic (Kellaris et al., 2018; Snijders Blok et al., 2015). This further indicates that *DDX3Y* can only partially complement mutations in *DDX3X* function in the brain. Conversely, loss of *DDX3Y* results in a male infertility phenotype despite robust expression of DDX3X in male germline tissues, indicating that *DDX3X* cannot complement loss of *DDX3Y* in the male reproductive system (Kotov et al., 2017; Ramathal et al., 2015; Rauschendorf et al., 2014).

Using both siRNA depletion and an auxin-inducible degron, we have previously demonstrated that DDX3X is required for translation initiation on transcripts with structured 5' leader sequences (Calviello et al., 2020). In this study, we examined the global translation profiles when cells depleted of endogenous DDX3X are

complemented with cDNAs of either DDX3X or DDX3Y. We could not detect significant changes of translation or steady state RNA levels (**Figure 2**). We used *in vitro* translation experiments with a luciferase reporter to confirm 5' leaders of genes that are sensitive to either depletion of DDX3X or inactivating mutations show no significant differences in translation in extracts containing either DDX3X or DDX3Y (**Figure 3, Figure S2-S3**). In fact, the genes that show decreased translation (TE_down) upon depletion of DDX3X show an increased change in the relative ribosome occupancy in the 5' leader compared to the CDS, a signature that is lost while comparing DDX3Y to DDX3X (**Figure 2**). This suggests that the two human paralogs of DDX3 perform equivalent functions in translation, at least in the context of our experimental system.

At least four models could potentially explain the inability of the DDX3 paralogs to complement each other. First, the two paralogs of DDX3 could have distinct target complements and affect the translation of different sets of transcripts. Second, the two paralogs could have the same target propensity but distinct subcellular localizations, and thus differ in their effects. These possibilities are partially rebuffed by the gain of DDX3Y expression complementing for loss of DDX3X in certain lymphoid malignancies (Gong et al., 2020) and in BHK21 hamster cells (Sekiguchi et al., 2004). Additionally, a genome-wide CRISPR screen for essential genes identified DDX3Y as being essential only in Raji cells (which are also of hematopoietic origin), which had suffered a truncating mutation in DDX3X, further indicating genetic complementation (Wang et al., 2015). However, genetic complementation might mask a subtle fitness defect or gene expression changes that could be important in other cellular states. In this study, we quantitatively demonstrate that the translation profile of DDX3X and DDX3Y-expressing cells are functionally identical, arguing against the two scenarios laid out above.

A third model to explain the lack of paralog complementation at the organism level is tissue specific expression patterns of the paralogs. At the RNA level, *DDX3Y* expression is sporadic in the different cell types of the brain, as well as all tissues excepting those of the male reproductive system (**Figure 4**). It has previously been suggested that specific transcriptional and translational regulation contribute to increased DDX3Y protein levels in the testes (Jaroszynski et al., 2011; Rauschendorf et al., 2011). One potential explanation for the inability of *DDX3Y* to complement *DDX3X* in the brain could be that the protein is expressed in insufficient quantities. Conversely, although DDX3X is detectable in adult male testes, multiple layers of transcriptional and translational control restrict its expression to post-meiotic

spermatids (Rauschendorf et al., 2014). The absence of DDX3X protein in premeiotic spermatids could explain its inability to rescue the infertility phenotype conferred by *DDX3Y* loss. A recent analysis of tissue specific expression of sex-linked paralogs correcting for multi-mapping short sequencing reads estimated that while the cumulative expression of DDX3 is statistically identical across tissues for individuals with XX and XY genotypes, the *DDX3Y* expression makes up only a small minority of total DDX3 (Godfrey et al., 2020). This lends further support to the idea that while the two paralogs are functionally identical, spatiotemporal differences in their expression precludes complementation in a number of scenarios. It is important to note that this model requires future work to be confirmed. Lineage-specific complementation of DDX3 paralogs *in vivo* would formally test this hypothesis, and there are already hints of paralog-specific developmental effects (Patmore et al., 2020).

A fourth model that cannot be formally ruled out is that there are specific context-dependent factors in neurodevelopment and germ-cell development that cause differential translational regulation by the X- and Y-linked DDX3 paralogs, and that these contribute to their inability to complement each other in these contexts – despite the gene products of *DDX3X* and *DDX3Y* having redundant functions. Some evidence in support of this model comes from the observation that in mice, while *Ddx3y* is able to compensate for the neurodevelopmental phenotypes caused by the loss of *Ddx3x* in neural lineages, it is unable to suppress the increase in hindbrain malignancies also caused by *Ddx3x* loss (Patmore et al., 2020). This would indicate some form of context-dependent accessory factor influencing the target complement of *Ddx3* homologs. However, it is worth noting that the ability of male mice with genetic lesions in *Ddx3x* to survive without neurological anomalies has not been recapitulated in humans as genetic variation in *DDX3X* in the human population is extremely low (Karczewski et al., 2020). We suggest that some combination of tissue-specific paralog and unknown co-factor expression may explain the sex bias of DDX3X associated developmental disorders, but that inherent differences in molecular function are unlikely. Further investigations in these directions could reveal specific targets or factors that could be exploited to mitigate the phenotypic consequences of genetic lesions in the *DDX3* paralogs.

Data access

Sequencing data can be retrieved using GEO accession number GSE157063. Source code for the analysis of ribosome profiling data was adapted from:

https://github.com/lcalviell/DDX3X_RPCLIP. Source code specific to the analysis in this manuscript is available at: https://github.com/srivats-venkat/DDX3X_3Y_comparison.

Author contributions

Conceptualization, S.V. and S.N.F.; Investigation, S.V. and L.C.; Writing – Original Draft, S.V.; Writing – Review & Editing, S.N.F., S.V. and L.C.; Resources, S.V. and K.W.; Funding Acquisition, S.N.F.; Supervision, S.N.F.

Disclosure declaration

S.N.F. consults for MOMA Therapeutics

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Figure 2: A. Western blot for degradation of endogenous DDX3X tagged with mini-AID (mAID) upon treatment with an auxin (indole-3-acetic acid) in HCT-116 human colorectal carcinoma cell line expressing OsTIR1 under the control of a CMV promoter (DDX3X^{degr}). **B.** Experimental schematic for ribosomal profiling after replacement of endogenous DDX3X with exogenous DDX3X or 3Y. **C.** Western blot for expression of exogenous FLAG-tagged DDX3X and DDX3Y in HCT-116 cells after degradation of endogenous DDX3X. **D. Left:** Full and **Right:** zoomed in plots of differential expression analysis of RNA and ribosome profiling changes upon complementation of DDX3X with either DDX3Y or DDX3X. Point size indicates p-value of differential translation. **E.** Fold-change in TE between DDX3Y and DDX3X expression in mRNAs classified based on DDX3-sensitivity (as determined by response in DDX3X depletion). All transcript classes show no change in TE between DDX3Y and DDX3X expression. **F.** The fold-change of the ratio in ribosome occupancy in the 5' UTR versus the coding sequence **Left:** DDX3-sensitive mRNAs (TE_{down} in DDX3X depletion) have increased ratio of ribosomes in the 5' UTR compared to the CDS. **Right:** The same mRNAs show no change in the ribosome occupancy ratio upon expression of DDX3Y compared to DDX3X. Effect size (Cliff's delta) between the "Not_significant" and "TE_{down}" groups is indicated.

Figure 3: A. Experimental schematic for *in vitro* translation after replacement of endogenous DDX3X with exogenous DDX3X or 3Y. **B.** Western blot for degradation of endogenous DDX3X tagged with mini-AID (mAID) and expression of exogenous FLAG-tagged DDX3X and DDX3Y in HCT-116 cells after degradation of endogenous DDX3X in *in vitro* translation extracts. **C.** Translation of *in vitro* transcribed reporter RNAs in control (DMSO) or DDX3X-degraded (auxin treated) HCT-116 cells. **D.** Translation of *in vitro* transcribed reporter RNAs in lysates from either FLAG-tagged DDX3X or DDX3Y on top of depletion of endogenous DDX3X. Translation of DDX3X sensitive RNAs show no change in reporter output in the presence of DDX3Y vs. DDX3X. **E.** Translation of *in vitro* transcribed Renilla luciferase reporter RNA fused to the ODC1 5' leader in lysates derived from HEK293T cells treated with and siRNA against DDX3X vs. control (top), or various inactivating DDX3X mutants compared to WT.

A.

Figure 4: A. Quantile-normalized expression of DDX3X and DDX3Y across a number of human tissues (data from GTEx). DDX3Y expression is particularly prominent in male-specific tissue types such as prostate and seminal vesicles, in the testes, and notably depleted in numerous tissues of the central nervous system.

Figure S1: Phylogenetic tree indicating distances between the sequences of DDX3X and DDX3Y in mammals (where sequence is available). Human DDX3X and DDX3Y are highlighted in yellow. The cluster of mammalian DDX3Y orthologs is indicated by red branches.

Figure S2: **A.** Differential expression analysis of RNA and ribosome profiling changes upon DDX3 degradation. Point size indicates differential translation **B.** Effects of transfection on ribosome occupancy and RNA levels in HCT116 cells. DDX3 paralogs and selected interferon response related genes are labelled. **C.** Principal component plots of **Left:** RNA levels and **Right:** ribosome occupancy in HCT116 cells transfected with DDX3 variants. The major source of variation is the transfection itself. **D.** GC content in the 5' UTR of the 100 genes with the greatest magnitude of increase (TE_{up}) or decrease (TE_{down}) in translation efficiency upon expression of DDX3Y vs. DDX3X or IAA vs. DMSO (without accounting for statistical significance). **E.** Effect size (Cliff's delta) between 5'UTR GC content of gene sets with indicated Ns selected as in (A). Number of genes indicated on the X-axis.

Figure S3: Scaled TPM expression of DDX3X and DDX3Y across a number of human tissues (data from GTEx).