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Translational compensation of gene copy number alterations by aneuploidy in *Drosophila melanogaster*

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ABSTRACT

Chromosomal or segmental aneuploidy—the gain or loss of whole or partial chromosomes—is typically deleterious for organisms, a hallmark of cancers, and only occasionally adaptive. To understand the cellular and organismal consequences of aneuploidy, it is important to determine how altered gene doses impact gene expression. Previous studies show that, for some *Drosophila* cell lines but not others, the dose effect of segmental aneuploidy can be moderately compensated at the mRNA level – aneuploid gene expression is shifted towards wild-type levels. Here, by analyzing genome-wide translation efficiency estimated with ribosome footprint data from the aneuploid *Drosophila* S2 cell line, we report that the dose effect of aneuploidy can be further compensated at the translational level. Intriguingly, we find no comparable translational compensation in the aneuploid Kc167 cell line. Comparing the properties of aneuploid genes from the two cell lines suggests that selective constraint on gene expression, but neither sequence features nor functions, may partly explain why the two cell lines differ in translational compensation. Our results, together with previous observations that compensation at the mRNA level also varies among *Drosophila* cell lines and yeast strains, suggest that dosage compensation of aneuploidy is not general but contingent on genotypic and/or developmental context.

INTRODUCTION

Whole-chromosome or large-segment aneuploidy—the gain and/or loss of whole or partial chromosomes—can severely impact cellular physiology, cause miscarriages and birth defects, and is a common hallmark of cancers (1,2). Occasionally, aneuploidy can facilitate adaptation. In yeast, for example, aneuploid changes improve response to stress (3) or resistance to drugs (4). In multicellular organisms, chromosome gain and loss has occurred many times as new sex chromosomes evolve (5). But how organisms tolerate such large-scale changes in karyotype is unclear. The main effect of aneuploidy is the presumed gross perturbation in gene expression that accompanies the altered gene doses (1,6). Investigating how gene expression is perturbed by chromosome-scale changes in gene copy number is the first step to understand the consequences of aneuploidy on cellular phenotype, organismal fitness and chromosomal evolution.

The effect of aneuploidy on gene expression has been studied at multiple levels. In *Drosophila melanogaster*, a triple-copy *Adh* gene, generated by gain of an extra copy of a quarter of chromosome arm *2L*, had mRNA expression levels and enzyme activity similar to that of a wild-type diploid (7) – the additional gene copy was compensated down. A genome-scale study of aneuploid strains in *D. melanogaster* found that segmental aneuploidy is compensated at the mRNA level (8). In the yeast, *Saccharomyces cerevisiae*, dosage compensation of aneuploidy is also observed, with ∼30% of the amplified genes in non-laboratory strains that harbor extra chromosomes being partially compensated at the mRNA level (9). Roughly 20% of amplified genes in laboratory yeast strains also show moderate compensation at the posttranslational (protein degradation) level (10). Together, these findings show that dosage compensation of aneuploidy can occur in multiple taxa (flies, yeast) and at multiple levels (mRNA, posttranslational).

The prevalence of dosage compensation of aneuploidy at the level of translation is relatively unexplored. A previous effort in yeast failed to find evidence for translational compensation (10), whereas limited translational compensation of aneuploidy occurs in some human neuroblastoma cells (11). Given that mRNA translation plays a critical role in the level and dynamics of protein abundance, and given the growing evidence for the dynamic regulation of translation during development (12–14), it seems worth investigating the impact of aneuploidy on translational dynamics in cells from a multicellular organism. In this study, we tackle this problem by analyzing data on genome-wide translation efficiency (TE) (15,16) (*i.e.* inferred protein production rate per mRNA) in two *D. melanogaster* cell lines with extensive segmental aneuploidy, S2 and Kc167 (17,18). To estimate genome-wide TE, we used data from the ribosome footprint

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technique (19,20). Briefly, the technique captures translating ribosomes in an mRNA population by isolating and sequencing ribosome-protected mRNA fragments (RPFs) when mRNAs are digested (21). TE is then estimated by dividing RPF abundance with mRNA abundance measured by standard RNA-seq (15,16) (see Materials and Methods for details). Using public ribosome footprint data, we compare TE between copy-number altered and normal genes in the two *Drosophila* aneuploid cell lines.

MATERIALS AND METHODS

Gene copy numbers in aneuploid S2 and Kc167 cell lines

The gene copy numbers of S2 cells were obtained from two sources (17,18). In one case, the copy numbers of genomic intervals were downloaded from Supplementary Table S1 of the reference (18), and the genes in each interval (based on the Flybase FB2014_03 annotation (22)) were assigned the corresponding copy number. In the other case, gene copy numbers were directly extracted from the additional file 4 of the reference (17). For Kc167 cells, the copy numbers were also obtained from the additional file 4 of the reference (17). Following reference (18), we focus on the gene groups with copy number equal to 3, 4 and 5 (or 1, 2 and 3 for X-linked genes in S2 cells) as the numbers of genes in these groups are much larger than other copy number groups, and these groups comprise most of the genes (∼96% for S2 and ∼95% for Kc167, respectively) in the genome.

Genome-wide translation efficiency in S2 and Kc167 cell lines and in wild-type embryos

The TE is estimated as the ribosome density per mRNA in its protein-coding region (15), which is calculated as the ratio of two quantities: the abundance of ribosomes on the mRNA population of a gene and the abundance of the mRNAs of that gene (15). The relative ribosome abundance on an mRNA population can be determined by sequencing RPFs using the ribosome footprint technique (21), and the mRNA abundance can be determined by standard RNA-seq; both are expressed as RPKM, i.e. the number of reads per kilobase of coding or exon regions per million aligned reads, to account for the factors of gene length and library sequencing depth. The RPF abundances for the S2 cell line and for 0–2 h embryos were extracted from the supplementary tables of reference (19). For the S2 cell line, we also estimated RPF abundance directly from sequence reads by using our own pipeline (see below) and reached the same result (Supplementary Figure S12) as that by using the supplementary table of reference (19). The RPF abundances for 0–6 h embryos were extracted from the supplementary tables of reference (12). The RPF abundance for Kc167 cell line was estimated from the short sequence reads from EBI ArrayExpress database (accession: E-MTAB-2421, published by Miettinen *et al.* (20)). We used only the sample processed with RNase I other than MN because the RNase I data give a higher correlation with that from S2 cells. The mRNA abundances for the S2 and Kc167 cell lines were estimated from sequence reads published by the references (18,19). The detailed sources for the RPF and mRNA abundance data are listed in Supplementary Table S1.

To convert sequence reads to RPF and mRNA abundances, we trimmed the sequence reads of 12-nt adaptor sequences from the 5′ end (for the Kc167 ribosome footprint data only) using a Perl script, filtered out low quality reads using the fastx_toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html), and mapped remaining reads to the *D. melanogaster* genome from the Flybase release FB2014_03 (22) using TopHat 2.1.0. The number of reads mapped onto each mRNA or CDS region was counted by using htseq-count (23). The final RPF and mRNA abundances were expressed as RPKM, and TE is the ratio of RPF and mRNA abundances. All the processed data are available in the Supplementary Data set S1.

To reduce sampling errors in calculating TE, we excluded lowly expressed genes as in (24). For the analyzed samples, we chose the following numbers of mRNA reads as cutoffs to filter genes, >30 for both S2 and Kc167 cell lines and >50 for 0–2 h embryo, which gave the best balance between reducing sampling errors and maintaining generality of data analysis according to our previous study (24). Note that the S2 cell lines for RPF abundance (19) and for gene copy numbers (18) may be different: the former was a line maintained at the University of California, San Francisco, USA (personal communication with Joshua Dunn, UCSF) and the latter is the S2-DRSC cell line. Using different cell lines makes our S2 cell analyses in Figure 1 more conservative due to introduced data noise.

Comparison of TE within each protein complex

To control for possible functional differences between aneuploid and normal genes, we compare the aneuploid and normal genes in each protein complex, presuming that the gene members in a protein complex have similar functions. To this end, we downloaded protein complex data from the supplementary tables of the reference (25), which detected protein complexes in *Drosophila* S2R+ cells using affinity purification followed by mass spectrometry analysis. We then looked for the protein complexes containing both aneuploid and normal genes and calculated two ratios for each protein complex: median TE of copy-number decreased genes to that of normal genes; and the median TE of copy-number increased genes to that of normal genes. For protein complexes containing only one category of copy-number altered genes (decreased or increased), only one ratio could be computed.

Translation-affecting sequence features

In our previous study (24), we summarized that the stability of mRNA secondary structure in 5′UTR, the lengths of 5′UTR, CDS and 3′UTR, codon usage and the proportion of positively charged amino acids in a CDS may affect translation initiation or elongation and in turn ribosome density. To determine if any of these factors is associated with the difference in ribosome density or TE between aneuploid and normal genes, we estimated these metrics in the following ways:

We downloaded protein-coding gene annotations and fasta-formatted sequences of *D. melanogaster* from FlyBase release FB2014_03 (22) and excluded genes that contain in-

ternal stop codons or whose CDS lengths are not a multiple of 3. For genes with multiple splicing isoforms, the isoform with the longest CDS was chosen. We then extracted the lengths of CDS, 5′UTR and 3′UTR from the information lines of the corresponding fasta files using a Perl script. To reduce annotation errors in our analyses, we excluded any CDS shorter than 90 nucleotides, 5′UTR shorter than 50 nucleotides and 3′UTR shorter than 50 nucleotides. The numbers of positively and negatively charged amino acids were counted by examining the protein encoded by each CDS using a Perl script.

To calculate the codon usage metric tAI (tRNA Adaptation Index), we downloaded the genomic copy numbers of tRNAs from GtRNAdb (http://gtrnadb.ucsc.edu/, last accessed May 15, 2015) (26) and used them as a proxy for tRNA abundances. With these tRNA copy numbers, we used the software Codon Usage Analyzer (CUA; Zhang Z, unpublished, http://dx.doi.org/10.1101/022814) to calculate tAI for each CDS obtained above.

To estimate mRNA stability, we used the folding free energy (FFE) of mRNA secondary structure in a 42-nt window in the 5′UTR, starting at 49 nucleotides before the start codon in each mRNA, because the FFE in this window is the best correlated with TE in S2 cells (24). The lower the FFE, the more stable the mRNA structure. The FFE of each window was computed using the program *hybrid-ss-min* in the software UNAFold 3.7 (27).

Functional analysis based on gene ontology

To test for any functional differences between the aneuploid genes in S2 and Kc167 cell lines, we used the online tool FatiGO++ (http://babelomics.bioinfo.cipf.es/) (28) to compare the functional distributions in gene ontology between the two cell lines within each of the following gene categories: X-linked copy-number-increased genes, X-linked copy-number-decreased genes, autosomal copy-number-increased genes and autosomal copy-number-decreased genes.

mRNA expression variation in Drosophila population

mRNA expression variation was estimated as the between-line expression variance (i.e. determined by genetic variation) among 185 Drosophila Genetics Reference Panel (DGRP) fly strains (29) and extracted from Supplementary Table S5 of reference (29).

Statistical analyses

All the statistical analyses and plots were made in R (30), and the script for each analysis is available upon request.

RESULTS

In aneuploid S2 cells, copy-number decreased and increased genes have higher and lower translation efficiencies than normal genes, respectively

The S2 cell line is originally derived from embryonic cells and widely used in the *Drosophila* community. On average, S2 cells are diploid for the X chromosome and tetraploid for each autosome (or simply 2X:4A) (18), representing a doubled male-like genome of *D. melanogaster*, with ≥40% of its genome being segmental aneuploid (17). Consequently, genes in S2 cells can be classified into three groups according to their aneuploid status: matching the 2X:4A average karyotype (i.e. two copies for X-linked genes and four copies for autosomal genes), copy-number decreased (CND) and copy-number increased (CNI). For simplicity, we hereafter refer to the first group as 'normal' genes (even though a wild-type male in *D. melanogaster* is 1X:2A) and the latter two groups collectively as aneuploid genes. We also separate X-linked and autosomal genes in all analyses as they systematically differ in translation efficiency in Drosophila (24).

One strategy to test for dosage compensation of aneuploid genes is to compare their expression levels to those of normal genes (17,18), assuming that normal genes are representative of the overall expression status of the aneuploid genes before the establishment of aneuploidy. Using this strategy, previous studies showed that the mRNA levels of aneuploid genes in S2 cells are closer to those of normal genes than expected from differences in gene dose (17,18), especially for CNI genes. Here, we confirm that mRNA expression is weakly compensated for the CNI genes (17); notably, the compensation seems restricted to autosomal genes only or is too weak to detect for X-linked genes (Supplementary Figure S1A). Next, using ribosome footprint data (19), we turn to dosage compensation at the level of translation. We find that CND and CNI genes have significantly higher and lower average TEs, respectively, than normal genes (Figure 1A; Wilcoxon rank sum test (WRST), $P_{\mathrm{WRST}} < 0.022$). These data show that TE is inversely correlated with the gene copy number, further dampening the effect of gene dose anomalies stemming from aneuploidy. This result is robust: the same pattern largely holds (i) for both the X chromosome and the autosomes (Figure 1A); (ii) when genes on each of the major chromosomal arms are examined separately (Supplementary Figure S2A and Supplementary Table S3; though the magnitudes and statistical significance vary among chromosomal arms); (iii) when using gene copy numbers estimated from a different study (17) (Supplementary Figure S3); (iv) when mutation-harboring genes are excluded from the analysis (Supplementary Figure S4); and (v) when genes encoding members of the same protein complex are compared (Supplementary Figure S5A and B; Supplementary Table S4 for complex counts). Note that the last result further suggests that the different TEs observed in Figure 1A are not due to functional differences among genes because genes in the same protein complex presumably serve the same function. These findings show that aneuploid gene doses are compensated at both mRNA and translation levels.

To characterize the combined effects of compensation at both the mRNA and translational levels for aneuploid genes, we examine the translation rate (i.e. inferred protein production rate per gene), calculated as ribosome density in the coding region of a gene (expressed as RPKM) (19) and equal to the product of TE and mRNA abundance. We find that the average translation rate per gene is the same among the CND, CNI and normal genes (Figure 2, $P_{\mathrm{WRST}} \geq 0.26$). Thus while mRNA abundances are incompletely

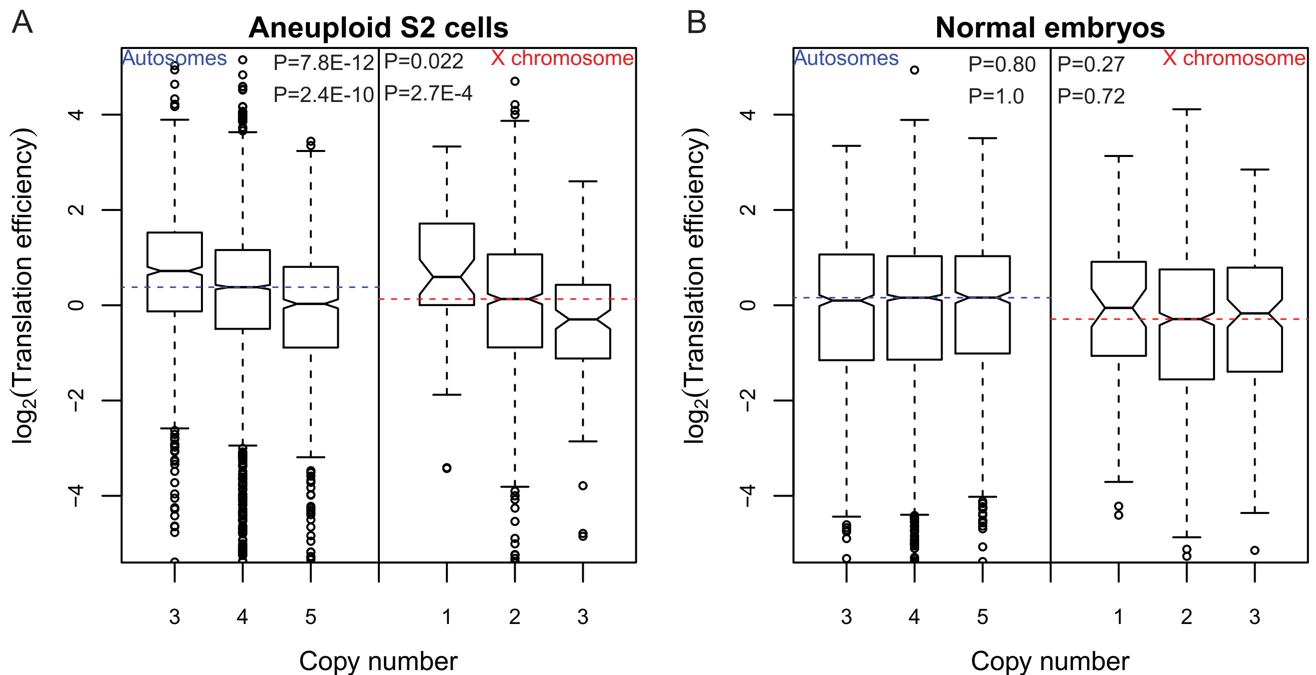

Figure 1. Aneuploid genes in the S2 cell line show altered translation efficiencies (TEs) (estimated as the ratio of ribosome-protected fragment (RPF) and mRNA abundances) that are inversely correlated to gene copy number (A). The same sets of genes do not show this pattern in wild-type 0–2 h embryos in which these genes have normal copy numbers (B). In the top of each panel, two *P*-values using Wilcoxon Rank Sum Test are given for the comparisons between copy-number decreased *versus* normal genes (e.g. 3-copy versus 4-copy for autosomal genes and 1-copy versus 2-copy for X-linked genes) and between copy-number increased versus normal genes (e.g. 5-copy versus 4-copy for autosomal genes and 3-copy versus 2-copy for X-linked genes), respectively. The dashed line in each panel marks the median value of the normal gene group.

compensated—they remain significantly different among the gene groups (Figure 2, $P_{\mathrm{WRST}} \leq 0.00057$)—the added contribution of translational compensation results in overall complete dosage compensation of aneuploid genes in S2 cells at the protein synthesis level. These results suggest that translational compensation plays an important role in dampening gene dose effects on gene expression, pulling the expression of aneuploid genes towards normal levels in S2 cells.

The different TEs are not inherent properties of aneuploid genes

The observation of higher and lower TEs for CND and CNI genes, respectively, is consistent with the hypothesis of dynamic translational compensation, but alternative hypotheses exist. Aneuploid genes might be inherently different from normal genes in translation, regardless of their aneuploid status. If, for example, CND and CNI genes have shorter and longer 5′UTRs, respectively, they would be expected to have higher and lower TEs, respectively (24). To test such possibilities, we first examined six sequence features known to be associated with mRNA translation (24) but found none that can explain the observed TE difference among gene groups (Supplementary Figure S6). We next performed a more direct test, comparing TEs in wild-type 0–2 h embryos among genes classified according to their aneuploid status in S2 cells. Again, we find no difference in TE among the gene groups (Figure 1B, $P_{\mathrm{WRST}} \geq 0.27$; Supplementary Figure S5C and D), except for the CND genes on chromosomal arm *3L* that show higher TE than normal genes (Supplementary Figure S2B). The same pattern is observed using the TE data from embryos representing different developmental stages (0–6 h) (Supplementary Figure S7, $P_{\mathrm{WRST}} \geq 0.12$). Overall, these findings suggest that no inherent differences in translation contribute to the difference between aneuploid and normal genes in S2 cells. It is therefore more likely that aneuploid genes experience dynamic translational compensation in S2 cells.

Aneuploid and normal genes do not differ in TE in Kc167 cells

To determine if dynamic translational compensation of aneuploidy is general, we applied the same analyses to recently available ribosome footprint data for the Kc167 cell line (see Materials and Methods). The Kc167 cell line is also derived from embryonic cells but has a karyotype 4A:4X, representing a tetraploid genome of a wild-type *D. melanogaster* female. Similar to S2 cells, Kc167 is also subject to extensive aneuploidy, and the aneuploid genes are moderately compensated at the mRNA level, especially for CND genes (17), which we confirm here for autosomal genes but not X-linked genes (Supplementary Figure S1B, $P_{\mathrm{WRST}} = 0.001$ and 0.23 for autosomal CND and CNI genes, respectively; $P_{\mathrm{WRST}} \geq 0.22$ for X-linked aneuploid genes). However, in contrast to S2 cells, we find no evidence for translational compensation in Kc167 cells: TEs are similar among the CND, CNI and normal genes (Figure 3; $P_{\mathrm{WRST}} \geq 0.10$). The same conclusion is reached when gene functions are controlled for by comparing genes encoding proteins that

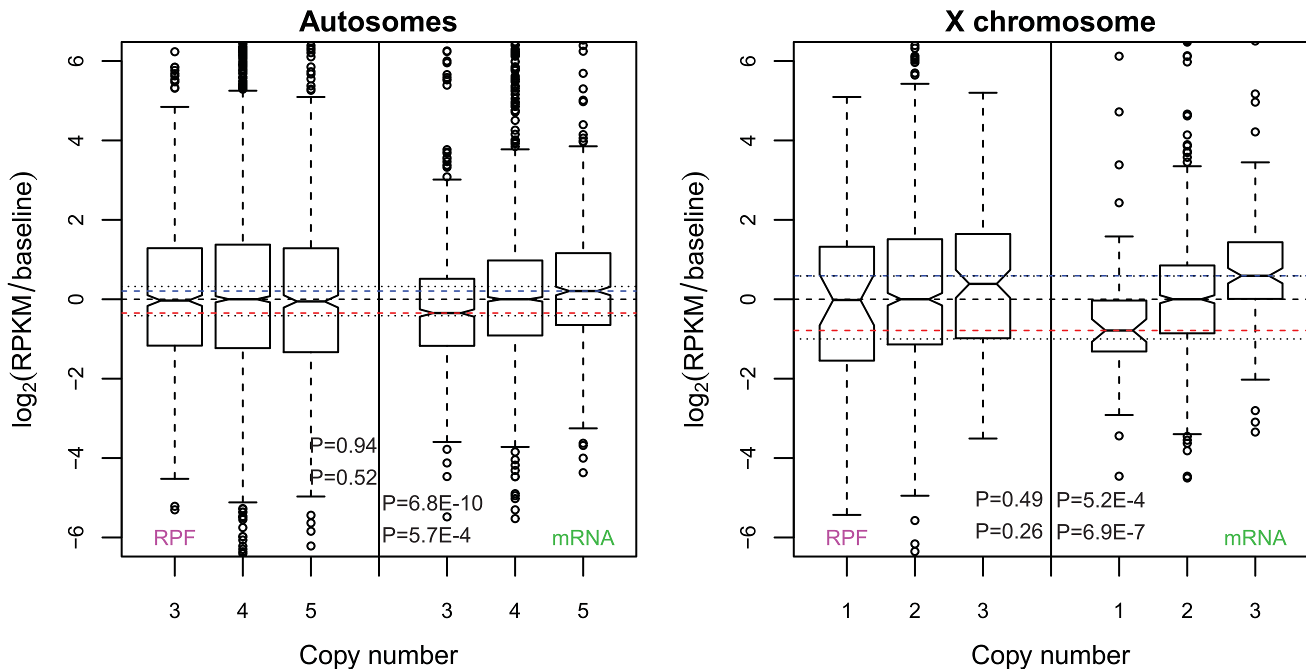

Figure 2. Combined compensation from both mRNA and translational levels leads to similar overall translation rates (protein production rate *per gene*) between aneuploid and normal genes. Translation rate is estimated by ribosome density, i.e. the RPKM of ribosome-protected fragments (RPFs), and equals the product of mRNA abundance and TE, and thus reflects the compensation at both mRNA and translation levels. For comparison, mRNA abundance (i.e. mRNA RPKM), which reflects only mRNA compensation, is also presented in the right panel of each plot. The two dotted lines mark the expected expression levels (scaled to gene doses) for low- and high-copy gene groups, respectively, when no compensations occur. The three dashed lines mark mRNA RPKM medians of the 3-, 4- and 5-copy (for X chromosome 1-, 2- and 3-copy) gene groups, respectively (note the X-chromosome 3-copy median line overlays the top dotted line, i.e. no mRNA compensation). RPF RPKM and mRNA RPKM are in different scales and thus divided by the respective medians of the 'baseline' normal groups (4-copy and 2-copy for autosomal and X-linked genes, respectively) to facilitate comparison of the combined and mRNA-level-only compensations. As in Figure 1, in each panel, two *P*-values using the Wilcoxon Rank Sum Test are given, comparing copy-number decreased and increased versus normal genes, respectively. RPKM = Reads Per Kilobase of coding or exon region per million aligned reads.

function within protein complex (Supplementary Figure S8, $P_{\mathrm{WRST}} > 0.125$).

We tested the possibility that translational compensation in Kc167 cells may be obscured by variation in basal TEs among gene groups. Specifically, if the genes experiencing aneuploidy in Kc167 cells have different basal TEs from normal genes, say, genes experiencing copy-number decrease and increase in Kc167 have lower and higher wild-type basal TEs, respectively, then after translational compensation in Kc167 cells, aneuploid genes might achieve TEs comparable to normal genes. We tested this hypothesis by comparing TEs in wild-type 0–2 h embryos for the same groups of genes assuming that the translation in embryos reflects basal rates. We find similar rates among the gene groups in embryos (Supplementary Figure S9, $P_{\mathrm{WRST}} \geq 0.32$). Furthermore, we find no evidence that the different gene groups differ in sequence features predicted to affect translation (Supplementary Figure S10), with two exceptions: the mRNA stability of 5′UTRs in autosomal CND genes is weaker and the 5′UTR length in autosomal CNI genes is longer than normal genes. These differences however predict higher and lower basal TEs for CND and CNI genes, respectively (24), contrary to the hypothesis that they might have lower and higher basal TEs, respectively.

The autosomal aneuploid genes in S2 and in Kc167 cells differ slightly in selective constraint on gene expression

We have confirmed that both S2 and Kc167 cell lines show moderate compensation at the mRNA level (Supplementary Figure S1) (17), but we find that only the S2 cells show dynamic translational compensation. We also find that sequence features that affect translation are unlikely to cause the difference, as aneuploid genes from the two cell lines are similar in sequence features relative to their respective normal genes (Supplementary Figure S6 and 10). Similarly, based on gene ontology, the gene functions of the aneuploid genes are similar between the two cell lines (Supplementary Table S2).

Given the degrees of compensations observed at the mRNA and translational levels in S2 and Kc167 cells (and assuming no posttranslational compensation), the ultimate protein abundance of aneuploid genes should be less compensated in Kc167 cells than in S2 cells. If so, one possibility is that the expression deviation of the aneuploid genes in Kc167 cells may happen to be less disruptive to cell function than the set of aneuploid genes in S2 cells. Genes with wide tolerances on variability in gene expression levels should correspond to those with relatively weaker selective constraints on mRNA abundance. We explored this possibility using mRNA expression data from the population of the DGRP (29). We assume that the selective constraint on mRNA abundance is positively correlated to the con-

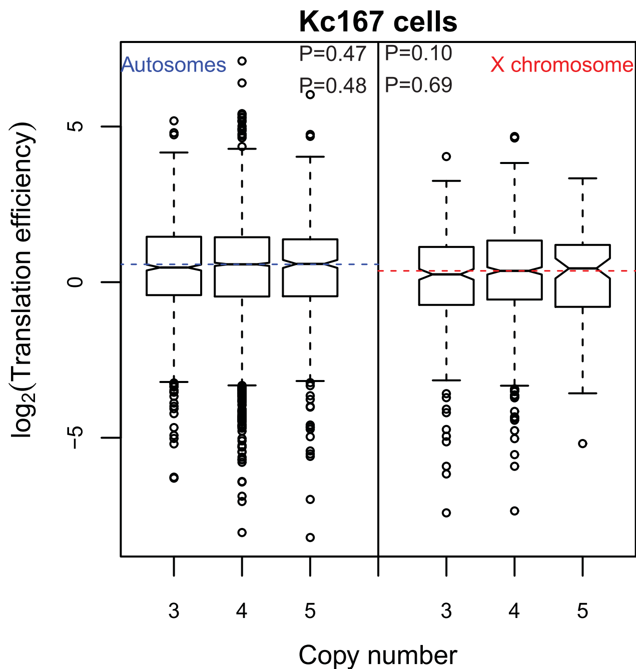

Figure 3. No difference in translation efficiency (TE) between aneuploid and normal (4-copy) genes in the Kc167 cell line. As in Figure 1, in each panel, two *P*-values using the Wilcoxon Rank Sum Test are given for comparing copy-number decreased and increased versus normal genes, respectively. The dashed line in each panel marks the median value of the normal group.

straint on mRNA translation. Then, as a surrogate for selective constraint on expression variation, we used the mRNA expression variation among the DGRP strains: the higher the variation, the weaker the selective constraint. We found that, in Kc167 cells, the autosomal aneuploid genes show slightly higher expression variation than the normal genes (Supplementary Figure S11A and S11B, $P_{WRST} = 0.025$ and 0.079 for male and female data, respectively), but X-linked genes do not ($P_{WRST} \geq 0.47$). In S2 cells, no significant difference is observed in any comparison (Supplementary Figure S11A and B, $P_{WRST} \geq 0.33$). The result suggests that the difference in selective constraint on gene expression could contribute to, but not explain, the difference in the translational compensation of aneuploidy between Kc167 and S2 cells.

DISCUSSION

Previous studies show that compensation of aneuploidy can occur at the mRNA (8,9,17,18) and at the posttranslational levels (10). Our results show that compensation of aneuploidy can also occur at the translational level. These observations together suggest that cells and organisms can employ different approaches to buffer the effects of imbalanced gene doses caused by aneuploidy. Our finding has two implications for understanding sex chromosome evolution and cancer progression. First, translational compensation may facilitate sex chromosome evolution by alleviating the reduced dosage caused by the degeneration of a sex chromosome in the heterogametic sex (e.g. degeneration of Y chromosome in males). Specifically, if in the earliest stage of sex chromosome evolution the mRNA level is not fully compensated (31), dynamic translational compensation may mitigate the reduced gene dose effect. Given that compensatory evolutionary changes between mRNA abundance and translation efficiency have been observed in yeast (32), translational compensation of the reduced gene dose during sex chromosome evolution seems possible. Second, cancer cells may exploit dynamic translational compensation to counteract aneuploidy, which could be crucial for a cancer cell's fate (33). Indeed, translational compensation of aneuploid genes has been found in some human neuroblastomas (11). In the future, it will be intriguing to see how common translational compensation is in different types of cancers and which genes tend to be compensated. The results may prove valuable in the development of new cancer therapies.

Although translational compensation can play an important role in cellular physiology, it does not seem to be general (present in S2 cells but not Kc167 cells). While S2 and Kc167 cells are both derived from Drosophila embryos, the genotypes, sexes and developmental stages from which they were derived differ. It is of course possible that sex or developmental stage differences contribute to the observed difference in translational compensation, but that determination cannot be made from such limited samples (a single cell line from each condition). We nevertheless considered other possibilities to understand why translational compensation is present in S2 but not Kc167 by comparing the properties of their respective aneuploid genes. The results suggest that selective constraint on gene expression, but neither gene functions nor sequence features, may partly contribute to the disparity. Similar inconsistency in the translation compensation occurs among human-derived neuroblastoma cell lines (11). Drosophila S2 cells, which have been propagated in labs for decades, and cells derived from high-risk neuroblastomas have experienced qualitatively similar histories of selection for proliferation. It will therefore be important to determine the extent to which translational compensation reflects an intrinsic property versus a secondary adaptation to enable cell proliferation. A similar inconsistency in aneuploid compensation extends to the mRNA level, with aneuploid compensation at the mRNA level varying among *Drosophila* cell lines (from none to strong compensation) (17) and between non-laboratory and laboratory yeast strains (the former, but not the latter, shows compensation) (9,10). More interestingly, even for the same gene, the different ways of increasing its copy number leads to different outcomes: increasing the copy number of *Adh* by duplicating ∼25% of chromosome arm *2L* (7), but not the whole chromosomal arm (34), leads to dosage compensation at the both mRNA and enzyme levels. Taken together, these results strongly suggest that compensation of aneuploidy varies among genotypes. With RNA-seq and translation profiling data from more tissues and genotypes than the two sampled here, it will become feasible to determine if, and to what extent, translational compensation depends on developmental or genetic context.

The molecular mechanism of dynamic translational compensation is unclear. Previous studies suggested that, at the mRNA level, autosomal dosage compensation can be achieved by two means: global factors that compensate all

aneuploid genes, such as the protein POF, which can compensate a whole 4th chromosome in *D. melanogaster* (8); or, more commonly, feedback regulation on a single-gene basis (8,35). At the translational level, compensation may also be achieved through mechanisms that are shared by all aneuploid genes and that vary among genes. Although global mRNA translation regulation is possible (12,36), the challenge is to understand how cells might sense and specifically regulate mRNAs from aneuploid genes. In contrast, the mechanisms of single-gene translational compensation likely vary among genes. For example, some genes may be through self-feedback regulation just as RPL32 can bind its own mRNAs to tune its translation in yeast (37). Revealing the mechanisms of translational compensation will of course clarify why compensation varies among cells and organisms.

In conclusion, our results suggest that aneuploidy can be compensated at the translational level, but the underlying mechanism and the reason for its disparity among cells need further exploration.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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