Keeping the Proportions of Protein Complex Components in Check

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How do cells maintain relative proportions of protein complex components? Advances in quantitative, genome-wide measurements have begun to shed light onto the roles of protein synthesis and degradation in establishing the precise proportions in living cells: on the one hand, ribosome profiling studies indicate that proteins are already produced in the correct relative proportions. On the other hand, proteomic studies found that many complexes contain subunits that are made in excess and subsequently degraded. Here, we discuss these seemingly contradictory findings, emerging principles, and remaining open questions. We conclude that establishing precise protein levels involves both coordinated synthesis and post-translational fine-tuning via protein degradation.

Introduction

Multiprotein complexes consist of different proteins that assemble with defined stoichiometries (Marsh and Teichmann, 2015). Too few of any one subunit limits the number of assembled complexes that can satisfy biological functions. Conversely, excess subunits outside their designated complexes are often nonfunctional and may have adverse effects (Goldberg, 2003; Juszkiewicz and Hegde, 2018). Understanding whether and how cells maintain precise proportions of individual complex components can offer fundamental insights into protein homeostasis, cellular economy, and consequences of regulatory perturbations.

In general, the steady-state abundance of proteins depends on both their synthesis and degradation rates (Box 1). Independent evidence from global quantification of both protein production and decay using ribosome profiling and metabolic pulse labeling experiments has culminated in a conserved principle that the proportion of complex components is indeed carefully maintained. On one hand, analysis of protein synthesis by ribosome profiling (see Brar and Weissman [2015] for a review of the methodology) showed broad concordance between subunit synthesis rates and their stoichiometry in the complex (Taggart and Li, 2018). On the other hand, metabolic labeling coupled to mass spectrometry (see Grandi and Bantscheff [2019] for an in depth discussion on the experimental approach) revealed that some subunits are produced in excess and subsequently removed by degradation (McShane et al., 2016). Although both studies point to the importance of keeping the proportions of protein complex components in check, they may be perceived to paint different views on how this control is achieved, i.e., whether it is at the level of production or decay (Figure 1). To resolve the apparent contradiction, we briefly review published evidence for proportional synthesis and post-translational buffering, take a close look at both ribosome profiling and proteomic data, and integrate both types of measurements toward a unified view that both coordinated production and

post-translational degradation are required to keep the proportions of protein complex components in check. We end by highlighting remaining open questions.

Evidence for Proportional Synthesis

Until recently, it has been difficult to quantitatively assay synthesis rates across the proteome, and as such, our understanding of such rates has been limited to a handful of individual cases. Well-characterized complexes demonstrate both proportional synthesis, as in the case of tubulin and globulin (Cleveland et al., 1981; Lodish, 1974), and imbalanced synthesis with active degradation, as seen for spectrin (Lehnert and Lodish, 1988). In the last decade; however, ribosome profiling has emerged as a powerful tool to measure synthesis globally by capturing the density of ribosomes on each mRNA (Ingolia et al., 2009; Li, 2015). This approach has revealed remarkable concordance between subunit stoichiometry and relative synthesis rate for nearly all *E. coli* protein complexes (Li et al., 2014). Although different subunits of the same complex are often co-transcribed from the same operon, additional transcriptional and post-transcriptional controls are put in place to precisely tune the relative synthesis rates of these subunits (Lalanne et al., 2018). This observation suggests that in prokaryotes, protein synthesis and degradation rates are tuned such that proportional synthesis is the dominant mechanism by which balanced protein stoichiometry is maintained.

Accurate quantification of proportional synthesis in eukaryotes is more difficult because of the increased abundance of paralogous genes and relative paucity of comprehensive biochemical characterization, but it is possible with protein complex curation and careful data analysis (Table 1) (Taggart and Li, 2018). Rigorous definition of the set of protein complex subunits is critical because any subunit that is transiently associated, not strictly required for complex function, or possesses complex-independent activity is not necessarily expected to be stoichiometrically abundant. In

> **Box 1. Dynamic Range of Protein Synthesis and Decay Rates**
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> To understand how cells control protein abundance, it is important to first consider the dynamic ranges of protein synthesis and decay that can be achieved physiologically. Most proteins in exponentially dividing tissue culture cells have half-lives that are considerably longer than cell doubling times (Schwanhäusser et al., 2011). The effective decay rates of protein concentrations are thus mainly determined by their dilution half-life, which equals the cell doubling time. In this regime, the protein steady-state levels are primarily controlled by the rates of protein synthesis, that is, the rate of protein synthesis integrated over the cell-cycle time is the steady-state level. In most organisms, synthesis rates as set collectively by transcription, mRNA stability, and translation span at least 100,000-fold across different proteins, with the upper bounds estimated to be 10^5 molecules per 20 min in bacteria and 10^7 molecules per 24 h in human cells (Ingolia et al., 2011; Schwanhäusser et al., 2011).
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> For proteins degraded within a cell cycle, the steady-state levels depend on both the rates of synthesis and degradation. If there is a single rate-limiting step for degradation, the decay kinetics is expected to follow a single exponential decay, and the steady-state level is simply the ratio of synthesis and decay rates. Because the effective decay rate is bounded by the dilution rate, the dynamic range of decay rate is limited to 1,000-fold for most cultured human cells (a few minutes to 24 h) and even lower for rapidly growing bacterial cells (a minute to 20 min). In this regime, there exist distinct ways to maintain two subunits at equal abundances. One approach is to have already matched synthesis rates followed by equal decay, i.e. "proportional synthesis." It is also possible to choose from a large number of combinations of synthesis and decay rates as long as they have the same ratio.
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> Beyond the simplest approximation, protein abundance can also be maintained by surveillance systems that selectively remove unassembled subunits. Such active feedback can lead to two decay paths specifically for the subunits that have higher production rates compared with their binding partners—a rapid pathway for the fraction made in excess and a canonical one for the fraction that assemble into complexes, i.e. "non-exponential decay." In this case, protein production does not have to be precise. Overall, understanding which strategy is followed by most protein complex components is critical to understand cellular economy and the consequences of dysregulation.

the best-characterized budding yeast, improved bioinformatic analysis of ribosome profiling data revealed that the majority of complex members are synthesized at rates that typically differ by no more than 1.29-fold (spread of synthesis rates within the 25–75th percentile of subunits) after correcting for stoichiometry. The distribution of mRNA levels for these complex subunits, measured by RNA sequencing, is similarly proportional to protein synthesis rates, with only marginal improvement from translational efficiency. This agrees with a narrow range of translational efficiencies in yeast (Weinberg et al., 2016) and is in contrast to the broader range needed to achieve proportional synthesis in bacteria (Li et al., 2014). A small number of exceptions to the rule may be influenced by factors such as protein complex localization, as the synthesis of mitochondrial complex components adhere less strictly to their stoichiometry on average. These synthesis rates are not generally subject to feedback regulation, as haploid yeast strains with a duplication of one chromosome show proportional increases in mRNA abundance and protein synthesis (Dephoure et al., 2014; Taggart and Li, 2018).

In primary cells from higher eukaryotes, quantification of protein components of the ribosome and proteasome suggest that they are synthesized at similarly close rates: The spread of synthesis rates within the 25–75th percentile for members of the human ribosome, 20S proteasome, and 19S proteasome are 1.57-fold, 1.42-fold, and 1.32-fold, respectively, even though these distributions may be artificially inflated by sub-stoichiometric incorporation of some nonessential subunits (Dahlmann et al., 2000; Emmott et al., 2019). Taken together, these data suggest that despite fundamental differences in the regulation of protein production and the vast space of biologically achievable protein synthesis and degradation rates, both prokaryotic and eukaryotic systems have evolved near-stoichiometric production of most protein complexes (Figure 1A).

Evidence for Post-translational Buffering

A long-standing concept is that proteins are stabilized by complex formation, whereas individual subunits that fail to assemble into the complexes (so-called "orphans") are degraded (Goldberg and Dice, 1974). This concept has garnered substantial support during the last decade (see review by Juszkiewicz and Hegde [2018]). For example, yeast and human cells carrying an extra copy of an individual chromosome typically show a proportional increase of corresponding mRNA levels. However, they usually do not accumulate free unassembled protein complex subunits that are encoded on the additional chromosomes. Instead, the surplus subunits are either degraded or form cellular aggregates (Brennan et al., 2019; Chen et al., 2019; Dephoure et al., 2014). Hence, while protein synthesis is not stoichiometric in aneuploid cells, the ratios of many subunits are restored post-translationally via degradation of orphaned subunits. Similarly, proteogenomic studies of cancer tissues show that a large fraction of gene-copy number variations are attenuated at the protein level (Geiger et al., 2010; Sousa et al., 2019).

Several lines of evidence indicate that protein degradation is also critical for complex stoichiometry in unmanipulated cells. Historically, most proteins have been assumed to be degraded by exponential kinetics (Schimke and Doyle, 1970). According to this view, young and old proteins have the same decay probability, and steady-state protein levels would simply reflect the ratio of synthesis and exponential decay rates (Box 1). However, a recent study found that >10% of proteins in near-diploid human cell lines show non-exponential degradation profiles (McShane et al., 2016). Specifically, the newly synthesized proteins were found to be less stable than their older counterparts. Most of these non-exponentially degraded proteins are subunits of protein complexes, and they tend to be produced in super-stoichiometric quantities. Hence, the emerging picture is that cells do overproduce specific subunits. For these
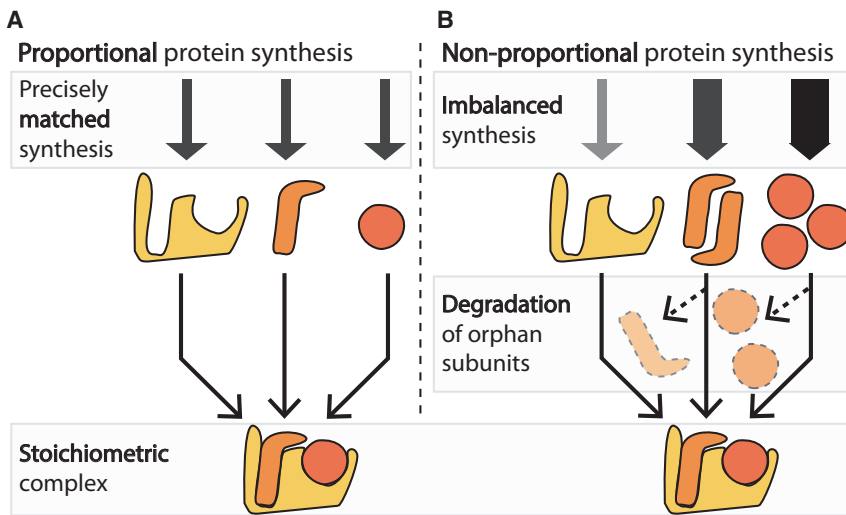
A

Proportional protein synthesis

Precisely **matched** synthesis

Stoichiometric complex

B

Non-proportional protein synthesis

Imbalanced synthesis

Degradation of orphan subunits

Figure 1. Two Models of How the Cell Produces Stoichiometric Protein Complexes
(A) In the proportional synthesis model, only the number of subunits that are required for the final complex are made. For the illustrated complex with a 1:1:1 stoichiometry, equimolar amounts of all subunits are produced.
(B) In a non-proportional synthesis model, more molecules of certain subunits are produced relative to a rate-limiting subunit. The surplus "orphaned" subunits that are not incorporated into the fully assembled complex are degraded. Both models result in the same steady-state proportion of subunits as the stoichiometry of the complex.

subunits, only a fraction of the newly synthesized protein molecules is subsequently stabilized by complex formation, whereas the rest is degraded. It therefore appears that non-proportional protein synthesis and subsequent post-translational buffering is common in unperturbed mammalian cell lines (Figure 1B).

Additional evidence for this model comes from the observation that protein degradation is typically faster in the subcellular compartment where newly synthesized subunits assemble into their respective holoenzymes, such that RNA polymerase subunits are more stable in the nucleus (where the complex is active) than in the cytosol (where the subunits are made and assembled) (Boisvert et al., 2012). Furthermore, proteogenomic analysis of outbred mice and normal human tissues revealed widespread stoichiometric buffering at the protein level (Chick et al., 2016; Sousa et al., 2019). Consequently, the spurious transcriptional coregulation of spatially close but functionally unrelated genes is buffered at the protein level (Kustatscher et al., 2017; Wang et al., 2017). Therefore, subunits of the same complex show better correlation at the protein than at the mRNA level (Kustatscher et al., 2019; Wang et al., 2017). It is worth noting that most studies reporting buffering at the protein level do not distinguish between regulation during and after synthesis. However, at least in yeast, very few protein complex subunits are dosage-compensated at the level of synthesis (Taggart and Li, 2018). Finally, a number of studies in different model systems concluded that 6%–30% of newly synthesized proteins are degraded within minutes of synthesis (Schubert et al., 2000; Vabulas and Hartl, 2005; Wang et al., 2013; Wheatley et al., 1980).

While the data above shows genome-wide trends, we now turn to the ribosome as a particularly well-studied example of imperfect stoichiometry. Microscopic and proteomic studies revealed that some ribosomal subunits are constitutively made in excess and degraded in several mammalian cell lines (Andersen et al., 2005; Lam et al., 2007; McShane et al., 2016). Hence, it appears that ribosomes are made by producing excess amounts of individual subunits (relative to other subunits and/or rRNAs), followed by the continuous degradation of unassembled molecules. Recently, a mechanism for selective degrada-

tion of overproduced ribosomal proteins has been uncovered: In yeast, excess subunits are tagged by the E3-ubiquitin ligase Tom1 for degradation by the ubiquitin proteasome system (Sung et al., 2016). In the absence of Tom1, free ribosomal subunits form protein aggregates, indicating that efficient clearing of orphaned subunits is vital. In humans, both the Tom1 homolog HUWE1 and the E3 ubiquitin ligase UBE2O have been shown to be involved in detecting orphaned ribosomal subunits and targeting them for degradation (Nguyen et al., 2017; Sung et al., 2016; Yanagitani et al., 2017). Recognition appears to be mediated by motifs in ribosomal proteins that are otherwise buried in assembled ribosomes, which explains the specific degradation of free subunits (Shemorry et al., 2013; Sung et al., 2016; Yanagitani et al., 2017).

In summary, eukaryotic cells possess post-translational buffering mechanisms to remove orphan subunits. A fraction of complex subunits is constitutively made in excess and subsequently degraded, suggesting that protein production is not always perfectly stoichiometric. The molecular details of this post-translational buffering mechanism are beginning to emerge.

Estimating the Extent of Post-translational Buffering

How much do proportional synthesis and post-translational buffering contribute to keeping the right proportions of protein complex components? Two recent studies, based on ribosome profiling and quantitative mass spectrometry, have come to surprisingly similar conclusions showing that 10%–15% of protein complex subunits are made in excess, whereas others are closer to proportional synthesis (McShane et al., 2016; Taggart and Li, 2018). In this section, we take a close look at these two published studies to estimate the frequency and extent of post-translational buffering on steady-state subunit levels. Finally, we directly compare the data obtained by the two complementary technologies.

We first review the fraction of complexes and subunits that exhibit post-translational buffering. Observed protein degradation profiles in a near-diploid human cell dataset show that 14% of annotated complex components (CORUM database, Giurgiu et al., 2019) exhibit non-exponential decay (Figure 2A) (McShane et al., 2016). Assuming that non-exponential degradation is due to overproduction, this result suggests that one of seven subunits is produced in excess to other subunits of the

Table 1. Technical or Biological Concerns and Best Practices in Genome-Scale Quantitative Studies of Obligate Protein Complex Components

Potential Caveat	Suggested Solution
Most databases do not distinguish obligatory, stable subunits from transiently interacting ones. The same complex can show up multiple times in a database, associated with different substoichiometric or temporary interaction partners. For example, complexes frequently associated with the cell membrane are commonly annotated once with and once without beta-actin as a "subunit". This is a caveat in the analysis shown in Figure 3B.	Manually curate the database of choice by literature searches. See (Taggart and Li, 2018) for an example.
Most databases do not define the stoichiometry of protein complexes. For example, the popular CORUM protein complex annotation database does not supply complex stoichiometries (Giurgiu et al., 2019). This is a caveat in the analysis shown in Figure 3B.	Although most complexes are thought to exist in a 1:1 stoichiometry (Marsh et al., 2015), only the complexes that have well-documented stoichiometry (e.g. from crystal structures) should be included for careful analysis.
Protein complex databases often do not include extra-complex functions of subunits. For example, subunits may homo-oligomerize or retain catalytic activity outside of the annotated complexes, which would alter expectations for stoichiometric abundance.	Use published biochemical studies to exclude subunits that have been shown to self-associate or do not strictly require complex formation for activity.
Most cells lines displaya neuploidy. Most, if not all, human cell lines have chromosomal aberrations, which include multiplication of certain genomic locations leading to increased transcript levels and protein production from genes encoded on these regions.	Use primary cells to avoid gene copy number variations. If cell lines have to be used, sequence the genome and/or karyotype the cell lines. It is also recommended to work with certain chromosomally stable cell lines (e.g., RPE-1 and HCT116 cells), which when passaged a limited number of times have fewer genetic issues (Stingele et al., 2012). It is worth noting that even after correcting for gene copy numbers, gene expression is often inherently perturbed in aneuploid cells because of imbalanced proteomes.
Eukaryotic genomes contain many highly similar genes and proteins. For example, accurate quantification of protein complex subunits encoded by paralogs can be difficult in ribosome profiling and mass spectrometry studies because of the short length of a ribosome footprint or because of redundant peptides, respectively.	Combine signal from paralogous proteins whenever possible (e.g., when proteins have identical function or are both constitutively associated with the complex). Use data analysis approaches equipped to distinguish highly similar genes. An example is the masking strategy described for ribosome profiling analysis described in Taggart and Li, (2018).

same complex. These 14% of subunits are concentrated in 33% of ~1,300 annotated complexes. In particular, 68% of ribosomal subunits exhibit non-exponential decay, as do 11% and 29% of the subunits of the 19S and 20S proteasomes, respectively. These estimates are conservative because of stringent cutoffs applied in the definition of non-exponentially degraded proteins and due to incompleteness of complex annotation. Nevertheless, the fractions of complexes and subunits that exhibit post-translational buffering are consistent with results from ribosome profiling data for yeast and human foreskin fibroblast cells; although the majority of complex subunits are synthesized within a narrow range relative to global protein synthesis ranges, a subset of complexes has overproduced subunits that show more rapid degradation (Taggart and Li, 2018).

Next, we estimate the extent of post-translational buffering for the overproduced subunits. Using data from a Markov chain-based mathematical model of protein decay (McShane et al., 2016), we can compute the fraction of protein molecules that is rapidly degraded early after synthesis. In the model, proteins are synthesized into an unstable state A, from where the proteins are either rapidly degraded or transferred to a second state (state B) where the proteins are more stable. By comparing the degradation rate in state A to the transfer rate from state A to state B for all of the non-exponentially degraded

proteins that are members of a complex, we can calculate the fraction of proteins that are degraded in state A (and the fraction that escapes to the more stable state B) (Figure 2B). We found that the median degree of initial degradation is 61% among the non-exponentially degraded subunits in human RPE-1 cells. In other words, for the newly synthesized molecules in this category, the populations that are rapidly removed and that are stabilized by complex assembly are typically of the same order of magnitude. This result is again consistent with ribosome profiling data for yeast and completely euploid cells from higher eukaryotic species, showing that the spread of complex-centered synthesis rates is typically much less than 2-fold and, for the complexes that disobey proportional synthesis, no more than an order of magnitude (Taggart and Li, 2018).

The above agreements are drawn from synthesis and degradation rates measured in different cell types and organisms, but it is also possible using data from these two studies to roughly estimate the degree of post-translational buffering in the same cell type. Based on the fact that non-exponential degradation of proteins is to some extent evolutionarily conserved and cell type independent, proteins that are non-exponentially degraded in nearly diploid human RPE-1 cells (McShane et al., 2016) are also expected to be overproduced in human foreskin fibroblasts (Taggart and Li, 2018). We
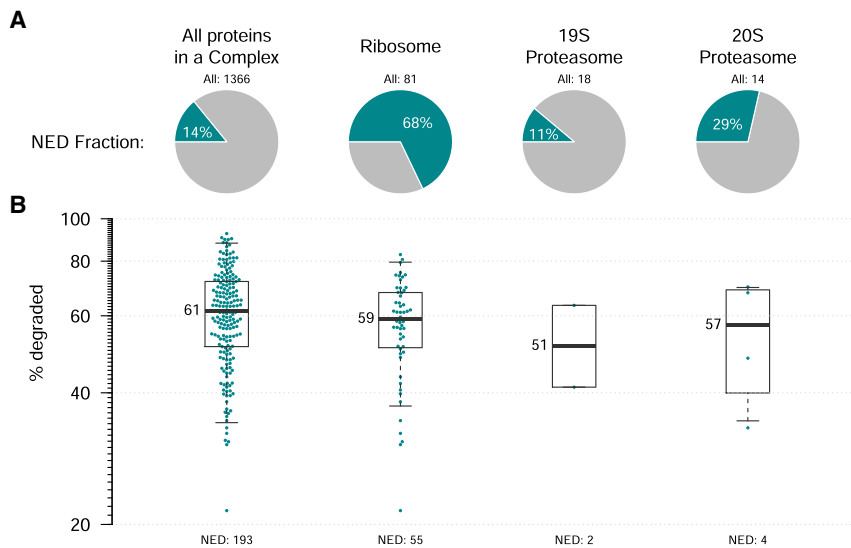
A

Figure 2. Extent of Post-translational Buffering via Protein Degradation

(A) The fraction of non-exponentially degraded (NED) proteins that are members of an annotated protein complex are depicted for all proteins in the CORUM core protein complex set, cytosolic ribosomes, 19S proteasome, and 20S proteasome.

(B) The fraction of degraded NED subunits is calculated from the fraction of the protein molecules that are degraded in the first state of the Markov chain-based 2-state model ($k_A/(k_A+k_{transfer})$). The median fraction of degraded proteins (in percent) is depicted next to each boxplot. All data used for this analysis are from McShane et al. (2016) and complex definitions from CORUM (see Table S1). Whiskers indicate the 5–95th percentiles of the data.

therefore group the proteins in the ribosome profiling data according to their degradation profiles (Figure 3). To minimize problems derived from imperfect complex annotation or moonlighting functions, we first restricted this analysis to three large, well-characterized complexes (80S ribosome, 20S proteasome, and 19S proteasome). We find that proteins with non-exponential degradation kinetics (in RPE-1 cells) are typically overproduced in human foreskin fibroblasts by about 20% (median, Figure 3A). Extending this analysis to all CORUM core complex subunits confirms this overall trend (Figure 3B), with a median overproduction of about 55% among the non-exponentially degraded subunits. We note that the overall larger spread of synthesis rates in the CORUM complexes is due to several confounding factors (summarized in Table 1): first, the relative synthesis rates for subunits are not corrected for stoichiometry because of lack of information, and second, the CORUM database includes many transiently associated subunits and complexes as well as subunits with moonlighting functions, which are not expected to be synthesized in proportion to stoichiometry. Nevertheless, the observation that non-exponentially degraded and exponentially degraded subunits in RPE-1 cells (as measured by mass spectrometry) have statistically significantly different distributions in fibroblasts (as measured by ribosome profiling) highlights the general consistency between ribosome profiling and mass spectrometry data, pointing to the rapid degradation of excess subunits produced for a non-negligible subset of mammalian protein complex components.

A Unified View on the Role of Translational versus Post-translational Adjustment of Protein Complex Stoichiometry

Precisely enumerating the contribution of translational versus post-translational adjustment is complicated by a number of factors (See Table 1 for recommendations on how to handle these caveats for future explorations). For example, the composition of many complexes is poorly defined outside of bacteria and yeast, the stoichiometry of their subunits is often not well annotated, individual subunits can have moonlighting functions

(Mani et al., 2015), and protein complex databases like CORUM (Giurgiu et al., 2019) have a high degree of redundancy. Furthermore, complexes long considered to have a defined composition later turned out to be more heterogeneous, such as the proteasome (Dahlmann et al., 2000; Huber et al., 2012) and the ribosome (Emmott et al., 2019). Another confounding factor for mammalian cell lines is the often unknown or unreported chromosomal state: as discussed above, gene copy number gains will increase mRNA abundance and total translation, thus causing unphysiological protein overproduction. Several of the studies discussed above were performed in HeLa cells, which have a complex karyotype (Landry et al., 2013). Even for near-diploid cell lines, local copy number variations of transcription factors and other regulators may perturb expression of genes located on diploid chromosomes. Finally, any experimental methods, such as quantitative mass spectrometry (McShane et al., 2016) and ribosome profiling (Ingolia, 2016), are affected by measurement noise and biases. Therefore, it is not clear how much of the observed variability reflects true differences in synthesis and degradation rates.

Despite these challenges, the genome-wide studies of both synthesis and degradation presented above converge to the consensus that although many protein complexes follow proportional synthesis, a non-negligible fraction of protein complexes are produced disproportionally. Thus, both coordinated protein synthesis and the degradation of orphan subunits cooperate to ultimately achieve the right proportions of protein complex subunits. To put things into perspective, it is instructive to consider the different scales of both processes (see also Box 1): protein synthesis rates in mammals as set by mRNA levels and translation span at least 100,000-fold across different proteins (Ingolia et al., 2011; Schwanhäusser et al., 2011). By contrast, the degree of post-translational buffering for steady-state subunit levels is less than 10-fold even for the most extreme cases observed (Figure 2B), i.e., virtually no subunit is made in vast excess. Thus, the emerging picture is that coordinated synthesis defines the right order of magnitude, whereas degradation is often essential for fine tuning. This view is also consistent with the observation that protein degradation plays a minor role in adjusting the huge dynamic range of different cellular protein copy numbers (Jovanovic et al.,
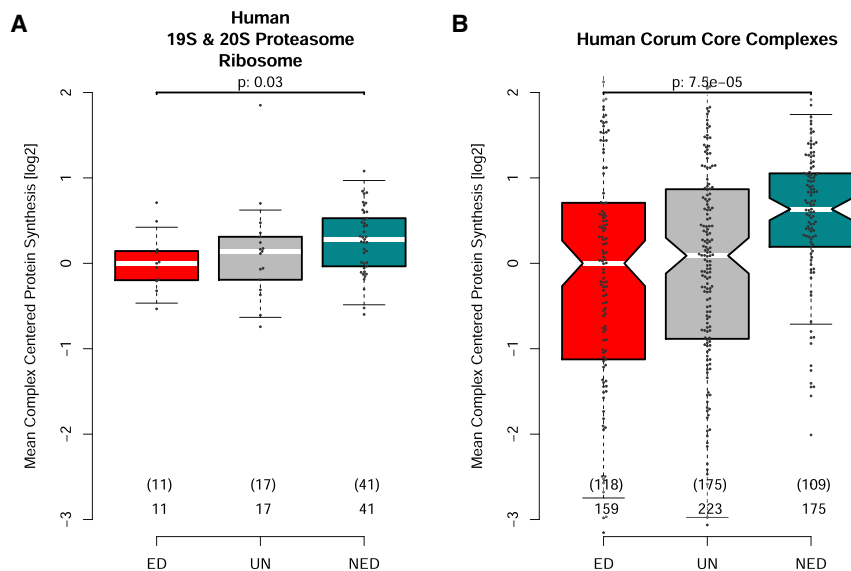
Figure 3. Comparison of Complex-Centric Ribosome Profiling Reads of Proteins with Exponential Degradation, Non-exponential Degradation, or Undefined Degradation Profiles
(A) Proteins from the 19S and 20S proteasome and the cytoplasmic ribosome (as reported in Taggart and Li [2018]).
(B) Complexes from the human CORUM database (excluding binary complexes). To reduce redundancy in CORUM, the database was filtered by selecting only the complex with the highest number of subunits among similar complexes (see Table 1). Mean complex-centered RPF values from remaining duplicated proteins were averaged (median). Overproduction of non-exponential degradation (NED) proteins in comparison to exponential degradation (ED) proteins was tested for significance using a one-sided Wilcoxon rank-sum test (p value depicted in the figure). Numbers of all or unique (in parentheses) proteins are depicted below each boxplot. All data used for this analysis are from Taggart and Li (2018) and McShane et al. (2016) (see Table S2). Whiskers indicate the 5–95th percentiles of the data.

2015; Schwanhäusser et al., 2011). Nevertheless, the overall degree of post-translational buffering is substantial, especially for certain complexes such as cytosolic ribosomes and mitochondrial complexes. Importantly, considering the high abundance of these complexes, it is clear that the quality control of their subunits and assembly is a major function of the cellular protein degradation machinery (Harper and Bennett, 2016).

Outlook
In summary, although ribosome profiling and quantitative mass spectrometry are fundamentally different technologies, the data support the same conclusion that the balance of protein complex components is achieved by the combined effects of coordinated synthesis and post-translational adjustment. Many open questions remain. For example, analysis of ribosome profiling data from zebrafish reveals that subunit production may be transiently more discordant during development than in the adult fish, presumably without any major consequences for the animal (Taggart and Li, 2018). By contrast, when proteasome activity naturally decreases during aging in Killifish, it leads to loss of complex stoichiometry and formation of protein aggregates in the fish brain (Sacramento et al., 2019). These two examples show that excess complex subunits occur during animal development and age-related disease. Understanding why and how the process can go awry will be of great importance.

Another open question is which feature(s) make certain complexes such as mitochondrial complexes and the ribosome more prone to non-stoichiometric subunit production than others (Isaac et al., 2018; Taggart and Li, 2018). Is it the fact that production of mitochondrial OXPHOS complexes is notoriously hard to coordinate, as the subunits are encoded on two different genomes separated by multiple membranes? In the case of ribosomes, it has been argued that overproduction of ribosomal proteins ensures that their levels are never rate limiting for ribosome assembly (Lam et al., 2007). One common feature

among many large protein complexes such as the mitochondrial OXPHOS complexes and the cytosolic ribosome is that they cannot be fully co-translationally assembled, a mechanism that has been proposed to facilitate complex assembly (Schwarz and Beck, 2019). Hence, non-proportional synthesis may reflect more complicated assembly pathways. Further studies will shed light on why cells do not produce all protein complex subunits in perfect proportions.

A final thought relates to the fact that all of the data presented are derived from bulk measurements of millions of cells. However, what really matters for complex assembly is the stoichiometry in individual cells rather than the population average. It is becoming increasingly clear that stochastic processes result in non-genetic heterogeneity of individual cells in a cell population (Nicholson, 2019). For example, it is well established that transcription occurs in bursts (Larsson et al., 2019) and that the levels of cytosolic mRNAs in mammalian cells fluctuate considerably over time (Battich et al., 2015), which should also give rise to variable protein synthesis rates. Therefore, protein synthesis in individual cells is expected to be less coordinated than bulk measurements indicate. Depending on the time scale of fluctuations, this may pose a significant challenge for cells, especially for intrinsically disordered proteins: such disordered proteins can engage in promiscuous molecular interactions when their concentration is increased and are therefore harmful when overproduced (Sopko et al., 2006; Vavouri et al., 2009). To counteract the toxic effects of disordered proteins, it might be advantageous for cells to produce an excess of their binding partners. This would ensure that disordered proteins are never alone, despite the inevitable cell-to-cell variability in protein synthesis rates. Consistent with this idea, the synthesis rates of disordered proteins are systematically lower than the synthesis rates of their binding partners (McShane et al., 2016). In a similar spirit, bacteria overproduce antitoxins to sequester toxins in their toxin-antitoxin modules (Li et al., 2014). Hence, the non-stoichiometric synthesis (in bulk measurements) may reflect an

evolutionary adaptation to prevent deleterious stoichiometric imbalances at the single-cell level. In the future, measuring protein synthesis and degradation at the single-cell level promises exciting insights.

AUTHOR CONTRIBUTIONS

J.C.T., H.Z., M.S., G.-W.L., and E.M. jointly conceptualized and wrote the manuscript, whereas the majority of the formal analysis and data curation was performed by J.C.T. and H.Z.

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