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Review

Ribosomal protein mutations and cell competition: autonomous and nonautonomous effects on a stress response

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Abstract

Ribosomal proteins (Rps) are essential for viability. Genetic mutations affecting Rp genes were first discovered in *Drosophila*, where they represent a major class of haploinsufficient mutations. One mutant copy gives rise to the dominant "Minute" phenotype, characterized by slow growth and small, thin bristles. Wild-type (WT) and Minute cells compete in mosaics, that is, $Rp^{+/-}$ are preferentially lost when their neighbors are of the wild-type genotype. Many features of Rp gene haploinsufficiency (i.e. $Rp^{+/-}$ phenotypes) are mediated by a transcriptional program. In *Drosophila*, reduced translation and slow growth are under the control of Xrp1, a bZip-domain transcription factor induced in *Rp* mutant cells that leads ultimately to the phosphorylation of eIF2α and consequently inhibition of most translation. *Rp* mutant phenotypes are also mediated transcriptionally in yeast and in mammals. In mammals, the Impaired Ribosome Biogenesis Checkpoint activates p53. Recent findings link *Rp* mutant phenotypes to other cellular stresses, including the DNA damage response and endoplasmic reticulum stress. We suggest that cell competition results from nonautonomous inputs to stress responses, bringing decisions between adaptive and apoptotic outcomes under the influence of nearby cells. In *Drosophila*, cell competition eliminates aneuploid cells in which loss of chromosome leads to Rp gene haploinsufficiency. The effects of Rp gene mutations on the whole organism, in Minute flies or in humans with Diamond-Blackfan Anemia, may be inevitable consequences of pathways that are useful in eliminating individual cells from mosaics. Alternatively, apparently deleterious whole organism phenotypes might be adaptive, preventing even more detrimental outcomes. In mammals, for example, p53 activation appears to suppress oncogenic effects of Rp gene haploinsufficiency.

Keywords: ribosomal protein, Minute mutation, cell competition, stress response, Xrp1, p53, protein translation, cell communication, cell nonautonomy, genetic mosaic

Minute mutations and their cell competition in *Drosophila*

One hundred years ago, the Morgan Drosophila lab at Columbia University described a set of mutations dominantly reducing bristle size, which they called "Minute" mutations (Bridges and Morgan 1923). The dominant Minute phenotype, which also includes developmental delay and reduced fertility and viability, was found to be associated with dozens of independent loci, later found to correspond almost entirely to the ribosomal protein (Rp) genes (Fig. 1a, b). The homozygous *M/M* genotypes are lethal (Kongsuwan *et al.* 1985; Andersson *et al.* 1994; Marygold *et al.* 2007). Prior to their molecular identification, it was thought that Minute mutations might affect systemic growth signals, because growth and maturation are under hormonal control. In 1975, Morata and Ripoll studied genetic mosaics to demonstrate that Minute genotypes in fact affected cell division rate cell-autonomously (Morata and Ripoll 1975). They also described a further, nonautonomous effect they called "cell competition". Specifically, they documented that Minute cells (cells heterozygous for Rp gene mutants, i.e. $Rp^{+/-}$ cells), in addition to being slow-growing, are selectively eliminated from mosaic imaginal discs and replaced by neighboring normal cells ($Rp^{+/+}$ cells) (Fig. 1c) (Morata and Ripoll 1975). This is interesting because the flies entirely heterozygous for mutations in Rp genes ($Rp^{+/-}$ or "Minute" flies) are viable, with near-normal size (Morata 2021). The cell nonautonomous influence of wild-type (WT) cells on $Rp^{+/-}$ cells measurably reduced their growth, whereas the growth of the nearby WT cells seemed to increase (Simpson 1979; Simpson and Morata 1981). It is important to note that the cell-autonomous growth differences between WT and $Rp^{+/-}$ cells also affect their representation in mosaic tissues (Martin *et al.* 2009).

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Multiple observations support the idea that cell competition represents a specific, active process, not just the passive effect of intrinsic differences in growth rate. One is the observation that cells do not compete across boundaries between compartments, the blocks of differently specified cells that construct the *Drosophila* imaginal discs, even when growing at different rates (Garcia-Bellido *et al.* 1973; Simpson 1979; Simpson and Morata 1981). The importance of relative differences is also highlighted by the observation that $Rp^{+/-}$ cells are no longer out-competed by WT cells during starvation (Simpson 1979). Although starved $Rp^{+/-}$ cells are even more slowly growing, apparently they are less distinct from starved $Rp^{+/+}$ cells, highlighting also the notion that cell competition depends on the relative cellular fitness. It was later found that cells with heterozygous mutations in Rp genes ($Rp^{+/-}$ cells) undergo apoptosis when surrounded by wild-type (WT) cells (Moreno *et al.* 2002). $Rp^{+/-}$ cell death is predominantly localized at the boundaries, adjacent to $Rp^{+/+}$ cells (Li and Baker 2007). Alterations in cell survival due to the proximity of other cells is a further indication of the active nature of cell competition.

Besides *Rp* mutations, other genetic differences are also now known to lead to cell competitions, not only in *Drosophila*, but also in mammals (Baker 2017; Maruyama and Fujita 2017). One apparently conserved example, described first in *Drosophila*, is competition between cells that express different levels of the Myc transcription factor that are too modest to have much effect on growth or development by themselves. Cells that have an extra copy of the Myc genomic locus, or use the tubulin promoter to drive modest transcription of a dMyc transgene, become "super-competitors" that are able to eliminate the nearby normal cells (de la Cova *et al.* 2004; Moreno and Basler 2004). During Myc driven super-competition, winners shift their metabolism, not only to outgrow but also to eliminate adjacent WT cells (de la Cova *et al.* 2014; Banreti and Meier 2020). Competition between cells expressing different levels of Myc has also been observed in mammals, in embryonic and adult tissues (Claveria *et al.* 2013; Sancho *et al.* 2013; Villa del Campo *et al.* 2014; Ellis *et al.* 2019). Super-competition is also reported between WT cells and cells with increased activity of Yorkie (either due to mutations in Hippo pathway or expressing higher levels of Yki), Wg, or JAK/STAT activity (Tyler *et al.* 2007; Neto-Silva *et al.* 2010; Vincent *et al.* 2011; Rodrigues *et al.* 2012). Differences in Hippo pathway can also lead to cell competition in mammals (Hashimoto and Sasaki 2019; Moya *et al.* 2019). The fact that Myc and other genes implicated in super-competition are oncogenic in different types of cancers supported the notion that cell competition might contribute to tumor development in mammals, as has now been demonstrated in several examples (Suijkerbuijk *et al.* 2016; Di Giacomo *et al.* 2017; Patel *et al.* 2017; Liu *et al.* 2019; Madan *et al.* 2019; Moya *et al.* 2019).

In other contexts, cell competition has a tumor-suppressive role. For example, cells with mutations in genes that are involved in apicobasal polarity (e.g. *scribble*, *discs large*) are eliminated from the *Drosophila* tissues when they are adjacent to WT cells, but in the absence of wild-type cells, tissues comprised entirely of polarity deficient cells form large tumors (Brumby and Richardson 2003; Igaki *et al.* 2009; Menendez *et al.* 2010; Tamori *et al.* 2010). Whether elimination is truly a consequence of proximity to WT cells is currently controversial. Recent study has suggested a role for systemic signals (de Vreede *et al.* 2022). In mammals, cells that have loss of Scribble and are cocultured with WT cells undergo p53-dependent apoptosis (Wagstaff *et al.* 2016). Accordingly, competition of Scribble mutant cells might be a conserved phenomenon, although the role for p53 appears unique to mammals.

There is considerable interest in the molecular mechanisms of cell competition, the physiological consequences of cell competition, and how it may contribute to diseases such as cancer, or be exploited in regenerative medicine. Many of the studies aimed at understanding cell competition mechanisms have also proven informative regarding cell-autonomous aspects of the Minute phenotype. In this review, we will discuss the molecular basis of both the Minute phenotype, and of cell competition of Minute cells, primarily in the *Drosophila* context but bringing in findings from *Rp* mutations in yeast, nematodes, zebrafish, mouse, and humans when it is informative to do so. We hope that a deep dive into the relationship of cell competition to one particular mutant syndrome will reveal concepts that may be applicable to other genotypes also.

Genes required for the *Drosophila* Minute phenotype and for cell competition

We and others have described genetic screens that particularly identified two genes that contribute to both the Minute phenotype and to the competition on Minute mutant cells in *Drosophila* (Fig. 2) (Tyler *et al.* 2007; Lee *et al.* 2016, 2018; Baillon *et al.* 2018; Kale *et al.* 2018; Boulan *et al.* 2019; Ji *et al.* 2019). One gene encodes RpS12, a Rp of the small subunit and an essential protein. Unusually, *rpS12* does not belong to the Minute class of *Rp* gene loci, because loss of one *rpS12* gene copy does not lead to the dominant Minute phenotype, unlike haploinsufficiency for 66 of the 79 *Rp* loci (Marygold *et al.* 2007). A genetic screen recovered a missense point mutation of *rpS12* that substitutes glycine 97 with aspartic acid (Tyler *et al.* 2007; Kale *et al.* 2018). This $rpS12^{G97D}$ allele does not affect the viability of homozygously mutant flies, but prevents the competitive elimination of $Rp^{+/-}$ cells mutated at other, dominant Minute Rp gene loci. Further studies conclude that, in addition to its essential role in the ribosome, the RpS12 protein has a second function as a sensor of Rp imbalance, and that this second role helps initiate the Minute phenotype. That is, haploinsufficiency for any of the 66 *Rp* genes that lead to a Minute phenotype appears to result in an increased, or novel, RpS12 activity that is responsible for aspects of the Minute phenotype (Kale *et al.* 2018). While the molecular basis of this signaling is not yet known, it is clear that it serves to activate expression of the second gene discovered to mediate the Minute phenotype, encoding the transcription factor Xrp1 (Lee *et al.* 2018; Boulan *et al.* 2019; Ji *et al.* 2019).

Mutations at the *Xrp1* locus were recovered from at least four independent genetic screens, two targeting genes required for cell competition of *Rp* mutant cells (Lee *et al.* 2016; Baillon *et al.* 2018), one seeking genes required for the developmental delay caused by Rp depletion (Boulan *et al.* 2019), and one seeking modifiers of a amyotrophic lateral sclerosis (ALS) disease model in *Drosophila* (Mallik *et al.* 2018). Xrp1 had been described previously, and presumably received the name X-Ray induced P53-dependent #1, as the major transcriptional target of p53 following irradiation (Brodsky *et al.* 2004; Akdemir *et al.* 2007). *Xrp1* encodes a AT-hook, bZip-domain protein that binds DNA as a heterodimer with Irbp18, another bZip protein that is the *Drosophila* C/EBP protein (Reinke *et al.* 2013; Francis *et al.* 2016). Xrp1/Irbp18 is also part of the protein complex binding to DNA sequences of the P element transposon (Francis *et al.* 2016).

In otherwise wild-type flies, Xrp1 null mutant animals are viable and morphologically normal, but Xrp1 is required in many aspects of Minute phenotype, including cell competition (Baillon

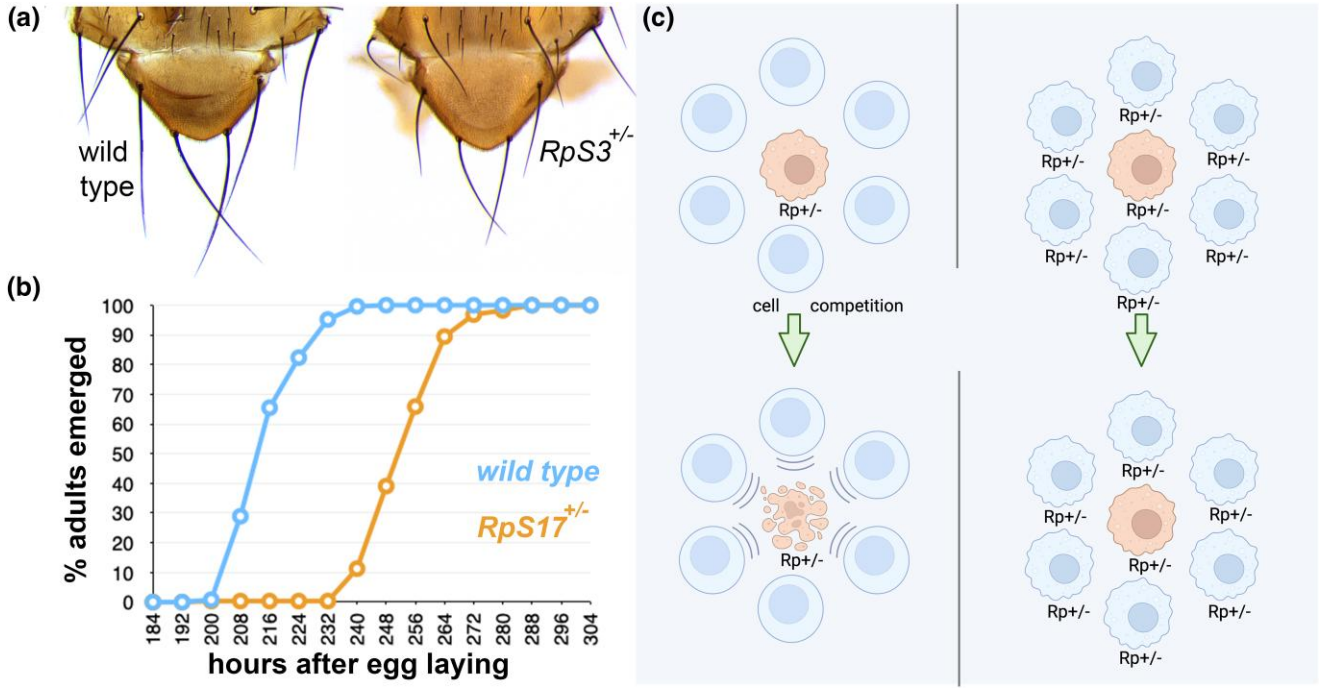

Fig. 1. The "Minute" phenotype of *Drosophila melanogaster*. a) Mutations in many *Rp* genes were first recovered as mutations causing a dominant reduction in the length and thickness of bristles on the adult body. Here, for example, the dorsal thoraces of wild-type and *M(3)95C* heterozygous flies (heterozygous for a mutation of the *RpS3* gene) are similar in size, but the *M(3)95C/+* bristles are shorter and thinner. b) *Rp* gene mutations also cause a dominant developmental delay, illustrated here as a delay of ~40 h in the emergence of *M(3)67C/+* adults (heterozygous for a mutation in the *RpS17* gene) compared to the wild-type controls (these data are for female flies; males exhibit a similar delay). c) Cartoon illustrating how the fate of a single $Rp^{+/-}$ imaginal disc cell (orange) depends on its neighbors. On the left, an $Rp^{+/-}$ cell exposed to $Rp^{+/+}$ neighbors undergoes apoptosis, whereas the rate of apoptosis is much lower for $Rp^{+/-}$ cells surrounded by other $Rp^{+/-}$ cells (right). Selective apoptosis results in the competitive elimination of $Rp^{+/-}$ regions form mosaics and their replacement by wild-type, $Rp^{+/+}$ cells. Created with BioRender.

et al. 2018; Lee *et al.* 2018). Xrp1 is also responsible for the reduced growth of $Rp^{+/-}$ imaginal disc cells, and contributes significantly to the developmental delay of $Rp^{+/-}$ flies, since the rate of development is substantially restored when Xrp1 is mutated (Lee *et al.* 2018; Boulan *et al.* 2019). Xrp1 is even responsible for the reduced translational rates in $Rp^{+/-}$ cells (Lee *et al.* 2018). Therefore, reduction in the rate of the bulk protein synthesis of the cell is not a direct effect of Rp haploinsufficiency, as might easily be predicted, but depends largely on a regulatory response coordinated by the Xrp1 transcription factor (Lee *et al.* 2018; Ji *et al.* 2019). Importantly, Xrp1 protein is almost undetectable in imaginal discs from the wild type, but mRNA levels are elevated and protein expressed in $Rp^{+/-}$ cells, downstream of the RpS12 activity that occurs (Lee *et al.* 2018).

How RpS12 is responsible for Xrp1 induction in Rp mutant cells is not yet certain. It could be that RpS12 in the ribosomal small subunit (SSU) particularly contributes to translational control of Xrp1 expression. If this is the case, the only way that extra copies of the *rpS12* gene locus could enhance Xrp1 expression, developmental delay, and elimination of Rp mutant cells, would seem to be if two pools of SSU normally exist, one with and one without RpS12, so that *rpS12* expression levels could affect the proportions of the two SSU species (Kale *et al.* 2018). This is potentially an example of the specialized ribosome hypothesis, which posits that all ribosomes may not share identical compositions, with structural variation contributing to functional specificity (Genuth and Barna 2018). Alternatively, RpS12 might be present in all SSU but have an additional function outside the ribosome that promotes Xrp1 expression (Kale *et al.* 2018). Many such extra-ribosomal functions have been described for other ribosomal proteins, including transcriptional and post-transcriptional regulation (Warner and McIntosh 2009).

Xrp1 expression, and cell competition, also result from multiple other genetic insults, besides mutating Rp genes. This includes reduction in rRNA transcription (by knock-down of TAF1B) (Kiparaki *et al.* 2022), impaired ribosome function (by knock-down of multiple initiation, elongation, and recycling factors) (Kiparaki *et al.* 2022), mutation in Helicase at 25E (Hel25E) (Ochi *et al.* 2021), inhibition of proteasome activity (Langton *et al.* 2021; Kumar and Baker 2022), depletion of the E3 ubiquitin ligase mahj/DCAF1 (Langton *et al.* 2021; Kumar and Baker 2022), and endoplasmic reticulum (ER) stress (Langton *et al.* 2021; Ochi *et al.* 2021; Kiparaki *et al.* 2022). Thus, *Xrp1* appears to be a sensor of multiple insults that thereby share a common transcriptional response leading to reduced translation and growth and elimination of affected cells by cell competition (Kiparaki *et al.* 2022; Kumar and Baker 2022) (Fig. 2).

Gene regulation by Xrp1

Xrp1 has been confirmed to be a sequence-specific transcription factor, which focusses attention on the transcriptome of Minute cells (Kiparaki *et al.* 2022). RNA-seq indeed reveals a transcriptional signature of Minute wing imaginal discs, comprising several hundred genes with altered mRNA accumulation (Kucinski *et al.* 2017). More than 80% of these mRNA changes depend on Xrp1 and RpS12 function (Lee *et al.* 2018; Ji *et al.* 2019). These Xrp1-dependent transcriptional changes are likely to contribute to the Minute phenotype. One strong candidate is Dilp8, which is transcriptionally upregulated ~10× in the $Rp^{+/-}$ genotypes, and

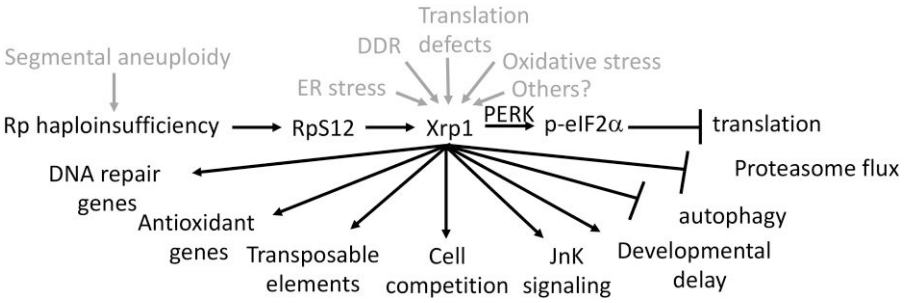

Fig. 2. A transcriptional stress response in *rp* mutant cells genetic screens and analyses have revealed that *rp* mutations activate expression of a transcription factor, Xrp1, by means of a special activity of the ribosomal protein RpS12 that is independently mutable from its essential function. Xrp1 activates PERK to phosphorylate eIF2α, and so suppresses most cellular translation. Xrp1 forms a heterodimer with the ubiquitous Irbp18 protein, constituting a sequence-specific transcription factor that regulates several hundred single copy genes, as well as some mobile elements. These include genes involved in the DDR as well as antioxidant genes implicated in the response to oxidative stress. It is presumed that these transcriptional targets, together or individually, lead to the Xrp1-dependent properties of *Rp* mutant cells, which include developmental delay, cell competition, JnK signaling, and defects in proteostasis and autophagy. In addition to *Rp* gene haploinsufficiency, Xrp1 is also activated by multiple other challenges, including endoplasmic reticulum (ER) stress, DNA damage, defective ribosome function, and oxidative stress. Thus, Xrp1 is a central player in a shared transcriptional response to multiple cellular stresses, at least some of which also lead to cell competition.

is likely contributing to the developmental delay of Minute larvae (Lee *et al.* 2018; Boulan *et al.* 2019; Ji *et al.* 2019). Which specific aspects of the transcriptional response lead to reduced translation, cellular growth, and cell competition is not yet certain. Overall, the Minute transcriptional response is enriched in DNA repair genes and antioxidant genes (Kucinski *et al.* 2017; Lee *et al.* 2018). There is no clear evidence for DNA damage or oxidative stress as yet (Ferrus 1975; Gladstone *et al.* 2012; Kucinski *et al.* 2017). The gene expression signatures possibly reflect shared roles of Xrp1 in other processes. That is, Xrp1 is induced as the major transcriptional target of p53 following DNA damage (Brodsky *et al.* 2004), and is thought to play a role in the DNA damage response (DDR), based on increased frequency of loss of heterozygosity in Xrp1 mutants following irradiation (Akdemir *et al.* 2007). The preponderance of DNA repair genes among Xrp1 targets may be related primarily to this aspect of Xrp1 function. Similarly, Xrp1 is induced by overexpression of Nrf2, the master regulator of oxidative stress (Langton *et al.* 2021), and so might play a role in oxidative stress. Certainly, Xrp1 mediates induction of antioxidant genes following ER stress (Brown *et al.* 2021). ER stress is tightly linked to oxidative stress, because oxidation of cysteine residues to form disulfides within the ER requires proper redox state (Cullinan *et al.* 2003; Harding *et al.* 2003; Tu and Weissman 2004). How these DNA repair-like and oxidative stress-like transcriptional programs contribute to reduced translation and growth, or to cell competition, remains to be determined.

Xrp1 regulates transposable element transcription

Transposable elements (TE) represent another potentially important target of Xrp1. Xrp1, as a heterodimer with Irbp18, binds to inverted repeats of the P element and appears to facilitate DNA repair after transposase cleavage, since it is required for full P element activity (Francis *et al.* 2016). Xrp1/Irbp18 is also the major transcriptional regulator of the retroelement Copia, whose transcription is elevated ~10× in Rp mutant wing discs in an Xrp1-dependent manner (Kiparaki *et al.* 2022). Increased TE expression and mobility are linked to age-associated phenotypes and to pathological conditions (Goodier 2016; Sun *et al.* 2018; Burns 2020). They are mutagenic, and also the main vectors of horizontal genetic information transfer, which are speculated to occur in neurodegenerative diseases and within cancer microenvironments (Schaack *et al.* 2010; Brettschneider *et al.* 2015; Kawamura *et al.* 2017; Chang and Dubnau 2019). p53 normally restrains retrotransposons both in *Drosophila* and in mammalian cancers (Goodier 2016; Kastenhuber and Lowe 2017; Tiwari *et al.* 2018, 2020). It is also speculated that somatic transpositions could be part of cellular diversity and neuronal plasticity mechanisms (Bourque *et al.* 2018), contributing to mosaicism and cell competition.

Xrp1 and pathways of neurodegenerative disease

Xrp1 is also known as a suppressor of a *Drosophila* model of ALS. Increased expression of Xrp1 mediates the toxic neuronal and muscular effects of mutations in the *cabeza* gene that encodes a FUS homolog implicated in the genesis of ALS (Mallik *et al.* 2018; Catinozzi *et al.* 2020). A hexanucleotide repeat expansion of the C9orf72 gene is the most common cause of familial ALS. *Xrp1* and two of its targets shared in $Rp^{+/-}$ cells, *Arc1* and *Gadd45*, are upregulated in a C9orf72 dipeptide repeat ALS *Drosophila* model (Xu *et al.* 2019). Interestingly, knocking down either *Gadd45* or *Arc1* was sufficient to ameliorate the neurodegenerative phenotypes of this model, but the potential role of Xrp1, which could be the most upstream regulator of this response, was not investigated in this study (Xu *et al.* 2019). Additionally, a recent study reports that the C9orf72 dipeptide activates a p53 transcriptional program driving neurodegeneration in multiple models, including *Drosophila* (Maor-Nof *et al.* 2021). These two studies strongly suggest that Xrp1 could drive a significant part of the C9orf72 neurodegenerative phenotype.

The suppression of translation in Minute mutants

As might be expected, global translation is reduced in *Rp* mutant cells, in *Drosophila* as in mice (Boring *et al.* 1989; Oliver *et al.* 2004). Our analysis had revealed that Xrp1 is responsible for the decreased rate of global protein synthesis in $Rp^{+/-}$ cells (Lee *et al.* 2018). Subsequently, at least four groups identified the basis of reduced translation in *Drosophila* as phosphorylation of the

translation factor eIF2α (Baumgartner *et al.* 2021; Ochi *et al.* 2021; Recasens-Alvarez *et al.* 2021; Kiparaki *et al.* 2022). Phosphorylation of eIF2α is a well-known mechanism of translation initiation that globally inhibits cap-dependent translation initiation in response to ER stress, amino-acid starvation, and, in mammals, infection with certain viruses, and heme deficiency (Farrell *et al.* 1977; Wek 2018; Wang and Proud 2022). The kinase phosphorylating eIF2α in Minute cells is PERK, a transmembrane kinase that is best known for its activation by ER stress (Ochi *et al.* 2021; Kiparaki *et al.* 2022). Accordingly, depletion of PERK, which has negligible effect on wild-type wing discs, restores normal levels of eIF2α activity and overall translation, and the same is seen upon overexpression of PPP1R15, the sole eIF2α phosphatase known in *Drosophila* (Ochi *et al.* 2021; Kiparaki *et al.* 2022). It is remarkable that the overall reduction in translation that is a typical of Rp mutant cells is due, not to a reduction in ribosome numbers, which are not affected by Xrp1 mutations (Kiparaki *et al.* 2022), but to regulation of a translation initiation factor by a transcription factor.

Consensus on the role of eIF2α phosphorylation in reducing translation in Minute cells is not yet accompanied by understanding of the mechanisms of eIF2α phosphorylation, or of the relationship between eIF2α phosphorylation, reduced translation, and cell competition. Although RNA-seq analysis shows that Xrp1 alters mRNA levels of PERK and other ER stress proteins, it is uncertain whether these changes are sufficient to explain the altered PERK activity observed (Kiparaki *et al.* 2022). Two groups have demonstrated reduced proteasomal and autophagic flux in Rp mutants, leading to accumulation of ubiquitinylated protein aggregates and autophagosomes (Baumgartner *et al.* 2021; Recasens-Alvarez *et al.* 2021). The protein aggregates and autophagosome accumulations seen in mutants affecting the SSU are downstream of Xrp1 activity and could contribute to the Xrp1-dependent PERK activation in those cells (Kiparaki *et al.* 2022). Interestingly, $RpL27^{+/-}$ mutant cells do not show the same increase in protein aggregation over WT cells, even though Xrp1 and PERK are still activated (Kiparaki *et al.* 2022). This suggests that protein aggregation might not be equally prevalent in all Minute mutants. Although no obvious distinction has previously been made between *Drosophila* phenotypes resulting from mutations affecting the SSU or large subunit (LSU) (Marygold *et al.* 2007), responses to defects in subunit biogenesis differ in yeast (Cheng *et al.* 2019). PERK is a transmembrane protein whose regulatory domain within the ER interacts with luminal chaperones and unfolded proteins, so it is unlikely PERK is directly activated by proteotoxic stress in the cytoplasm. It is possible that PERK could be activated indirectly, because cytoplasmic and luminal proteins compete for proteasomal destruction (Nishitoh *et al.* 2002). All in all, the molecular steps between Xrp1 expression and PERK activation remain to be established.

There is also debate over the contribution of eIF2α phosphorylation to cell competition. It was shown several years ago that differences in translation and growth between cells do not necessarily stimulate competition. Wild-type cells are not significantly affected by neighboring cells growing more rapidly due to CycD/Cdk4 activity, or due to activation of the PI3K pathway (de la Cova *et al.* 2004). Similarly, cells experiencing reduced global translation due to overexpression of 4E-BP are not eliminated by competition with nearby WT cells, showing that reduced global translation is not sufficient for cell competition (Baumgartner *et al.* 2021). Consistent with this, cells depleted for translation factors including eIF4G, eIF5A, and eEF2 are not eliminated by competition from nearby wild-type cells as long as they are prevented from expressing Xrp1, even though they exhibit significantly reduced global translation (Kiparaki *et al.* 2022). These findings indicate that lowered translation does not seem to be sufficient for cell competition. Accordingly, depletion of PPP1R15 is not sufficient to induce cell competition in the absence of Xrp1 (Kiparaki *et al.* 2022). There is uncertainty, however, whether lowered translation might be necessary for cell competition, despite not being sufficient. This should be testable by restoring global translation rate to *Rp* imaginal disc cells by PERK depletion or by overexpression of PPP1R15. One group concluded that PERK depletion could suppress cell competition of *Rp* mutant cells, consistent with a contribution of reduced translation to cell competition (Ochi *et al.* 2021). However, we found that mutating PERK did not prevent elimination of *Rp* mutant cells, indicating that other aspects of Xrp1 function must be required (Kiparaki *et al.* 2022). This was in agreement with Xrp1 being necessary for competition of cells having defects in other steps of translation (such as initiation, elongation, etc.), whose elimination was not regulated by PERK and PPP1R15 (Kiparaki *et al.* 2022). The reasons for these contrasting results, and the contribution of changes in translation to cell competition, remain to be resolved.

The molecular mechanism of competitive cell interactions

The transcriptome changes wrought by Xrp1 in Rp mutant cells number in the hundreds of genes, and the changes in translation efficiency due to eIF2α phosphorylation are likely to be numerous also. The exact nature of the critical difference(s) between wild-type and *Rp* mutant cells that trigger local elimination of the latter are uncertain, although there are many theories. It has been proposed that WT and *Rp* mutant cells compete for Dpp signaling (Moreno *et al.* 2002), and are induced to express different isoforms of the putative Ca channel flower (Rhiner *et al.* 2010). It was proposed, and also disputed, that apoptotic corpse engulfment pathways somehow enhance apoptosis in *Rp* mutant cells (Li and Baker 2007; Lolo *et al.* 2012). *Rp* mutant cells are proposed to activate genes that also function in innate-immune pathways (Meyer *et al.* 2014). It is proposed that activity of Nrf2, which is the transcriptional master regulator of the oxidative stress response, is a trigger for elimination of *Rp* mutant cells, even though oxidative stress was not detected (Kucinski *et al.* 2017). Another hypothesis is that *Rp* cells near to WT cells activate autophagy, which is pro-apoptotic in the context of chronic Jnk signaling that all *Rp* mutant cells experience (Nagata *et al.* 2019). All these suggestions require more investigation. It should also be pointed out that most of these hypotheses do not specify whether or how a specific recognition of *Rp* mutant cells by WT cells takes place. In the case of WT cells undergoing elimination by super-competitor cells with elevated Myc, a cell competition mechanism thought to be distinct from that between WT and *Rp* mutant cells, the current model is that imbalances in expression of secreted innate-immune regulators and transmembrane receptors leads to local, pro-apoptotic innate-immune signals, without specific recognition of out-competed cells (Alpar *et al.* 2018). There is also the possibility that mechanical stress, due to differential growth of WT and *Rp* mutant cells, may contribute to cell elimination (Matamoro-Vidal and Levayer 2019). One potential insight into the mechanism of elimination is that, unlike most apoptotic processes in *Drosophila* development, which depend predominantly on a single initiator caspase, Dronc, competitive apoptosis of *Rp* mutant cells involves little Dronc activity and can also be initiated by the little-known caspase Dream/Strica (Kale *et al.* 2015).

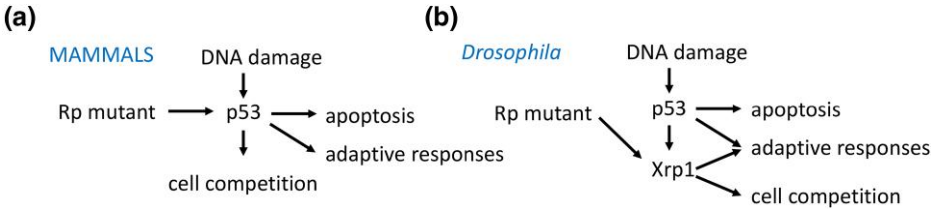

Fig. 3. Comparing the DNA damage response and IRBC in mammals and in *Drosophila*. a) In mammals, DNA damage activates p53, leading to adaptive responses and/or apoptosis. P53 is also activated by *Rp* haploisufficiency, through the Impaired Ribosome Biogenesis Checkpoint. Although it is not yet demonstrated that mosaic DNA damage, or mosaic *Rp* mutation, lead to cell competition in mammals, differences in p53 activity levels between mammalian cells do lead to competitive elimination of cells in many contexts with relatively higher p53 activity. b) *Drosophila* DNA damage activates p53, leading to adaptive responses and/or apoptosis. P53 also activates Xrp1 transcription, which contributes to adaptive responses to irradiation. *Rp* mutations do not activate p53 in *Drosophila* imaginal discs, but instead activate expression of Xrp1, a p53 target, which is required for cell competition.

Unfortunately, this does rather little to clarify the mechanism of competitive cell death for now, since little is known regarding mechanisms of Dream/Strica activation.

"Minute" phenotypes and pathways in other eukaryotes

As might be expected, Rp genes are essential, and often have haploinsufficient mutant phenotypes, in organisms from yeast to mice and man. Unexpectedly, clear homologs of Xrp1 are difficult to find outside Dipteran insects, and are very divergent even there (Blanco *et al.* 2020). The rapid evolution does not indicate genetic drift due to lack of selective value, but instead occurs because *Drosophila* Xrp1 is evolving at a rapid rate under the strong influence of positive selection for evolutionary change (Blanco *et al.* 2020). It is interesting that Xrp1 interacts with at least two TEs, facilitating both P element transposition and transcription of Copia elements, because evolutionary arms races with pathogens are one possible cause of such rapid evolution (Francis *et al.* 2016; Kiparaki *et al.* 2022).

The difficulty recognizing *Xrp1* homologs raises the question of whether *Rp* mutant phenotypes are different in other organisms, or mediated by divergent or distinct transcription factors. Remarkably, evidence is emerging that *Rp* mutant phenotypes in yeast, zebrafish, and mice do also depend on transcription, as seen for *Drosophila*. In yeast, acute *RP* depletion results in activation of the ribosome assembly stress response, rapidly interrupting *RP* gene transcription by depleting a transcription factor IFH1 from *RP* gene promoters (Albert *et al.* 2019; Tye *et al.* 2019). Chronic RP reduction, due to deletion of one paralog of the many duplicated pairs of *RP* genes in yeast, leads to reduced growth and changes in ribosome profiles that are almost entirely explained by changes in mRNA abundance, not by translation efficiency (Cheng *et al.* 2019). Zebrafish haploinsufficient for *rp* gene mutants show a cancer predisposition with ageing that is related to activation of the transcription factor p53 in *rp* mutants, and its inactivation in tumors (Amsterdam *et al.* 2004; MacInnes *et al.* 2008). In humans, mutations in many *RP* genes lead to Diamond-Blackfan Anemia, characterized by erythropoietic defects, as well as reduced growth, delayed maturity, skeletal malformations, and increased cancer predisposition (Vlachos *et al.* 2012, 2018; Ulirsch *et al.* 2018). As in zebrafish, p53 is activated in human and mouse cells heterozygous for *RP* mutations, and is responsible for aspects of the phenotype in $Rp^{+/-}$ mutant mice. The case can be made that mammalian p53 carries out functions similar to those of Xrp1, although there are also similarities to the bZip-domain protein DDIT3/CHOP, as explained further below.

Xrp1, P53, and DDIT3/CHOP

p53 is unrelated to Xrp1 by sequence and structure. The proteins are related functionally, however, because Xrp1 is a p53 target in the *Drosophila* DDR (Brodsky *et al.* 2004), and is believed to play a role in DDR because loss of heterozygosity following irradiation increases in the absence of Xrp1 (Akdemir *et al.* 2007). Xrp1 transcription is also increased after perturbation of the spindle-assembly checkpoint (Baillon *et al.* 2018). P53 is not required for $Rp^{+/-}$ phenotypes in *Drosophila* (Kale *et al.* 2015), but mammalian p53, by contrast, is part of the impaired ribosome biogenesis checkpoint (IRBC). Whenever mammalian ribosome biogenesis is disrupted, the 5S RNP, a component of the 60S large ribosomal subunit that comprises the 5S rRNA, RpL5, and RpL11, binds and inhibits HDM2 (the main ubiquitin ligase for p53 in humans: MDM2 in mice), resulting in p53 stabilization (Fig. 2a) (Zhang and Lu 2009; Bursać *et al.* 2012; Donati *et al.* 2013; Pelletier *et al.* 2020) (Gentillela *et al.* 2017; Pelletier *et al.* 2018). Recent studies have linked the reduced translation rates in $Rps6^{+/-}$ mouse cells to p53, comparable to the role of Xrp1 in the reduced translation rates of $Rp^{+/-}$ cells in *Drosophila* (Tiu *et al.* 2021). The IRBC is activated not only by *Rp* mutations but also by other nucleolar stresses, and by expression of oncogenes such as Myc, even though oncogenes enhance ribosome biogenesis (Fig. 2a) (Derenzini *et al.* 2017; Morcelle *et al.* 2019). We suggested that the mammalian IRBC may be replaced in *Drosophila* by a pathway in which RpS12 replaces RpL5/RpL11, activating Xrp1 in place of p53 (Fig. 3) (Baker *et al.* 2019).

DDIT3/CHOP came to attention as a potential functional correspondent of Xrp1 through studies of the *Drosophila* C/EBP protein Irbp18, the only known heterodimer partner of *Drosophila* Xrp1 (Reinke *et al.* 2013; Francis *et al.* 2016). Xrp1 functions in Minute genotypes also depend on Irbp18 and are thought to be mediated by the Xrp1/Irbp18 heterodimer (Blanco *et al.* 2020). Mammalian heterodimer partners of C/EBP are therefore candidates to replace Xrp1 functionally in mammals. Accordingly, DDIT3/CHOP is a C/EBP partner that is also induced after irradiation, and promotes cell death, reminiscent of Xrp1 (Luethy *et al.* 1990; Yang *et al.* 2017). Ectopic hDDIT3 expression in *Drosophila* leads to a phenotype similar to that of Xrp1 overexpression, and shows some dependency on Irbp18 (Blanco *et al.* 2020). In mammals, DDIT3/CHOP particularly couples ER stress to apoptosis (Zinszner *et al.* 1998; Marciniak *et al.* 2004; Yamaguchi and Wang 2004; Ohoka *et al.* 2005; Li *et al.* 2014; Tian *et al.* 2019). DDIT3/CHOP protein expression is induced by Atf4, a transcription factor whose expression is enhanced by ER stress, because Atf4 is encoded by one of the few transcripts whose translation is enhanced when eIF2α is phosphorylated (Palam *et al.* 2011). In *Drosophila*, Xrp1 protein

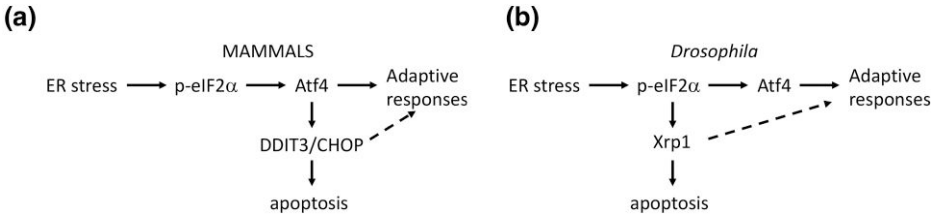

Fig. 4. Aspects of the ER stress response in mammals and in *Drosophila*. a) In mammals, ER stress leads to eIF2α phosphorylation by PERK. This inhibits most cap-dependent translation, but paradoxically enhances translation of a select subset of mRNAs, containing unusual 5′-untranslated region (5′-UTR) structures. One of these encodes Atf4, a transcription factor controlling multiple aspects of the ER stress response. Atf4 also activates transcription of DDIT3/CHOP, a bZip-domain protein similar to Xrp1. DDIT3/CHOP is particularly involved in inducing apoptosis in response to ER stress. b) ER stress in *Drosophila* activates expression of Xrp1 protein independently of Atf4, perhaps because some Xrp1 mRNAs contain 5′-UTR structures that are typical of transcripts translated when eIF2α is phosphorylated. Xrp1 can induce expression of genes that contribute to ER stress adaptation and is a potent inducer of apoptosis.

expression is also induced by ER stress and eIF2α phosphorylation. This was discovered both by the Igaki group, who found that ER stress leads to cell competition, which they then found to be Xrp1-dependent (Ochi *et al.* 2021), and also by the Ryoo group, who were inspired by the possibility that Xrp1 and DDIT3/CHOP might share similar functions to discover that Xrp1 was responsible for Atf4-independent transcriptional responses to ER stress in *Drosophila* (Brown *et al.* 2021). In the course of assessing whether eIF2α phosphorylation lies upstream or downstream of Xrp1 in the cell competition pathway, we and others additionally found that eIF2α phosphorylation stimulated Xrp1 expression, and cell competition (Langton *et al.* 2021; Kiparaki *et al.* 2022). All in all, somewhat related pathways of ER stress can be drawn for *Drosophila* and for mammals, whereby ER stress is coupled to cell death by Xrp1 in flies and by DDIT3/CHOP in mammals (Fig. 4). Accordingly, it is plausible that multiple other stresses besides *Rp* mutations, especially those leading to eIF2α phosphorylation, might promote cell competition if they occur in sporadic somatic cells, as has already been shown is the case for cells experiencing ER stress (Ochi *et al.* 2021; Kiparaki *et al.* 2022).

Optimizing stress responses through cell competition?

These recent studies reveal that Minute cells can be considered to exhibit a transcriptional stress response that is responsible for multiple aspects of the Minute phenotype, mediated by the bZip-domain transcription factor, Xrp1. The ribosome assembly stress is related to the unfolded protein response, the DDR, and other stress responses. One feature of many stress responses, including the unfolded protein response and the DDR, is their bifunctional role in either promoting cell adaptation and repair in the face of stress, or promoting cell death (Roos and Kaina 2013; Sano and Reed 2013; Green and Levine 2014; Navarro-Yepes *et al.* 2014). It is usually believed that stress responses effectively calculate, within each cell, the degree of stress-induced damage. This determines whether it is better to attempt to repair the damage and protect against ongoing stress or to eliminate the severely damaged cells by apoptosis. Removing the most damaged cells is expected economize on resources that could better be marshaled elsewhere, and perhaps to minimize transformation or other deleterious effects of highly damaged cells. It seems evident that a purely cell-autonomous system can only crudely optimize the decision to repair or replace. If damage is variable between cells, then a cell with any particular level of damage might either be the least damaged cell in the tissue, which it would presumably

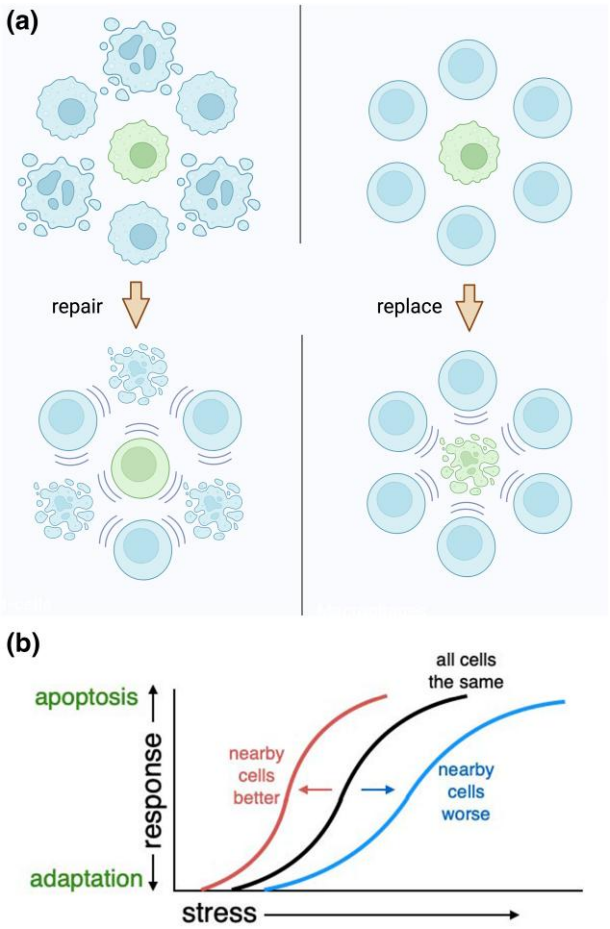

Fig. 5. Model for optimizing stress responses. a) The cartoon illustrates the fate of cells exposed to differing amounts of stress. In both the left and right panels, the central cell experiences identical stress levels. On the left, all the surrounding cells are at least as badly affected, or worse. In this situation, it would be most advantageous to preserve the less-damaged central cell, and repair it to the extent possible, as a resource for repopulating the tissue after removal of more severely damaged cells. On the right, the central cell is more stressed than it neighbors. Under these circumstances, it might be preferable to remove this cell, using the neighbors to provide a pool of replacements. Created with BioRender. b) The decision to repair or replace damaged cells could become context-dependent if the probability of apoptosis is affected both by the cell-autonomous stress level and by the status of the neighbors that are potential replacements. Such a system could result in progressive elimination of a more-stressed population in a mosaic, as seen in cell competition.

be imperative to preserve, or the most damaged, which might best be removed and replaced. Accordingly, a greater optimization of resources should be achieved if the status of other, nearby cells is factored into the repair/replace decision. Eliminating and replacing damaged cells should be a more attractive proposition when less-damaged replacements will be available nearby. By contrast, protecting and repairing damaged cells might be the only viable option when the status of other nearby cells is equally bad or worse. We propose that cell competition provides just such a mechanism to adjust the outcome of stress responses to the status of nearby cells (Fig. 5). The familiar image of genetically identical $Rp^{+/-}$ cells largely surviving en masse but predisposed to apoptotic death near to $Rp^{+/+}$ cells may represent a bias of stress responses toward eliminating $Rp^{+/-}$ cells when more healthy cells are available nearby as a source of replacements.

It may be interesting in this regard to compare the phenotypes of *Rp* gene mutations in organisms where cell competition is or is not possible. In the yeast *Saccharomyces cerevisiae*, for example, a unicellular organism where no selective advantage accrues from programmed cell death, no p53 gene or apoptosis pathway are found. Interestingly, *RP* gene mutations protect yeast from ER stress and extend replicative lifespan (which is not to say that these yeast strains are healthy in other respects) (Chiocchetti *et al.* 2007; Steffen *et al.* 2012). In *Drosophila* and in mammals, *Rp* mutations delay growth and increase mortality (Lambertsson 1998; Ulirsch *et al.* 2018). It could be that these deleterious consequences of *Rp* gene mutations in whole animals are collateral damage of the capacity to remove and therefore replace individual stressed and damaged cells by cell competition. It is notable, therefore, that like in yeast, *rp* mutations also extend lifespan in *Caenorhabditis elegans*, a multicellular animal where replacing apoptotic cells is not generally possible because of the inflexible cell lineage (Hansen *et al.* 2007). It will be interesting to see whether Rp mutation are generally more deleterious, on a whole animal basis, in multicellular organisms with regulative development where cell competition in response to mosaic mutation is possible, and less deleterious in unicellular organisms or those with mosaic development where death of individual damaged cells is less likely to be adaptive for the organism.

Physiological functions of cell competition

This review has not yet explicitly addressed how cell competition is advantageous for the organism. In *Drosophila*, multiple specific advantages of cell competition have been suggested. These include ensuring reproducible organ size in the face of variations of cell growth (Simpson and Morata 1981; de la Cova *et al.* 2004), eliminating spontaneous developmental defects, and thereby extending longevity (Merino *et al.* 2015), and eliminating preneoplastic cells before tumors can form (Brumby and Richardson 2003; Tamori *et al.* 2010). It is not certain whether *Rp* mutant cells are eliminated by cell competition in mammals (Oliver *et al.* 2004), but competitive elimination of cells expressing lower Myc levels is proposed to maintain pluripotency in early mouse embryogenesis(Díaz-Díaz *et al.* 2017), as well as shown to maintain epidermal function (Ellis *et al.* 2019).

Now that the pathway eliminating $Rp^{+/-}$ mutant cells is partially known, its contribution to these processes can be investigated in *Drosophila*. *Xrp1* mutant adults do not exhibit obvious developmental defects, or shortened lifespan (Lee *et al.* 2018; Mallik *et al.* 2018), suggesting that this cell competition pathway may not be relevant to the removal of developmentally aberrant cells in the same way as has been suggested for other cell competition genes (Merino *et al.* 2015). Recently, however, *Rp* gene loci and the Xrp1 pathway have been found to help remove aneuploid cells (Ji *et al.* 2021).

A role of cell competition in removing aneuploid cells was first suggested based on the evidence that aneuploid cells resulting from DNA damage are eliminated from *Drosophila* imaginal discs, the presence of *Rp* loci all over the genome, and the known competitive elimination of *Rp* haploinsufficient cells (Titen and Golic 2008; McNamee and Brodsky 2009). Consistent with the predictions, cells with heterozygous chromosomal deletions are eliminated during development when the deletions included a Rp gene, but only in mosaics with WT cells, and depending on the *rpS12* and *Xrp1* genes, implicating cell competition (Ji *et al.* 2021). By contrast, cells heterozygous for chromosomal deletions that did not affect any Rp genes were generally able to proliferate and survive to differentiate adult tissues. The one exception encountered was that cells haploinsufficient for the *eIF2γ* gene were also lost by cell competition. The explanation may be that haploinsufficiency for *eIF2γ*, encoding a component of the eIF2 translation initiation factor, reduces eIF2 function much as phosphorylation of eIF2α does, and activates Xrp1 and cell competition by the same mechanism (Ji *et al.* 2021). Thus, the copy number of Rp genes, and possibly a few select other genes encoding proteins that act in the same pathway like *eIF2γ*, are spread across the genome and serve as sensors for some examples of aneuploidy. Eliminating aneuploid cells is likely to be a beneficial function of cell competition (Ji *et al.* 2021; Baker and Montagna 2022).

In humans, aneuploidy is responsible for birth defects and miscarriages, and is a hallmark of ageing, cancer and neurodegeneration (Sheltzer and Amon 2011; Lopez-Otin *et al.* 2013; Yurov *et al.* 2019; Ben-David and Amon 2020). Removing sporadic aneuploid cells should therefore be advantageous in humans (Baker and Montagna 2022). Eliminating aneuploid cells from mammalian embryos exhibiting mosaic aneuploidy, which are surprisingly common, is likely to reduce miscarriage and birth defects, for example (Hook 1981; van Echten-Arends *et al.* 2011; Bazrgar *et al.* 2013; Greco *et al.* 2015). Mouse p53 is shown to mediate the elimination of aneuploid embryonic cells before, during and after implantation (Singla *et al.* 2020), consistent with the role of P53 activity differences in cell competition between cells in multiple other mammalian tissues (Bondar and Medzhitov 2010; Dejosez *et al.* 2013; Wagstaff *et al.* 2016; Fernandez-Antoran *et al.* 2019). It is also hypothesized that aneuploidy can sometimes be adaptive, where various stresses (e.g. ER stress, heat) lead cells to rearrange their chromosomes in order to adapt and survive (Beaupere *et al.* 2018; Chunduri and Storchova 2019).

Adaptive value of the Minute phenotype

If much of the effect of *Rp* gene haploinsufficiency reflects a transcriptional response, the question arises why such an apparently deleterious response has evolved. The findings in *Drosophila* raise the possibility that Xrp1 activity in Minute cells is adaptive because it enables cell competition to eliminate aneuploid cells, and cells with some other stresses. In this view, reduced translation and growth retardation would be adaptive for the organism when they occur only in sporadic cells, because they enable cell competition (Lee *et al.* 2018). It is possible that reduced translation and growth retardation serve no useful purpose when the whole animal is of the *Rp* mutant genotype, but represent the unavoidable price to be paid for the cell competition pathway. If is true of humans, Diamond-Blackfan Anemia could also be an unfortunate consequence of pathways that are adaptive when activated

only in sporadic *Rp* mutant cells, but collateral damage when expressed in the whole organism.

An alternative hypothesis is that the consequences of *Rp* mutations on the whole animal are in fact adaptive, and that the deleterious appearance of reduced translation and growth is only superficial. This idea is encouraged by recent findings concerning *mahj*/Dcaf1, another mutant genotype that is eliminated by Xrp1-dependent competition. Although clones of *mahj*/Dcaf1 cells survive better in the absence of *Xrp1*, codepletion of *mahj* and *Xrp1* from some tissues is lethal for the organism (Kumar and Baker 2022). Thus, *Xrp1* function might be adaptive for *mahj* mutant cells, in addition to facilitating their elimination by cell competition (Kumar and Baker 2022).

There is, as yet, little evidence that the Minute phenotype is advantageous in *Drosophila*, but the IRBC may be adaptive in mammals. Mutating p53 prolongs survival of $Rps6^{+/-}$ embryos from E5.5 to E12.5, and suppresses morphological defects in mice with *Rpl24* mutations, consistent with the notion that the IRBC is deleterious for whole animals (Panić *et al.* 2006; Barkić *et al.* 2009). On the other hand, mutating p53 increases embryonic lethality of $Rpl24^{Bst/+}$ mice, indicating that IRBC is protective overall for survival of this genotype (Barkić *et al.* 2009). It may be worth noting that p53 also protects *Drosophila* cells expressing elevated Myc, which are at a disadvantage in mosaics and eliminated unless p53 is expressed (de la Cova *et al.* 2014).

It can also be argued that mammalian IRBC is adaptive through tumor suppression. *Rp* point mutations are recurrent in some cancers, indicating an oncogenic role (Sulima *et al.* 2017). Initially it was thought, based on cancer predisposition of *Rp* mutant zebrafish, that *Rp* mutations promote cancer because chronic p53 activation due to IRBC creates a growth advantage for p53 mutant cells, which are then oncogenic (Amsterdam *et al.* 2004; MacInnes *et al.* 2008). More recent evidence points in another direction. A mouse mutant of MDM2, C305F, blocks the IRBC and prevents p53 activation. Instead of preventing tumorigenesis in a Myc overexpression mouse model, MDM2(C305F) accelerates it, indicating that the IRBC in fact protects against an oncogenic consequence of Myc overexpression (Macias *et al.* 2010; Liu *et al.* 2017). Further evidence comes from *RPL5* mutations found in human tumor sequences. RpL5 is one of the HDM2-inactivating proteins that normally activates IRBC; this is prevented by the specific tumor alleles. These *RPL5*-mutant tumors generally contain WT p53 alleles (Oršolić *et al.* 2020). If tumors containing *MDM2* or *RPL5* mutations that do not activate IRBC and create no selection for secondary p53 mutations are tumorigenic, this argues strongly that *RP* mutations are intrinsically oncogenic. They do not need p53 mutations to cause cancer, rather the p53 activation normally induced by IRBC is tumor-suppressive.

Only a small proportion of overall cancers contain *RP* point mutant drivers, but the potential significance of tumor suppression by the IRBC is greatly enhanced by the possibility that *RP* genes are important sensors of aneuploidy in humans, as well as in *Drosophila*. A study of gene expression and proteomic changes in immortalized human retinal pigmented epithelium cell lines that lost one or another chromosome suggested that the main effect of monosomy is haploinsufficiency for *RP* genes (Chunduri *et al.* 2021). The same authors propose that human cancers that lose p53 tend to have chromosome losses rather than chromosome gains, consistent with the idea that chromosome loss may be oncogenic through *RP* gene haploinsufficiency, which is suppressed by p53 activation (Chunduri *et al.* 2021).

The oncogenic mechanism implied for *Rp* mutations is not yet clear although models have been suggested (Sulima *et al.* 2017, 2019; Girardi *et al.* 2018). One can imagine changes in translation that occur as a direct consequence of altered Rp levels, accumulation or turnover of Rp left unused when another Rp is limiting, or accumulation or turnover of unused rRNA or rRNA-derived species. A 10–30% reduction in ribosomal subunits occurs in *Drosophila* $Rp^{+/-}$ mutants (Lee *et al.* 2018; Kiparaki *et al.* 2022). This might be sufficient to affect translation of specific messages. There is little evidence of this in yeast, however, where ribosome profiling shows that changes in translation after reduced *RP* gene transcription are mostly explained by changing mRNA abundance, not changes in translation efficiency (Cheng *et al.* 2019). It may seem surprising that a 50% reduction in *Rp* gene transcription leads only to 10–30% reduction in ribosome number. Ribosomes are required for cell growth and so reduced ribosome numbers slow changes in cell volume and mass, compensating for ribosome numbers (Kiparaki *et al.* 2022). Longstanding analyses of cellular resource allocation lead to a similar conclusion (Maaloe 1979; Scott *et al.* 2014; Metzl-Raz *et al.* 2017; Shore and Albert 2022). Basically, cell growth requires Rp synthesis for ribosome biogenesis as well as expression of many other proteins. Because total protein synthesis capacity is finite, cells cannot double synthesis of the Rp proteins to compensate for haploinsufficiency in one *Rp* gene without reducing translation of other proteins required for growth. Conversely, if cells do not compensate for diminished ribosome biogenesis in *Rp* mutant heterozygotes, then the growth-related translation products cannot be utilized. Accordingly, cells find an intermediate state where ribosome numbers are partially compensated, while also partially reducing resources devoted to translating other growth-related genes.

Interestingly, gene expression changes typical of mutations affecting the yeast LSU differ from those affecting the SSU (Cheng *et al.* 2019). In particular, mutations affecting SSU biogenesis tend to promote accumulation of mRNA encoding Rp and ribosome biogenesis factors, suggesting a compensatory response, whereas mutations affecting LSU biogenesis tend to promote accumulation of mRNA encoding proteasome and autophagy functions, suggesting an adaptive response to enable turnover of unused ribosome components. The mRNAs encoding Rp and translation factors are reduced in *Drosophila Rp* mutants lacking *Xrp1* (Ji *et al.* 2019), and the same is true in monosomic human cells lacking p53 (Chunduri *et al.* 2021). Thus, Xrp1 in *Drosophila*, and p53 in human cells, regulate ribosome biogenesis mRNAs oppositely to yeast deficient for SSU *RP* genes.

Unused ribosome components, or the consequences of their turnover, present a further molecular mechanism by which *Rp* mutations affect cells. In yeast, acute depletion of *RP*, or *rRNA* biogenesis leads to aggregation of orphan Rp (Albert *et al.* 2019; Tye *et al.* 2019). This has been proposed to occur in *Drosophila* (Baumgartner *et al.* 2021; Recasens-Alvarez *et al.* 2021) but the protein aggregates detected so far, as well as the reduced autophagic flux and proteasome activity reported, occur downstream of Xrp1, and apparently not as a direct effect of orphan Rp (Kiparaki *et al.* 2022). It is also interesting that, in *Drosophila*, decreased autophagic flux and increased proteotoxic stress have been reported predominantly from *Minute* mutations affecting the SSU (Nagata *et al.* 2019; Baumgartner *et al.* 2021; Recasens-Alvarez *et al.* 2021; Kiparaki *et al.* 2022). The dependence of Xrp1 expression on RpS12, however, does indicate a role for at least this Rp in triggering the Minute phenotype. Although the subject of little investigation so far, rRNA turnover is also likely to increase in *Rp* mutants, with potential effects on activity of the exosome, which is thought to be generally responsible for turnover of unused rRNA (Sinturel *et al.* 2017).

Concluding remarks

Recent advances are transforming our understanding of the Minute syndrome in *Drosophila*, caused by haploinsufficiency for *Rp* genes, both with respect to the cell-autonomous consequences for translation and growth, and the nonautonomous process of cell competition. It has been remarkable and unexpected to find that many aspects of the Minute phenotype in *Drosophila* depend on a transcriptional stress response. Evidence is emerging for similar conclusions in yeast and mammals. In the latter case, mediated at least in part by p53, rather than by Xrp1 as in *Drosophila*. Connections between the Minute phenotype and other stress responses including ER stress, the DDR, and oxidative stress make it possible to envisage cell competition as the consequence of nonautonomous inputs into cell death and survival outputs of stress responses. It is now demonstrated in *Drosophila* that cell competition can play an important role removing sporadic segmental aneuploid cells, individual cells experiencing ER stress, and perhaps cells experiencing other related stresses. Many details remain to be resolved, for example including how PERK is activated in *Rp* mutant cells, and the molecular nature of the interactions presumed to occur between competing cells. An important question remains whether an adaptive contribution of cell competition to maintaining optimal tissue constitution during development is sufficient to offset the apparently deleterious consequences of Minute mutations for nonmosaic flies, or whether the nonmosaic Minute phenotype could also be beneficial for another reason. Given the potential significance of *Rp* protein haploinsufficiency to human cancer, arising either through *Rp* point mutants or through chromosome monosomies, the question of what molecular mechanisms result directly from *Rp* haploinsufficiency, and how they may be suppressed by Xrp1 or p53 activity respectively, may be an important question for the future.

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Conflicts of interest

The authors declare no conflict of interest.

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