Aneuploidy, or an aberrant karyotype, results in developmental disabilities and has been implicated in tumorigenesis. However, the causes of aneuploidy-induced phenotypes and the consequences of aneuploidy on cell physiology remain poorly understood. We have performed a metaanalysis on gene expression data from aneuploid cells in diverse organisms, including yeast, plants, mice, and humans. We found highly related gene expression patterns that are conserved between species: genes that were involved in the response to stress were consistently upregulated, and genes associated with the cell cycle and cell proliferation were downregulated in aneuploid cells. Within species, different aneuploidies induced similar changes in gene expression, independent of the specific chromosomal aberrations. Taken together, our results demonstrate that aneuploidies of different chromosomes and in different organisms impact similar cellular pathways and cause a stereotypical antiproliferative response that must be overcome before transformation.

Results

Aneuploid Strains of Budding Yeast Share a Chromosome-Independent Stress Response. We previously reported that disomic yeast produced via chromosome transfer exhibit an ESR, in which genes related to RNA processing and the ribosome are downregulated, and genes involved in protein folding, detoxification of reactive oxidative species, and various other processes are upregulated (13). We sought to determine whether aneuploid cells generated in other ways also exhibited a stress and/or slow-growth response similar to that observed in the disomes. For all subsequent analyses, aneuploid chromosomes were excluded from consideration so as to avoid artifacts due to normalization. We first compared the disomes to 23 aneuploid strains generated during the construction of the yeast deletion collection (22). These aneuploidies include multiple chromosome gains and losses, and thus display more complex karyotypes than those present in the disomes. We found that the Pearson correlation coefficient (PCC) between the mean expression levels in these strains and in the disomic yeast was 0.37 (P < 10^-187), while the PCC between these strains and a wild-type strain was 0.02 (P > 0.05), which demonstrated highly significant transcriptional similarity between these aneuploid populations (Fig. 1A and B).

We hypothesized that the correlation between strains was due to a shared underlying stress or slow-growth response. To test this, we compared the aneuploid strains obtained from the deletion collection to gene expression data from three cdc mutant strains: cdc28-4 and cdc23-1, which divide slowly and exhibit a significant ESR at the permissive temperature, and cdc15-2, which proliferates at a wild-type rate at the permissive temperature and does not display an ESR (13). Both cdc28-4 and cdc23-1 exhibited significant correlations with aneuploid strains from the deletion collection (r = 0.30, P < 10^-114, and r = 0.31, P < 10^-116, respectively), while cdc15-2 was uncorrelated with the aneuploid strains (Fig. 1A and B; r = 0.02, P > 0.05). Next, we quantified the ESR (19, 20). No such common signature has been reported among aneuploid cells in any other organism. Additionally, it has been suggested (21) that the phenotypes detected in the disomic strains may be a unique consequence of the method that was used to generate aneuploidy, in which genetic markers were used to select for rare chromosome transfer events between nuclei (13). To further our understanding of aneuploidy, we examined gene expression data from aneuploid cells from diverse organisms. We detected a conserved transcriptional response that was associated with stress and decreased cell proliferation that was apparent in aneuploid yeast, plants, mice, and humans. These data suggest that aneuploidy in various species is detrimental to cell fitness, and that many consequences of aneuploidy are a common response to chromosomewide dosage imbalances.
the intensity of the stress response in each disomic strain by averaging the expression levels of genes annotated to the ESR (Fig. S1). We found that 15 out of 16 disomes exhibited significant pairwise correlations with the average expression level in the aneuploid strains obtained from the deletion collection, and the strength of the correlation increased with the intensity of the stress response in the disomes (Figs. S2A and S2B; \(r = 0.64, P < 0.01\)). Disomes that did not display an ESR (e.g., disome I) exhibited minimal correlations with the deletion collection aneuploid strains, and disomes that displayed significant ESRs (e.g., disome IV) tended to exhibit stronger correlations. These results are consistent with our hypothesis that a shared transcriptional stress response underlies the similarity between different aneuploid populations.

We note, however, that the various deleted genes within the deletion collection are likely to be at least partially responsible for the stress phenotype. Two observations suggest that aneuploidy is also a relevant cause of the similarity with the disomic transcriptomes. First, among individual deletion strains, there was a significant positive correlation between the percent of the genome that was aneuploid and the PCC with the disomes (Fig. S3A; \(r = 0.54, P < 0.01\)). Second, we found that the stress response gained intensity as the number of genes on aneuploid chromosomes increased (Fig. S3B; \(r = 0.49, P < 0.05\)). This relationship was true for both aneuploid strains from the deletion collection and disomic strains that we constructed (\(r = 0.58, P < 0.0005\)). Thus, it is likely that aneuploidy contributes to the similarities in gene expression between these sets of strains.

We next sought to identify aneuploidy-responsive genes in yeast. We sorted the disomes and aneuploid deletion strains according to the number of genes present on aneuploid chromosomes, and then calculated correlation coefficients between the expression levels of each gene and the degree of aneuploidy across the panel of strains. There were 446 genes identified whose expression levels were significantly correlated or anticorrelated with the degree of aneuploidy across all strains (Fig. S4; PCC > 0.5 or PCC < −0.5; \(P < 0.002\)). Among transcripts that were positively correlated with increasing aneuploidy, Gene Ontology (GO) term analysis revealed an enrichment of genes related to oxidative stress (\(P < 10^{-10}\)) and protein refolding (Table S1; \(P < 10^{-8}\)). Transcripts that decreased with increasing aneuploidy were enriched for non-coding RNA processing (\(P < 10^{-11}\)) and ribosome biogenesis genes (\(P < 10^{-8}\)). Importantly, there was highly significant overlap among aneuploidy-responsive genes and the ESR (\(P < 10^{-13}\), hyper-geometric test). Furthermore, among the 446 genes, 414 of them exhibited a codirectional change in the slow-growing cdc mutants. Discordant transcripts (i.e., those that increased with aneuploidy but were expressed at less than wild-type levels in the cdc strains, and vice versa) were not significantly enriched for any GO terms.

Taken together, these data indicate that most (but not all) transcriptional changes caused by aneuploidy are related to stress and/or slow growth, and that increasing degrees of aneuploidy generally exert increasing degrees of stress on cell homeostasis.

Next, we obtained gene expression data from seven aneuploid strains that were derived via triploid meiosis (21). The average correlation between these strains and the disomes and five out of seven pairwise correlations with the disomes were highly significant (Fig. 1A and Fig. S3C). There was a mild anticorrelation between the aneuploid products of triploid meiosis and cdc15−2 (\(r = −0.05, P < 0.001\)), but a significant positive correlation with the ESR-exhibiting mutants cdc28−4 and cdc23−1 (\(r = 0.21, P < 10^{-5}\), and \(r = 0.30, P < 10^{-13}\), respectively). Among the five aneuploid strains that were correlated with the disomes, four showed a significant stress/slow growth response relative to a euploid strain (Fig. S3D). We conclude that a shared transcriptional response is a common although not obligate consequence of aneuploidy in yeast, and this response is independent of the mechanism by which aneuploidy is generated.

Aneuploidy Causes a Stress Response in Fission Yeast. We next sought to determine whether aneuploidy causes a stress response in other organisms. We averaged gene expression data from two aneuploid strains of the fission yeast Schizosaccharomyces pombe, then identified upregulated and downregulated genes using a ±1.3-fold change (FC) cutoff (23). GO term analysis of upregulated genes revealed that the most enriched functional category was the response to stress (Table S2; \(P < 10^{-25}\)). Downregulated genes included many terms associated with the ribosome, including ribosome biogenesis (\(P < 10^{-14}\)) and the nucleolus (\(P < 10^{-15}\)). Similar GO term enrichments were obtained using rank products, a cutoff-independent method of identifying differentially expressed genes [Table S3 (24)]. We noted that these GO terms are a hallmark of the budding yeast ESR, suggesting that aneuploidy in different yeasts causes a similar stress-related transcriptional response. Indeed, an environmental stress response has also been described in S. pombe (25), and out of 236 genes that constitute the fission yeast ESR, 203 genes exhibited codirectional transcriptional changes in the aneuploid S. pombe (Fig. S5).

To determine whether aneuploidy caused genomewide similarities in gene expression in different species, we identified one-to-one orthologs between Saccharomyces cerevisiae and S. pombe and then calculated the PCC between the averaged aneuploid strains in each organism. The correlation coefficient between disomic budding and fission yeast strains was highly significant (Fig. 2A; \(r = 0.30, P < 10^{-47}\)). In addition, there was a weak correlation between aneuploid fission yeast and cdc15−2 (\(r = 0.04, P < 0.05\), but stronger correlations with cdc28−4 and cdc23−1 (\(r = 0.22, P < 10^{-29}\), and \(r = 0.31, P < 10^{-52}\)). We found that 14 out of 16
individual disomes also exhibited significant pairwise correlations with S. pombe, and these transcriptional similarities were particularly striking when genes annotated to GO terms affected by aneuploidy were compared (Fig. 2B and Fig. S2C). Moreover, the PCC between individual disomes and S. pombe tended to increase based on the intensity of the stress response in each disomic strain ($r = 0.60$, $P < 0.02$; Fig. S2D). Last, we sought to determine whether specific groups of genes exhibited coordinate changes in expression in both species. Orthologous genes that were upregulated in both organisms were significantly enriched for those involved in the response to oxidative stress ($P < 10^{-14}$) and the response to heat ($P < 10^{-14}$), and downregulated genes were enriched for ribosome biogenesis factors ($P < 10^{-14}$) and those associated with the nucleolus (Table S4; $P < 10^{-14}$). We conclude that aneuploidy in different fungal species induces a highly related stress response.

Aneuploidy Causes a Stress Response in Arabidopsis thaliana. Based on the conserved transcriptional response to aneuploidy among different fungi, we hypothesized that aneuploidy in higher organisms could also result in a stress/decreased proliferation response similar to that seen in aneuploid yeast. To test this, we analyzed gene expression data from Arabidopsis thaliana plants that were trisomic for chromosome 5 (26). GO term enrichment analysis revealed that many of the same pathways were perturbed by aneuploidy in plants as in yeast (Tables S5 and S6). “Response to chemical stimulus” and “response to stress” were among the most upregulated GO terms ($P < 10^{-12}$ and $P < 10^{-10}$, respectively), and the cytosolic ribosome and ribosome biogenesis were highly enriched among downregulated genes ($P < 10^{-11}$ and $P < 10^{-8}$, respectively). Furthermore, we identified one-to-one orthologs between budding yeast and A. thaliana, and found that trisomic plants and disomic yeast exhibited a significant genomewide transcriptional correlation (Fig. 2A; $r = 0.26$, $P < 10^{-15}$). There was no correlation between trisomic plants and cdc15-2 ($r = 0.02$, $P > 0.05$), but significant correlations with cdc28-4 and cdc23-1 ($r = 0.17$, $P < 0.002$ and $r = 0.20$, $P < 0.0002$, respectively) as well as 11 out of 16 individual disomes (Fig. 2C and Fig. S2E). As with S. pombe, the correlation coefficient between the yeast disomes and trisomic A. thaliana increased with the intensity of the stress response in the budding yeast strains (Fig. S2F; $r = 0.69$, $P < 0.003$). We conclude that a shared stress response underlies significant transcriptional similarity between aneuploid budding yeast and A. thaliana.

Aneuploid Mouse and Human Cells Share Slow Growth-Related Changes in Gene Expression. We next analyzed expression data from mouse embryonic fibroblasts trisomic for one of four chromosomes (chromosome 1, 13, 16, and 19) that were normalized to MEFs obtained from their euploid littermates (15). We first sought to determine whether different trisomies caused similar changes in gene expression. We found highly significant overlap among differentially expressed genes across the trisomies: in 12 out of 12 pairwise comparisons, a gene that was up- or downregulated in one trisomic cell line was significantly more likely to exhibit a similar change in expression in a different trisomy (Fig. 3A and B). For instance, ~6% of all genes on euploid chromosomes in trisomy 16 were upregulated at a 1.5-FC cutoff, but among genes that were upregulated in trisomy 19, 20% were also upregulated in trisomy 16 ($P < 10^{-25}$, hypergeometric test). Significant similarities were also observed when differentially expressed genes were identified using $t$-tests or more stringent FC cutoffs (Fig. S6A–D). To determine whether the same genes were affected across the trisomic cell lines, we applied a permutation test, in which gene expression values were randomized within each trisomy. Although 78 genes were upregulated and 168 genes were downregulated in three or more trisomies, no more than 37 and 92 genes were up- or downregulated, respectively, among 100,000 random permutations of the expression data (Fig. S7A and B). GO terms enriched among upregulated genes were highly variable and reflected perturbations in many aspects of cell physiology (Tables S7 and S8). Of note, we observed that many upregulated terms were related to stress and inflammation, including the response to wounding ($P < 10^{-13}$), the acute inflammatory response ($P < 10^{-10}$), and the response to stress ($P < 10^{-14}$). The most enriched GO term among upregulated genes was the extracellular region ($P < 10^{-15}$), which reflected increased transcript levels of cytokines as well as various matrix-related genes. Downregulated GO terms were more specific: the most downregulated term was cell division ($P < 10^{-14}$), and nearly all affected GO terms were directly related to progression through the cell cycle, including mitosis ($P < 10^{-13}$), DNA replication ($P < 10^{-14}$), and chromosome condensation ($P < 10^{-13}$). This is consistent with our previous finding that trisomic MEFs exhibit poor proliferative capacity relative to euploid cells (15). We conclude that trisomic MEFs display some chromosome-independent transcriptional similarities that are indicative of slow growth and cellular stress.

Does a chromosome-independent response to aneuploidy exist in humans as well? To test this, we examined gene expression data from four datasets that included trisomy 13, 18, and 21. Within each sample, we asked whether genes that are upregulated or downregulated in one trisomy were more likely to be up- or downregulated in another trisomy. In eight out of eight pairwise comparisons, the overlap between different trisomies was significantly more than expected by chance (Fig. 3C and D and Fig. S6E–H). To determine whether similar genes were affected across datasets, we performed a permutation test. Although 94 and 137 genes were up- or downregulated, respectively, in four or more trisomic samples, no more than 59 and 49 genes were up- or downregulated, respectively, among 100,000 random permutations of the expression data (Fig. S7 C and D). Surprisingly, dysregulated genes in human trisomies were enriched for many of the same GO terms as were found in

![Fig. 2. Aneuploidy causes a stress response in S. pombe and A. thaliana. (A) The correlation coefficients between aneuploid strains of either S. pombe (black bars) or A. thaliana (white bars) and the indicated strains of S. cerevisiae are displayed. (B and C) Heat maps of orthologous genes annotated to aneuploidy-related GO terms from aneuploid strains of (B) S. pombe or (C) A. thaliana and the indicated disomes are displayed.](https://www.pnas.org/doi/10.1073/pnas.1209227109)
CFC 21 samples displayed enrichments of stress-related and cell-cycle-related GO terms, and those GO terms were not enriched among differentially expressed genes when only data from fetal cerebra and amniotic fluid were considered (Table S13). Natural limits on cell division that exist in utero may partially mask the different proliferative capacities of trisomic and euploid cells, while unconstrained growth in culture highlights this disparity. For this reason, we used gene expression data from cultured trisomic human cells for subsequent comparisons.

The similarity between enriched GO terms in trisomic human and mouse cells suggested that aneuploidy causes a conserved transcriptional response across mammals. To test this, we identified one-to-one orthologs between humans and mice, then calculated the correlation between the average gene expression values from trisomic MEFS and cultured trisomic human cells. The PCC across all genes was moderate but highly significant \( r = 0.11, P < 10^{-20} \). In addition, we noted significant overlap between the sets of differentially expressed genes in trisomic mouse and human cells \( P < 10^{-17} \), hypergeometric test), which was particularly evident among cell-cycle transcripts (Table S14). Thus, aneuploidy induces a similar gene expression pattern indicative of slow growth and/or cellular stress in both mouse and human cells.

### Stress-Related Transcriptional Similarities across all Aneuploid Cell Types

The common stress response in aneuploid cells of highly divergent species raised the possibility that yeast and mammalian cells share a transcriptional response to aneuploidy. To test this, we identified one-to-one orthologs between yeast, plants, mice, and humans. We found that disomic yeast exhibited a small but statistically significant correlation with the averaged expression values of trisomic MEFS and of cultured trisomic human cells (Fig. 4A; \( r = 0.12, P < 10^{-5} \), and \( r = 0.10, P < 0.002 \), respectively). The significance of these correlations was also confirmed by permutation testing (Fig. S7 E–H). A majority of individual disomes also exhibited significant pairwise correlations with the trisomic mammalian cells (Fig. S8C). Furthermore, we observed significant correlations between the aneuploid strains of *S. pombe* and *A. thaliana* and the trisomic MEFS, as well as between *A. thaliana* and the trisomic human cells (Fig. 4A).

We hypothesized that the similarities in gene expression between the aneuploid transcriptomes were a consequence of the slow growth/stress response that was common among aneuploid cells of all species. Consistent with this hypothesis, there was a significant positive correlation between the stress-response intensities in each disome and their PCC with the trisomic mammalian cells (Fig. 4B). Moreover, *cdc28-4* and *cdc23-1* exhibited significant positive correlations with both trisomic mammalian cell types, and *cdc15-2* did not (Fig. 4A). Taken together, these findings suggested that conserved aspects of the transcriptional response to stress and/or slow growth, rather than aneuploidy per se, drives the expression correlations between aneuploid cell types. To test this, we compared gene expression data from aneuploid cells to chemostat-grown yeast disomes. In chemostats, the doubling times between disomic and euploid cells were equalized by nutrient titration, thereby masking the slow growth/stress response. Indeed, for each interspecies comparison, aneuploid cells were anticorrelated with chemostat-grown disomes (Fig. 4C). Next, we examined the set of yeast genes whose expression levels directly vary according to the rate of cell division (19). In batch culture, the correlation coefficients between the disomes and other species were significantly increased in three out of four comparisons when only orthologs of the 500 strongest growth-responsive genes in yeast were compared. These results demonstrate that conserved elements of a transcriptional stress or slow growth program in eukaryotes underlie the significant transcriptional similarities between aneuploid yeast, plant, and mammalian cells. The lesser predictive value of the growth-responsive yeast genes in comparisons with mammalian cells likely reflects the fact that ribosome synthesis is strongly downregulated by slow proliferation in yeast, while the most striking transcriptional changes among aneuploid mamm-
Fig. 4. Transcriptional similarities among all aneuploid cell types. (A) Correlation coefficients between the indicated cell type and either trisomic MEFs (black bars) or cultured trisomic human cells (white bars) are displayed. Asterisks indicate a statistically significant correlation \( (P < 0.05) \). (B) The stress-response intensity of the disomic strains is plotted against the pairwise correlations with the trisomic mouse and human cells. The black line is a linear regression plotted against the data excluding disome IV (SRI–1). (C) Correlation coefficients between the indicated aneuploid cell types and chemostat-grown disomes (black bars), batch-grown disomes (white bars), and 500 growth-responsive genes in batch-grown disomes (gray bars) are displayed.

Aneuploid cells were the downregulation of cell cycle transcripts (Tables S14 and S15 and Fig. S8).

A second prediction derived from these results is that aneuploid cells should exhibit similar gene expression changes to euploid cells experiencing exogenous stresses. Aneuploidy in budding and fission yeast affects known stress-response genes in those organisms (Figs. S1 and S5); therefore we examined stress-induced transcription in \( \text{Arabidopsis} \) and mammalian cells. We analyzed gene expression data from \( \text{Arabidopsis} \) grown under salt, reactive oxygen species (ROS), or drought stress (27), and from MEFs treated with salt, the protein synthesis inhibitor ansomycin, or TNF-\( \alpha \) (28). The transcriptionomes of stressed plants and MEFs were significantly correlated with the corresponding trisomic tissue (Fig. S9; \( r = 0.40, P < 10^{-300} \), and \( r = 0.11, P < 10^{-15} \), respectively). Furthermore, the PCC values were significantly higher when we examined only genes that changed codirectionally by a certain threshold under all stress conditions. As expected, many GO terms that were enriched among upregulated genes in both stressed and trisomic tissue were stress related, including the response to chemical stimulus \( (P < 10^{-20}) \) and the defense response \( (P < 10^{-14}) \) in \( \text{Arabidopsis} \) and the inflammatory response \( (P < 10^{-9}) \) and cytokine activity in MEFs (Tables S16 and S17; \( P < 10^{-4} \)).

Last, we sought to identify conserved changes in expression in response to aneuploidy among single-ortholog genes across species. We used relatively permissive criteria, and asked which genes changed codirectionally in aneuploid budding yeast, fission yeast, mouse, and human cells. We found that 254 single-ortholog genes changed codirectionally in all four species (240 down and 14 up), significantly more than expected by chance \( (P < 10^{-5}, \text{and} \ P < 0.007, \text{respectively, Fig. S7I and J}) \). The most affected GO terms among those genes were the nucleus \( (P < 10^{-14}) \) and nucleic acid metabolism (Table S18; \( P < 10^{-14} \)). These terms reflected the downregulation of ribosomal genes (primarily in fungi) and cell-cycle-related genes (primarily in mammalian cells; Fig. S8 and Table S18). Out of the 254 genes that exhibited codirectional changes across species, 230 of the genes exhibited a similar change in the yeast \( \text{cdc} \) mutants. Among discordant genes, no significant GO term enrichments were observed. We conclude that aneuploidy in different cell types induces a conserved transcriptional program that is also elicited by exogenous stress and/or slow growth.

Discussion

We have identified a stress/slow-growth-related transcriptional signature that is present in aneuploid cells of diverse organisms and is largely independent of the identity of the extra chromosome(s). Previous analyses have described an oxidative stress response and the downregulation of proliferation-related genes in human samples and mouse models of Down syndrome (29–32). The data presented here suggest that these phenotypes and others found in aneuploid cells may be a common consequence of aneuploidy, as eukaryotic cells appear to exhibit a stereotypical transcriptional response to chromosomewide gene dosage changes.

Why is aneuploidy associated with a stress response? First, aneuploidy increases a cell’s energy needs. This may result from the wasteful transcription, translation, and degradation of proteins encoded by extra chromosomes, and is evidenced by the decreased efficiency at which aneuploid cells convert nutrients into biomass (13, 15). Altered metabolism may also increase the production of reactive oxygen species (33), and ROS-related GO terms were commonly upregulated in aneuploid cells. Second, production of certain proteins may saturate key chaperones, prohibiting them from folding client proteins whose functions are required for viability. Proteins that escape proper folding or degradation may also form cytotoxic aggregates (34, 35). Last, although many aneuploidy-induced phenotypes appear to be independent of the identity of the extra chromosome, copy number changes of a few particularly dosage-sensitive genes may have direct consequences. For instance, budding yeast cells are exquisitely sensitive to tubulin levels, and a single extra copy of tubulin causes the lethality of disome VI (13, 36, 37). We posit that these factors contribute to limit the proliferative capacity of aneuploid cells, thereby resulting in the common downregulation of cell cycle and ribosomal genes.

It is interesting to note that euploid yeast strains that displayed a basal stress response \( (\text{cdc}23-1 \text{ and } \text{cdc}28-4) \) exhibited significant correlations with aneuploid cells in every organism, and the intensity of the ESR in disomic yeast predicted the strength of their transcriptional similarity with aneuploid cells in other organisms. The ESR was first described as a common transcriptional signature in yeast cells treated with multiple independent stresses, although later research demonstrated that it could also result from a slowed rate of cell division (18–20). Whether the ESR-like transcriptional changes observed in aneuploid higher eukaryotes result from stress, or whether they are also a byproduct of differences in growth rate, remains to be tested.
Not every aneuploid strain that we examined displayed a significant stress response. In many cases, this can be explained by threshold effects of dosage imbalance, as the degree of aneuploidy is proportional to the intensity of the transcriptional response (Fig. S1 B). Still, some outliers fail to follow this overall trend: although chromosome XII contains the third most ORFs of any yeast chromosome, disome XII displays the third lowest stress response (28:167–175).

Among higher eukaryotes, the shared transcriptional response of single-chromosome aneuploidy causes a transcriptional response indicative of increased cellular stress and decreased proliferative capacity. These results present aneuploidy as a complex phenomenon with potentially antitumorigenic properties. Although aneuploidy can contribute to transformation by altering the dosage of oncogenes and tumor suppressors, the stresses induced by aneuploidy on metabolism and protein folding limit growth potential. Yet, if cells adapt to aneuploidy by developing mutations, which improve their proliferative capacity (14), and it may be the case that cancer cells must develop similar genetic changes that allow them to tolerate aneuploidy and acquire robust proliferative capacity as well.

Materials and Methods

RNA collection, hybridization, and analysis of strains derived via triploid meiosis were performed as previously described (13). Other datasets were acquired from the Gene Expression Omnibus or were downloaded from the relevant publication. Further experimental details are described in SI Materials and Methods.

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Supporting Information

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SI Materials and Methods

**Yeast Expression Data.** Gene expression data from diploid and *cdc* mutant yeast strains were downloaded from (1). These data include gene expression values from yeast grown in batch culture, in which cells were allowed to reach logarithmic growth. Under these conditions, the yeast disomes display an ESR. This dataset also includes expression values from yeast grown under phosphate limitation in chemostats. Under this growth regime, the doubling time between euploid and disomic strains was equalized, thereby masking the ESR.

Gene expression data for strains derived via triploid meiosis were acquired as previously described and were deposited in the Gene Expression Omnibus (accession number GSE35853) (1). In strain A15, we detected extra copies of chromosome X (2), which were not present in the published karyotype. Gene expression data from 23 aneuploid strains from the deletion collection were downloaded from [http://hugheslab.ccb.rutgers.edu/](http://hugheslab.ccb.rutgers.edu/supplementary-data/ii). A few of these strains are believed to have developed aneuploidies because the deletion directly affects the spindle assembly checkpoint or chromosome segregation. Other strains gained chromosomes that carry a paralog of the deleted gene. However, in the majority of cases, the causes of aneuploidy in these strains are unknown (3). The set of genes that constitute the budding yeast ESR were downloaded from [http://genome-www.stanford.edu/yeast_stress/](http://genome-www.stanford.edu/yeast_stress/). Growth rate-responsive genes in yeast were acquired from (4).

For analyses involving *S. cerevisiae*, ORFs annotated as “dubious” and ORFs that were detected in fewer than 10 disomic strains were removed from consideration. In addition, YAR015W and YBR115C were excluded, as they were the sites of marker integration in the euploid strain; and YDR342C and YDR343C were excluded, as they were amplified at the DNA level in aneuploid strains (1). Three wild-type vs. wild-type replicates were used as a control. The percent of the genome that was aneuploid was calculated as the sum of the number of ORFs present on a chromosome that was gained or lost, divided by the total number of ORFs in a euploid cell of that ploidy. The intensity of the stress response in yeast was calculated as follows: for all genes *x* that are unregulated in the ESR and all genes *y* that are downregulated in the ESR, the stress-response intensity (SRI) of a given strain was calculated as:

\[
SRI = \frac{\sum_{i=1}^{m} y_i + \sum_{i=1}^{n} -y_i}{m + n}
\]

As the expression levels of most ESR genes change in response to growth rate, stress response intensities are not bimodal, but instead vary along a continuum. Strains were considered to exhibit an ESR if their SRI were positive and their SRI significantly differed (*P* < 10\(^{-5}\), Student *t* test) from that of an isogenic wild-type strain.

Gene expression data from aneuploid strains of fission yeast were downloaded from GEO (accession number GSE8782). Strain C16, which contained one additional copy of 163 ORFs, and strain Ch16+528, which contained two additional copies of the same 163 ORFs and one additional copy of 63 ORFs, were used for analysis. As these two strains contain very similar duplicated regions, they were averaged for subsequent comparisons. *S. pombe* genes involved in the fission yeast ESR were downloaded from [http://www.bahlerlab.info/projects/stress/](http://www.bahlerlab.info/projects/stress/).

**A. thaliana Expression Data.** Gene expression data for plants trisomic for chromosome 5 were downloaded from [http://bioinf.boku.ac.at/pub/trisomy2008/](http://bioinf.boku.ac.at/pub/trisomy2008/). Log2-FC values were calculated between aneuploid and euploid samples for all genes not on chromosome 5. Gene expression data from stressed plants were downloaded from [http://www.weigelworld.org/resources/microarray/AtGenExpress](http://www.weigelworld.org/resources/microarray/AtGenExpress). Log2-FC values were calculated between treated and mock-treated samples at the 24 h time point.

**Mammalian Expression Data.** Gene expression data from trisomic mouse cell lines were downloaded from (5). Probes were updated using Release 32 of the NetAffx probe annotations, and the set of probes classified as “expressed” were used in this study. Gene expression data from stressed mouse cells were downloaded from GEO (accession number GSE18320). Gene expression data from human aneuploidies were downloaded from GEO (accession numbers GSE6283, GSE1397, GSE16176, and GSE25634). For all mammalian aneuploidies, nonspecific probe sets and probe sets mapping to the X or Y chromosome were excluded from consideration. Probe sets that mapped to the same gene were collapsed by averaging. Within each dataset, log2-FC values were calculated between aneuploid and euploid samples.

**Data Analysis.** Gene expression data were analyzed in Excel, MATLAB, and Python using custom scripts. For all correlative studies, only gene expression values from euploid autosomes were considered, and multiple replicates of individual strains were averaged for comparisons. Aneuploid chromosomes were excluded because of the possibility that dosage compensation mechanisms decreased the transcription of a select number of genes present on extra chromosomes, which would introduce biases into subsequent analyses. Correlation values reported in the text represent the Pearson coefficient between two samples. Every comparison found to be significant via the Pearson method was also found to be significant when the Spearman coefficient was calculated instead.

GO term enrichment analysis was performed using GPprofiler with a Benjamini–Hochberg-corrected *P* value of 0.05 and a maximum *P* value of 10\(^{-3}\) (6). Enrichments were performed against the relevant background gene set, e.g., against all genes not on an aneuploid chromosome or against the set of one-to-one orthologs between species. Consistent with our previous methodology (1, 7), differentially expressed genes were identified using a ±1.3-FC cutoff in yeast and plants. We used a ±1.5-FC cutoff in multiple samples for mammalian expression data, a similarly low-stringency threshold that allowed us to detect expression changes in datasets that contained variable numbers of replicates. As a secondary method, we used the rank products algorithm, an FC cutoff-independent protocol that is particularly useful in identifying differentially expressed genes in datasets with small sample sizes (8–10). The rank products algorithm was implemented in TM4 using a *P* < 0.05 significance threshold for yeast and plant data and a *P* < 0.005 threshold for mammalian data (11). Values reported in the text are from GO term analysis using an FC cutoff.

For budding yeast, mice, and humans, orthologous genes were identified using GPprofiler (6). For *Arabidopsis* and fission yeast, orthologous genes were identified using InParanoid (12). All interspecies comparisons were between genes in which a one-to-one orthology relationship existed. Clustering was performed in Gene Cluster 3.0 (13) and visualized in Java TreeView (14). For clustering and visualization, the expression values of genes present on aneuploid chromosomes were replaced by the average value of that gene on euploid chromosomes in other strains.
Permutation tests to confirm the significance of transcriptional similarities were performed in Python. For each strain or species, blank cells were fixed in place. Blank cells usually resulted from the exclusion of an aneuploid chromosome, and these cells were locked so as to keep the number of relevant comparisons constant. Next, nonempty cells were randomly shuffled, and after each shuffle the relevant parameter was scored. \( P \) values reported for permutation tests represent maximum probabilities based on the number of permutations performed.

Fig. S1. The ESR in disomic strains of *S. cerevisiae*. (A) Strains were sorted according to their stress response intensity and genes annotated to the ESR were clustered. Genes that constitute the *S. cerevisiae* ESR were downloaded from [http://genome-www.stanford.edu/yeast_stress](http://genome-www.stanford.edu/yeast_stress). The stress-response intensity in each disome was calculated by averaging the expression levels of genes upregulated in the ESR and then subtracting the average expression level of genes downregulated in the ESR. (B) The stress-response intensities of each disomic and *cdc* mutant strain are displayed.
Fig. S2. Stress-related transcriptional similarities between aneuploid budding yeast, fission yeast, plants, and mammalian cells. The correlation coefficients between each disomic strain and (A) aneuploid strains from the yeast deletion collection, (C) aneuploid strains of *S. pombe*, (E) trisomic *A. thaliana*, and (G) and trisomic mammalian cells are displayed. The stress-response intensity of each disomic strain is plotted against its correlation coefficient with (B) aneuploid strains from the yeast deletion collection, (D) aneuploid strains of *S. pombe*, and (F) trisomic *A. thaliana*. We found that disomic strains that did not exhibit a stress response (e.g., disome I and XII) exhibited small or insignificant correlations with aneuploid cells in other species, while disomic strains that exhibited strong stress responses (e.g., disome IV and disome XIII) tended to exhibit high interspecies correlations. This is consistent with our hypothesis that stress-related transcription underlies the significant genomewide similarities observed between aneuploid cells in different species. A linear regression excluding disome IV (SRI∼1) is plotted against the data in each graph.
Fig. S3. Effects of aneuploidy in deletion collection strains and triploid meiotic products. (A) The correlation coefficients between individual aneuploid deletion collection strains and the disomes were plotted against the percent of the genome that was aneuploid in each strain (see Materials and Methods). A linear regression is plotted against the data. (B) The stress response intensities of disomic strains and aneuploid deletion collection strains were plotted against the percent of the genome that was aneuploid in each strain. Red and blue lines represent linear regressions plotted against the data. (C) The correlation coefficients between the disomic strains and aneuploid products of triploid meiosis were plotted. Karyotypes of these strains are: A2: 1N+II, XII; A3: 1N+I,II,XII; A9: 1N+II,XIII,XVI; A10: 1N+III,XII,XVI; A13: 1N+I,II,VII,XIII; A14: 1N+IX,XVI; and A15: 1N+II,III,VII,IX,X,XI,XII. (D) The stress-response intensities of aneuploid products of triploid meiosis were calculated. An asterisk indicates a statistically significant increase in the stress response relative to wild-type (P < 10^-5, Student t test).
Fig. 54. Aneuploidy-responsive genes in *S. cerevisiae*. Yeast disomes and aneuploid strains from the deletion collection were sorted according to the number of genes present on aneuploid chromosomes in each strain, normalized to the baseline ploidy of each strain. Then, a correlation coefficient was calculated between the degree of aneuploidy (measured as the percent change in gene content) and the transcript level of each gene. A cutoff PCC value of ±0.5, corresponding to a *P* value less than 0.002, was used to identify aneuploidy-responsive genes. GO term enrichment analysis of these gene sets are presented in Table 51, and examples of the different gene classes identified are displayed here. (A) The expression of *HSP104* (Left) and *ATG8* (Right) tend to increase as the degree of aneuploidy increases. (B) The expression of *RPL18B* (Left) and *RRP9* (Right) tends to decrease as the degree of aneuploidy increases. (C) The expression of *MTW1* (Left) and *BDH1* (Right) are not significantly affected by increasing degrees of aneuploidy.
Fig. S5. Aneuploid strains of *S. pombe* display a stress response. Genes that constitute the *S. pombe* ESR were downloaded from http://www.bahlerlab.info/projects/stress/ and the expression values of these genes in the two aneuploid strains were clustered. Of 132 genes that are upregulated in the ESR, 106 are upregulated when the aneuploid strains are averaged. Of 104 genes that are downregulated in the ESR, 97 are downregulated when the aneuploid strains are averaged.

Fig. S6. Expression similarity in trisomic MEFs and human cells. (A and B) Genes up- or downregulated in one trisomic MEF line are significantly more likely to exhibit a similar change in another trisomy. Gray bars indicate the percentage of all genes up- or downregulated at a 2-FC cutoff in the indicated cell line. White bars indicate the percentage of genes up- or downregulated in that trisomy that are also up- or downregulated in the trisomy represented with a gray bar. (C and D) Analysis as above, but differentially expressed genes were identified among replicates of Ts13 and Ts16 using a 1.5-FC and *P* < 0.05 (Student *t* test) cutoff. Note that only single replicates of Ts1 and Ts19 were analyzed in (S), precluding the use of a *t* test to analyze these cell lines. (E and F) Analysis as above using data from human trisomies. Differentially expressed genes were identified using a 2-FC cutoff. (G and H) Analysis as above, but differentially expressed genes were identified using a 1.5-FC and *P* < 0.05 (Student *t* test) cutoff. Asterisks indicate statistical significance (*P* < 0.05, hypergeometric test).
Fig. S7. Permutation testing of similarity significance. (A–D) To confirm that the number of similarly up- or downregulated genes was significant across mammalian aneuploidies, we randomized gene expression values and then calculated the number of similarly affected genes that occurred by chance. (A and B) 100,000 random permutations of gene expression data from trisomic MEFs. Bar graphs indicate the number of genes that are (A) upregulated or (B) downregulated in three or more cell lines. (C and D) 100,000 random permutations of gene expression data from human trisomies. Bar graphs indicate the number of genes that are (C) upregulated or (D) downregulated in four or more sample types. (E–H) To confirm the significance of Pearson correlation comparisons between mammalian cells and other eukaryotes, we randomized gene expression values and then calculated the PCC for each randomized sample. (E and F) 10,000 random permutations of disomic yeast gene expression values compared with (E) mouse and (F) human values. (G and H) 10,000 random permutations of disomic yeast gene expression values compared with (G) mouse and (H) human values. (I and J) 10,000 random permutations of disomic yeast gene expression values compared with (I) mouse and (J) human values. (G and H) 10,000 random permutations of disomic yeast gene expression values compared with (G) mouse and (H) human values. (I and J) 10,000 random permutations of disomic yeast gene expression values compared with (I) mouse and (J) human values.
random permutations of wild-type yeast gene expression values compared with (G) mouse and (H) human values. (I and J) To confirm the significance of the codirectional changes among 1:1 orthologs observed in aneuploid cells, we randomized gene expression values and calculated the number of co-directional changes observed in each randomized sample. The number of (I) upregulated and (J) downregulated genes observed among 100,000 random permutation are displayed.

Fig. S8. Cellular processes perturbed across species by stress and aneuploidy. Scatter plots of genes annotated to the indicated GO terms comparing expression levels in either (A and B) aneuploid budding and fission yeast or (C and D) trisomic mouse and human cells. Although the average transcripts in each category are expressed at levels less than euploid levels, cell-cycle transcripts (e.g., nuclear division genes) are expressed at lower levels in mammalian cells, and ribosomal and translational transcripts (e.g., ribosome biogenesis genes) are expressed at lower levels in fungi. Additionally, ribosome biogenesis transcript levels in trisomic MEFs and human cells are uncorrelated with one another, while nuclear division genes [and cell cycle genes generally (Table S14)] show a strong correlation between species. (E and F) Heat maps of single-ortholog genes in aneuploid budding yeast, fission yeast, mouse, and human cells. Genes that are downregulated by aneuploidy are also down-regulated in ESR-exhibiting yeast cdc mutants (the average of cdc28-4 and cdc23-1, Bottom).

Fig. S9. Similarities between stressed and aneuploid A. thaliana and MEFs. (A and B) The Pearson correlation coefficient between (A) trisomic and stressed A. thaliana and (B) trisomic and stressed MEFs are displayed. When stress-response genes were defined in these species by examining only those genes that changed ± a certain threshold under all stress conditions, the correlation coefficients significantly increased (P < 0.05).

Other Supporting Information Files

Tables S1-S18 (XLSX)