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Peer Review

Evaluation of Chen et al.: Overexpression of Protein Complexes and Aneuploidy

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One snapshot of the peer review process for "Overdosage of Balanced Protein Complexes Reduces Proliferation Rate in Aneuploid Cells" (Chen et al., 2019).

*Editor's Note: This is a first-round review of "*Overdosage of Balanced Protein Complexes Reduces Proliferation Rate in Aneuploid Cells*" by Ying Chen, Siyu Chen, Ke Li, Yuliang Zhang, Xiahe Huang, Ting Li, Shaohuan Wu, Yingchun Wang, Lucas B. Carey, and Wenfeng Qian. It was written for* Cell Systems *as part of the peer review process. We chose to feature it because it is a good example of providing a thorough critique while remaining constructive, with the goal of improving the paper.*

After the first round of review, Chen et al. (2019*, this issue of* Cell Systems*) was revised to take the reviewers' comments into account. The paper was then re-submitted, re-reviewed, accepted for publication, and now published in this issue of* Cell Systems*. For comparison, an earlier version of Chen et al. was deposited on bioRxiv ahead of review and can be found here:* https://doi.org/10.1101/376988*. Dr. Angelika Amon blinded her identity during the peer review process but has chosen to reveal it here. Chen et al. support the publication of this Peer Review; their permission to use it was obtained after their paper was officially accepted. This Peer Review was not itself peer reviewed. It has been lightly edited for stylistic polish and clarity. Figure callouts refer to the figures in the original submission. No scientific content has been substantively altered.*

In this manuscript, Chen et al. (2019) investigate the causes of fitness defects in aneuploid yeast strains and human cancers. Previous work had suggested that imbalances in protein complexes are a significant cause of fitness defects in aneuploid cells. The classic example studied in this context is alpha and beta tubulin. A single extra copy of the beta tubulin gene is nearly lethal. Introduction of an extra copy of the alpha tubulin gene suppresses this growth defect (Katz et al., 1990). A second hypothesis—not mutually exclusive with the balance hypothesis—suggested that synthesizing excess proteins and their degradation confers a fitness defect. The classic example supporting this hypothesis was the observation that mild overexpression of a misfolded URA3 or YFP protein causes fitness defects (Geiler-Samerotte et al., 2011). The authors here propose a different hypothesis, arguing quite strongly that neither protein complex imbalances nor increased protein synthesis and/or degradation burden confer fitness defects, but rather, overexpression of protein complexes does.

The authors start out by generating complex aneuploid yeast strains by sporulating pentaploid cells. Unfortunately, the authors chose non-isogenic parents to generate this pentaploid strain—which, in this reviewer's opinion, makes the data difficult, if not impossible, to interpret (see "experimental concerns", point 1). Also, at least some of the strains appear to be unstable, which complicates things even further (see "experimental concerns", point 2). The authors then correlate the proliferation rate of these strains with the number of imbalanced protein complexes or protein complexes where the entire complex is in excess. This analysis revealed that the proliferation defect of aneuploid strains is negatively correlated with the number of excess complete complexes rather than number of imbalanced protein complexes. The authors conclude that overexpression of protein complexes is responsible for the proliferation defects of aneuploid strains and term this discovery the "overdosage hypothesis."

The authors then set out to test their hypothesis by manipulating the dosage of three complexes. The problem is that the complexes they chose are known to affect proliferation. They are cell cycle control and transcription factor complexes that are known to be especially dosage sensitive. Not surprisingly, they get the answer they want. Overexpression of the entire complexes is more deleterious than expressing individual subunits of the complexes. It should be noted that individual subunits of these protein complexes are stable when overexpressed and hence unlikely to be subject to degradation. So, the analysis of these complexes excludes neither the balance nor excess burden hypothesis. It should also be noted that there are concerns with how these strains were constructed (see "experimental concerns", point 4). Additional controls are also missing. Why were the excess burden and balance hypotheses not tested in these strains? For example, the consequences of deleting excess ribosomal subunits to eliminate the excess burden hypothesis should be examined. The effects of expressing excess components of stable complexes that are known to have dominant negative effects when in excess should also be analyzed.

The authors then show that protein complexes that when overexpressed are enriched in cell cycle regulators. This makes sense. Cell-cycle regulators are well established to be dosage sensitive and their levels directly affect proliferation rate.

The paper ends with an analysis of the TCGA dataset and arrives at the conclusion that—in cancer, too—genes encoding complexes that inhibit cell cycle progression are underrepresented. This conclusion is sort of trivial. Of course,

cell-cycle inhibitory complexes will be underrepresented in cancer. I further suspect that the correlation the authors observe is driven by the p53 pathway. This reviewer also notes that another study of cancer genomes led to the conclusion that aneuploidies arise to rectify complex imbalances in cancer (see "other concerns", point 3).

In summary, (1) there are significant experimental concerns, and (2) the data are overinterpreted and prior literature is ignored. The notion that overexpression of protein complexes is the major driver of fitness defects in aneuploid cells is not supported by the data. It is based on correlations and the analysis of three complexes that are known to affect fitness of cells when overexpressed. Furthermore, there is ample evidence in the literature that stoichiometric imbalance and increased burden on protein synthesis and degradation pathways affect the fitness of aneuploid cells. Arguing that their contribution is minimal or non-existing is inappropriate.

Experimental concerns:

1) Strain construction: The strains used to construct aneuploid strains are not isogenic. It is therefore impossible to know whether the growth defects that are measured are due to aneuploidies or hybrid incompatibilities. This is truly a confounding problem that I do not know how to possibly address other than repeating the entire analysis with isogenic strains.
2) Stability of aneuploid strains: Many complex aneuploid strains are quite unstable. This is especially true in long-term growth assays such as growth competition experiments. The fact that at least some of the strains studied here are unstable is evident in Figure 1C (i.e., strains 2D and 3B).
3) Doubling time measurements: Normalizing doubling times of individual strains to the average proliferation of all aneuploid strains is not appropriate. This will greatly skew the data, especially because the spread of growth rates varies so greatly. Also, how were spores treated that did not grow at all? What growth rate value was given to them? Surely, it is inappropriate to exclude these strains from the calculations.
4) Doubling time measurements: The authors state that the data were further normalized to the fastest growing aneuploid strain. Why was this done? Doubling times of aneuploid strains should be compared to the euploid parents.
5) To test the overdosage hypothesis, the authors delete individual genes in aneuploid strains. No controls are shown that the strains still harbor the original karyotype following transformation, a process that could potentially cause changes in karyotype.

Other concerns:

1) The authors very strongly argue that dosage imbalances of protein complexes do not contribute to fitness defects of aneuploid strains, but rather, overexpression of entire complexes is the main determinant of fitness. How can the authors so categorically exclude the dosage imbalance hypothesis when they only analyze viable strains? The prime example supporting the balance hypothesis—the alpha-beta tubulin heterodimer—will not even grow up in the experimental set up chosen by the authors.
2) Correlation between overexpressed protein complexes and G1 delay: This reviewer does not know a single protein complex that, when overexpressed, delays cells in G1 in yeast. None of the protein complexes listed in Tables S3 and S4 cause a G1 delay when overexpressed, either. So clearly, events other than overexpressing cell cycle regulators are responsible for the G1 delay that is observed.
3) Using elegant statistical approaches, Ozery-Flato et al. (2011) showed that chromosome pairs are commonly gained or lost in cancer, indicating that aneuploid cancer cells employ chromosome gain or loss events to restore a balance in their altered protein ratios. This work is not even mentioned in the present manuscript and is directly at odds with the conclusions drawn here.
4) Figure 2F: The correlation between excess complexes and growth defects seems to be entirely driven by three or four outliers.
5) Figure 5B: What is the proliferation rate for strains in which the tubulins are unbalanced?

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