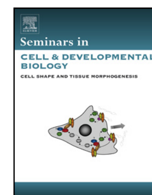
Seminars in Cell & Developmental Biology 28 (2014) 110–115

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb



Review

Aneuploidy and tumorigenesis in *Drosophila*

Marco Milán [a,b,*], Marta Clemente-Ruiz [b], Andrés Dekanty [b], Mariana Muzzopappa [b]

[a] ICREA, Spain
[b] Institute for Research in Biomedicine (IRB Barcelona), Baldiri Reixac, 10-12, 08028 Barcelona, Spain

ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form 3 March 2014
Accepted 10 March 2014
Available online 15 March 2014

Keywords:
CIN
p53
JNK
Delamination
Wingless
MMP1
Stress

ABSTRACT

Aneuploidy, described as an abnormal number of whole chromosomes or parts of them, has been observed in the majority of sporadic carcinomas, the most common type of cancer occurring in humans and derived from putative epithelial cells. However, the causal relationship between aneuploidy and tumorigenesis remains highly debated. On the one hand, aneuploidy has been shown to be a powerful driver of tumor progression, anticancer drug resistance, and tumor relapse. On the other hand, aneuploidy causes proteotoxic and metabolic stress, which compromises cell cycle proliferation and growth. Here we discuss the role of aneuploidy in tumorigenesis in light of the contribution of *Drosophila* epithelial cancer models and propose a stress-induced tumor-promoting role of aneuploidy.

© 2014 Elsevier Ltd. All rights reserved.

Contents

1. Introduction 110
2. Effects of aneuploidy on cellular and organismal fitness in *Drosophila* 111
3. CIN and tumorigenesis in *Drosophila* 112
4. Ionizing radiation and tumorigenesis in *Drosophila* 113
5. Does aneuploidy exert a stress-induced tumor-promoting activity? 114
Acknowledgements 114
References 114

1. Introduction

One hundred and twenty years ago, David von Hansemann reported aberrant mitotic figures, including multipolar mitosis, and asymmetric segregation of chromosomes in carcinoma samples [1]. A few years later, the zoologist Theodor Boveri proposed a causal relationship between the abnormal segregation of chromosomes and cancer [2]. Since then, aneuploidy, a state in which cells do not carry a multiple of the haploid DNA content, has been found in most tumors analyzed so far. A recent systematic analysis of 43,205 human tumors found that 68% of solid tumors are aneuploid [3]. Consistent with Boveri's proposal that chromosomes carrying oncogenes (*Teilungsfoerdernde Chromosomen*) are maintained in tumor cells, Benezra and colleagues found that chromosomes 7, 12 and 20—carrying EGFR, BRAF, SHH, KRAS, CDK4, MDM2, BCL2L1, E2F1, and CDC25B oncogenes—were preferentially gained in their tumor samples [3]. Similarly, and consistent with Boveri's proposal that chromosomes carrying tumor suppressor genes (*Teilungshemmende Chromosomen*) are lost in tumor cells, chromosome instability caused by insufficiency of the spindle assembly checkpoint (SAC) gene *Bub1* was reported to drive tumorigenesis through loss of heterozygosity of Rb and p53 tumor suppressor genes [4]. Chromosomal instability (CIN), the continuous change in chromosome number, constitutes a powerful driver of not only tumor progression but also the anticancer drug resistance and tumor relapse [5,6]. Thus, tumors that experience transient CIN recur at markedly elevated rates upon oncogene withdrawal [7].

While "whole chromosome" aneuploidy (gain or loss of whole chromosomes) results from chromosome segregation errors, "segmental or structural" aneuploidy (copy number changes affecting

* Corresponding author at: Institute for Research in Biomedicine (IRB Barcelona), Baldiri Reixac, 10-12, 08028 Barcelona, Spain. Tel.: +34 93 4034902; fax: +34 93 4037109.
E-mail address: marco.milan@irbbarcelona.org (M. Milán).

http://dx.doi.org/10.1016/j.semcdb.2014.03.014
1084-9521/© 2014 Elsevier Ltd. All rights reserved.

parts of chromosomes) is due mainly to DNA replication or repair defects (reviewed in [6]). However, the high levels of aneuploidy observed in most tumors do not appear to be caused by the acquisition of mutations in core genes directly involved in chromosome segregation or in DNA replication and repair. Thus, to date, only one cancer predisposition syndrome has been directly associated with mutations in mitotic checkpoint genes [8], and mutations in the SAC genes *Bub1*, *BubR1*, *Bub3*, *Mad1* and *Mad2* have been reported at a low frequency in various types of cancers (reviewed in [9]). Moreover, a systematic analysis of the sequences of 100 cell cycle checkpoints and DNA repair genes in early passage human colon cancer cell lines identified very few mutations (reviewed in [9]). The general idea nowadays is that germline mutations in p53, PTEN or BRCA2—all known to predispose to tumorigenesis—and somatic mutations in tumor suppressor genes (p53 and Rb) or oncogenes (c-Myc or Ras) directly or indirectly affect critical checkpoints that protect cells against genome DNA damage and lead to whole chromosome, segmental or structural aneuploidy.

Despite these observations, aneuploidy has been shown to be deleterious at the cellular and organismal level in all eukaryotes analyzed to date. Unbalanced genomes cause proteotoxic and metabolic stress, which compromises cell cycle proliferation and growth [10–12]. These harmful effects of aneuploidy might be consistent with the notion that high levels of CIN contribute to tumor suppression [13]. Alternatively, the acquisition of aneuploidy-tolerating mutations may support CIN-induced tumorigenesis *in vivo* [14]. Here we discuss these hypotheses in light of the contribution of aneuploidy-induced *Drosophila* cancer models and propose a stress-induced tumor-promoting role of aneuploidy in epithelial tissues.

2. Effects of aneuploidy on cellular and organismal fitness in *Drosophila*

Fruit flies bear only four pairs of chromosomes, and the fourth chromosome carries a small number of genes (Fig. 1A and B). Given the small number of chromosomes, *Drosophila* does not appear to be, in principle, a highly suitable model system in which to study the consequences of aneuploidy on cell and organismal viability. Surprisingly, however, research in *Drosophila* has greatly contributed to furthering the field. In the early years of last century, Calvin Bridges showed that increasing the dosage of the whole genome to three copies produced a viable genotype, while three copies of individual chromosomes (chromosome trisomies, Fig. 1D) were detrimental to the organism [15]. In the 1930s, Patterson and colleagues presented evidence that the fertility of segmentally trisomic flies (three copies of certain chromosomic regions) was inversely proportional to the length of the amplified piece [16]. In the 1970s, Dan Lindsley and colleagues analyzed the viability of segmentally aneuploid individuals [17]. They presented evidence that trisomies for large regions were lethal and that intermediate trisomies caused reduced survival. Remarkably, only one triplo-lethal locus was identified. Thus, "*the deleterious effects of aneuploidy are caused by the additive effects of genes that slightly reduce viability and not by the individual effects of a few aneuploidy-lethal genes*". A few years later, in studies on the impact of aneuploidy on cellular proliferation and viability *in vivo*, Pedro Ripoll demonstrated that the viability of aneuploid cells decreases as the size of the amplified chromosome fragment increases [18]. Taken together, these results indicate that altering the dosage of part of the genome is more deleterious than changing the doses of the whole genome and imply that the harmful effects of aneuploidies at the cellular and organismal level are due to the imbalance of a large set of genes rather than single genes.

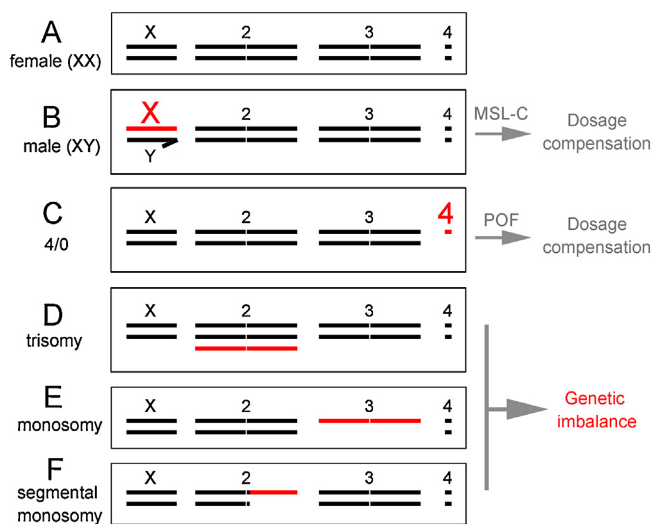


Fig. 1. Aneuploidies in *Drosophila*. Whereas monosomies for the X (B) and 4th (C) chromosomes are buffered by the MSL-C and POF systems, respectively, which increase gene expression of the corresponding chromosomes, segmental (F) or whole chromosome (D and E) aneuploidies for the chromosomes lead to genetic imbalance.

Remarkably, Bridges found that trisomies for the two major autosomes (chromosomes 2 and 3) were lethal, while individuals with different copies of the X or the diminutive 4th chromosome were viable [15]. Indeed, two chromosome-specific mechanisms have evolved to compensate for variations in the number of these chromosomes and to equalize their gene expression levels with respect to the two major autosomes. While the male-specific lethal complex (MSL-C) plays a fundamental role for dosage compensation of the only X chromosome present in males (reviewed in [19], Fig. 1B), painting of fourth (POF) stimulates the expression of the 4th chromosome in 4/0 tissues ([20,21], Fig. 1C). The observations that mutations in MSL-C lead to cell and organismal lethality in male (X/Y) tissues [22] and that loss of POF compromises the organismal viability of 4/0 individuals [20,21] reinforce the notion that the deleterious effects of aneuploidies result from the imbalance of a large set of genes (Fig. 1D–F). The discovery of POF and its role in compensating for variations in the number of 4th chromosomes contributed to the general idea of the presence of an autosomal dosage compensation mechanism in *Drosophila*. However, recent data indicate that the presence of 4th chromosome-specific regulation of gene expression is a possible remnant of its former life as a sex chromosome [23]. A POF-independent general segmental aneuploidy-buffering system has also been proposed to compensate for gene dose imbalances in autosomes 2 and 3. This notion is supported by the observation that genes present in segmental monosomies show on average a 1.6-fold reduction in expression levels rather than the expected 2-fold reduction [24,25] and that some genes in segmental trisomies show compensation [26,27]. However, this compensation appears to be lost in segmental aneuploidies of larger fragments. In this regard, careful analysis of segmental monosomies generated in the same genetic background indicate that autosomal dosage compensation is highly heterogeneous and gene-specific and that gene network interactions make a significant contribution to the process [28]. Thus, only the MSL-C and POF mechanisms appear to compensate for gene expression in hemizygous conditions for the X and 4th chromosomes. This notion is consistent with the finding that only trisomies for the two major autosomes (chromosomes 2 and 3) cause lethality [15]. A similar gene-specific buffering mechanism to that observed in segmental monosomies in *Drosophila* at the transcriptional level was also observed in disomic budding yeast and in trisomic human cell lines at the protein level [12,14]. Thus, while the correlation between gene copy number and protein level is generally strong in

these cells, members of protein complexes encoded on extra chromosomes are not expressed according to gene copy number and are adjusted toward normal levels [14].

3. CIN and tumorigenesis in *Drosophila*

Drosophila research has not only made a significant contribution to furthering our knowledge of the effects of aneuploidy on cell and organismal viability but also strengthened the idea of cancer as a genetic disease. Indeed, the first ever tumor-causing mutations were isolated in fruit flies [29,30]. Mary Stark, a PhD student in Thomas Morgan' lab, presented evidence that fly strains containing tumors were hereditary in origin, and she mapped them to certain chromosomes [31]. A few years later, she analyzed the origin and lethality of a variety of tumors, including those in the larval digestive tract, lymphosarcoma in the blood-forming organ, and melano-epithelioma in the skin epithelium. On the basis of her findings, she concluded that "*cells of lethal tumors, derived from cells embryonic in nature and without power of differentiation, have a greater capacity for growth and infiltration, important characteristics of malignancy*" [32]. Although the dispute about the commonalities between vertebrate and *Drosophila* tumors remains, the work of Elizabeth Gateff a few years later contributed to unraveling the striking similarities between *Drosophila* and vertebrate neoplastic tumors. These similarities included rapid growth, loss of differentiation capacity, increased invasiveness, lethality and, most interestingly, karyotypic abnormalities [33]. Since then, *Drosophila* has become an acknowledged model for research into the molecular and cellular mechanisms underlying tumorigenesis (reviewed in [34–36]). Both stem cell-derived and epithelial tumors have contributed to this advance in the last few years. On the one hand, several reports using the larval primordia of the adult eye and wing, highly proliferative and growing epithelial monolayers, have identified key aspects of oncogene-driven tumorigenesis [37] and have provided evidence of the cooperation between classical oncogenes, the production of ROS, and mutations in tumor suppressor genes [38–41]. In these cases, a JNK-dependent tumorigenic response of the tissue, in terms of growth and tissue invasiveness, appears to be at work. On the other hand, fly brains have provided useful model systems to reinforce the proposed role of stem cells as the cells of origin in malignant tumors [42,43]. This notion is based on the observation that defects in asymmetric divisions of larval neural stem cells lead to an expansion of the stem cell population, which causes over-proliferation and induces metastatic behavior and malignancy [43–45].

Is CIN causative of tumorigenesis in these two models? In epithelial tissues, the contribution of CIN to tumorigenesis is revealed when aneuploid cells are maintained in the tissue [46,47]. A thorough analysis of the effects of CIN on larval primordia reinforced the previous observations that aneuploidy is deleterious at the cellular level [18]. Depletion of SAC genes (*bub3*, *rod*) and genes involved in spindle assembly (*abnormal spindle* (*asp*)), chromatin condensation (*orc2*) and cytokinesis (*diaphanous* (*dia*)) induces CIN in epithelial cells and these cells die by apoptosis (Fig. 2A [46,47]). In contrast to mammalian cells, CIN-induced apoptosis in larval primordia is independent of the activity of the tumor suppressor gene Dp53 and dependent on the activation of the stress response JNK pathway [46]. The acquisition of aneuploidy-tolerating mutations has been shown to improve the fitness of multiple different aneuploidies [14] and proposed to contribute to CIN-induced tumorigenesis *in vivo*. Consistent with this notion, the maintenance of aneuploid cells in the epithelium by means of blocking the apoptotic pathway at different levels elicits CIN-induced tumorigenic behavior in terms of DE-cadherin delocalization, cell delamination, basement membrane degradation,

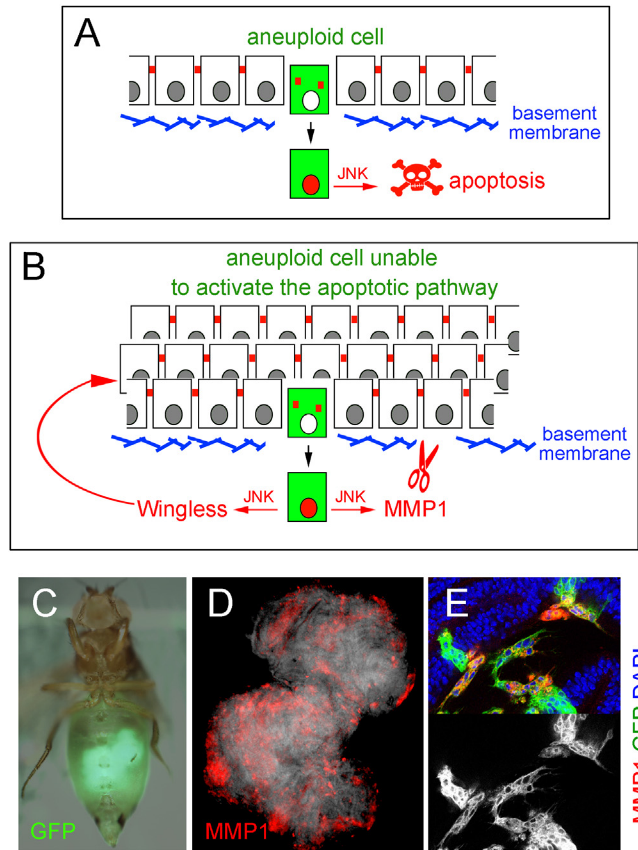


Fig. 2. CIN-induced tumorigenesis in *Drosophila* epithelia. Aneuploid cells delocalize DE-cadherin (in red), delaminate basally from the main epithelium and activate the JNK pathway (red nuclei). Whereas delaminating cells activate the apoptotic program in a JNK-dependent manner (A), JNK becomes pro-tumorigenic in delaminating cells unable to activate the apoptotic pathway (B). (C) Epithelial tumor (in green) generated by CIN, implanted in a fly host and visualized 20 days thereafter. (D) Tumorigenic implant extracted from the adult host and expressing MMP1 (in red). (E) Higher magnification of motile transformed cells (in green), expressing MMP1 (in red) and surrounded by normal epithelial cells (in blue).

and neoplasic overgrowth (Fig. 2B [46,47]). Similarly to what happens in *Drosophila*, mutations in *p53*, which drives CIN-induced apoptosis in mammalian cells, accelerates tumor development in two independent aneuploidy models [4].

CIN drives tumorigenesis in *Drosophila* epithelial cells by making use of a subversive role of JNK in these cells (Fig. 2B–E). Thus, activation of JNK, originally serving to remove aneuploid cells from the epithelium by apoptosis, plays a tumor-promoting role in tissues unable to enter programmed cell death. JNK exerts its functions by inducing the expression of the mitogenic molecule Wingless and the Matrix Metaloproteinase 1 (MMP1 [46], Fig. 2B, D, and E). A similar subversive role of JNK in driving tumorigenesis has also been reported in other epithelial tumors caused by the cooperation between Notch or Ras oncogene activation and loss of the *scribble* tumor suppressor [38,39,48]. While JNK-mediated apoptosis eliminates cells mutant for the Scribble polarity complex, JNK promotes tumors in these cells when they express an activated form of Ras (Ras^{V12}). JNK exerts its functions by inducing the expression of mitogenic molecules and MMP1, which are responsible for tissue overgrowth and basement membrane degradation, a pre-requisite for tissue-invasiveness. In CIN-induced tumors, cells with a significant level of aneuploidy delaminate from the epithelium—most probably as a result of DE-cadherin delocalization—activate JNK, and express Wg and MMP1 [46]. The tumorigenic response of the tissue to CIN is revealed upon inhibition of the apoptotic program at various levels, including the pro-apoptotic genes and the effector

caspases. Thus, cell delamination and JNK activation are not caused by entry into apoptosis, and CIN-induced tumorigenesis relies on highly aneuploid delaminating cells, which act as classical organizing centers to promote the growth of the non-delaminated tissue.

In fly brains, CIN does not cause tumorigenesis. Centrosome amplification is a common feature in many cancer cells, and it severely disturbs mitotic process and cytokinesis *via* the formation of more than two spindle poles, thus resulting in an increased frequency of chromosome segregation errors (chromosomal instability, CIN [49]). Based on these observations, Basto and colleagues analyzed the impact of centrosome amplification on CIN and tumorigenesis in fly tissues [50]. Unexpectedly, they found that fly brains with extra centrosomes showed bipolar (and not multipolar) mitoses and, consequently, presented low levels of aneuploidy. However, centrosome amplification generated tumors in brain tissues. This tumorigenic response appeared to rely on defects in the asymmetric division of neuroblasts and the consequent expansion of the stem cell population. Similarly, fly brains with dysfunctional centrosomes form tumors that show a high level of aneuploidy after serial transplantation [51]. However, aneuploidy is not the cause of this tumorigenic behavior, as fly brains mutant for genes involved in mitotic checkpoints induced aneuploidy but did not cause tumorigenesis. It is interesting to note in this context that mouse and human brains are genomically heterogeneous, and neural cells contain somatically generated mosaic aneuploidy (reviewed in [52]). These observations open up the possibility that neural cells are more resistant to the deleterious effects of aneuploidy and that JNK is not activated in fly brains subjected to CIN.

Remarkably, the context-dependent tumor-promoting role of CIN appears to occur not only in *Drosophila* but also in mammals. Thus, many CIN mouse models display an increased susceptibility to spontaneous tumor formation, specifically in lung epithelial cells and not in other tissues [6]. Whether the tumor-promoting role of CIN in lung cells relies on similar molecular and cellular mechanisms as in *Drosophila* epithelial tissues remains to be elucidated.

A recent report in *Drosophila* proposes that CIN does not drive the tumorigenic response of the SAC-deficient epithelial tissue upon additional blockade of the programmed cell death (PCD) pathway [53]. This notion is based on the following observations. While depletion of the SAC gene *bub3* caused overgrowth of wing disk cells unable to enter apoptosis with a remarkable 100% penetrance, the penetrance of tissue overgrowth upon depletion of SAC genes *mad2* or *BubR1* was much lower (10–30%). Moreover, depletion of *CENP-E*, which mediates precise interactions between kinetochores and microtubules of the mitotic spindle, or of *Nsl1*, which targets SAC proteins to the kinetochore [54], did not cause any detectable growth phenotype [53]. Although the authors state that these results present evidence that aneuploidy resulting from impaired SAC is not sufficient to drive tumorigenesis in epithelial tissues, the levels of aneuploidy, measured by cellular DNA content analysis or by karyotypic analysis of mitotic cells, were relatively heterogeneous in the different genetic backgrounds tested [53]. Depletion of *bub3* was able to induce by far the most significant levels of aneuploidy (around 30% of mitotic cells with aneuploidy) when compared to *mad2*-, *BubR1*-, *CENP-E*- or *Nsl1*-depleted cells (12–19%) [53]. These observations suggest that tissue overgrowth is obtained only when a given percentage of aneuploid cells is exceeded in the tissue. Consistent with this proposal, a subsequent analysis with independent dsRNA forms against CENP-E and Nsl1 induced levels of aneuploidy similar to those previously observed upon depletion of *bub3* (around 30% [46]), and under these circumstances tumor-like neoplasic overgrowth was found upon additional blockade of the apoptotic program [75]. This overgrowth was accompanied by the expression of MMP1 and Wg in delaminating cells, both in larval tissues and allograft transplants. These results reinforce the tumorigenic role of CIN in *Drosophila* epithelial tissues [46] and question the proposed SAC-independent roles of checkpoint proteins in suppressing tumorigenesis [53]. These results imply that tumor-like growth requires a minimum percentage of aneuploid cells in the tissue.

4. Ionizing radiation and tumorigenesis in *Drosophila*

In 1947, Hermann J. Muller was awarded the Nobel Prize for the discovery of the production of mutations by means of ionizing radiation (IR), including chromosome rearrangements [55]. Half a century later, John L. Haynie and Peter J. Bryant unraveled the deleterious effects of IR on the proliferation dynamics of *Drosophila* wing cells, and proposed that these effects were "*possibly due to radiation-induced aneuploidy*" [56]. IR-induced cell death was subsequently shown to be caused by the activation of the DNA damage response (DDR) pathway genes *dp53* and *chk2* [57,58]. The immediate (first few hours) apoptotic response of the tissue to the generation of double stranded breaks (DSBs) was found to depend on the capacity of these two genes to activate the JNK signaling pathway and induce the expression of pro-apoptotic genes [59–62]. In contrast, a late (2/3 days) apoptotic response of the tissue to IR was shown to be JNK-dependent [63] but Dp53-independent [64]. This late response was proposed to be a consequence of the production of chromosome rearrangements [63]. When IR-treated cells are maintained in the tissue by additional blockade of the apoptotic pathway, the tissue shows a late (2/3 days) JNK-dependent hyperplastic response [59]. This late activation of JNK, which most probably serves to remove aneuploid cells from the epithelium by apoptosis, plays a tumor-promoting role in tissues unable to enter apoptosis. This effect is achieved by inducing the expression of the mitogenic molecule Wingless.

The similarities between CIN and IR treatments in promoting tumorigenesis in *Drosophila* epithelia upon additional blockade of the PCD pathway are striking, and JNK activation might be caused in both cases by the production of aneuploid genotypes. Indeed, a thorough analysis of the potential pro-tumorigenic action of IR-induced DNA damage and the contribution of cell cycle arrest and DNA repair to tumor progression has indeed reinforced the notion that the tumorigenic action of IR is due to failure in DNA repair and that chromosome rearrangements, induced by imprecise repair of DSBs, are most probably the driving force in JNK-mediated tumorigenesis [65]. DSB repair is mediated in S and G2 by an error-free mechanism called homologous recombination (HR) and in G1 by an error-prone mechanism called non-homologous end joining (NHEJ). While DmRAD51 and the DEAD-like helicase DmRAD54 play essential roles in HR [66,67], NHEJ is executed by the activity of the ATP-dependent DNA ligase Lig4 [68]. The contribution of NHEJ to the repair of IR-induced DSBs is undetectable, and IR-induced JNK activation and tumorigenesis are largely unaffected by mutations in *lig4* [65]. In contrast, HR plays a major role in DSB repair, and depletion of elements involved in HR enhances the tumorigenic behavior of the tissue in terms of JNK activation and tissue overgrowth. Consistent with the role of HR in DSB repair, defects in G2 arrest upon IR treatment compromises the dynamics of DNA repair and enhances the tumorigenic response of the tissue. Remarkably, DDR-independent lengthening of G2 contributes to DNA repair and completely rescues IR-induced JNK activation. Most interestingly, the late apoptotic response of the tissue to IR, which was proposed to be a consequence of the production of aneuploid cells [63], is also rescued by DDR-independent lengthening of G2 [65]. Since impaired HR repair of DSBs induces genome rearrangements such as deletions [69,70], these results reinforce the deleterious effects of segmental aneuploidies (in this case induced by IR) and support the tumorigenic action of aneuploidy in tissues unable to enter the apoptotic pathway.

HR repair of DSBs also suppresses spontaneous tumor development in aged adult flies. Genome rearrangements, including translocations and deletions, are the most predominant type of mutations in aged adult flies [71], and aged flies develop tumors in proliferating tissues such as the testis and gut [72]. Impairment of HR repair in flies mutant for *DmBlm* (the *Drosophila* BLM ortholog, a RecQ helicase involved in unwinding the DNA double helix for replication or repair [73]) increases the frequency of genome rearrangements, and most interestingly, increases the frequency of tumor development at early ages in testis and gut tissues, and reduces longevity [74]. The incidence of spontaneous tumorigenesis is largely unaffected by mutations in *lig4* [74]. Thus, genome instability, in the form of chromosome rearrangements, also contributes to spontaneous tumor development in proliferative tissues of aged flies. All together, these results support the proposal that "segmental" aneuploidies (Fig. 1F) participate in both spontaneous and IR-induced tumorigenesis in *Drosophila* tissues.

5. Does aneuploidy exert a stress-induced tumor-promoting activity?

The experimental results summarized in this review highlight the deleterious effects, at the cellular and organismal level, of segmental and whole chromosome aneuploidy in *Drosophila* and unveil a context-dependent tumorigenic role of aneuploidy. Aneuploidy induces apoptosis in *Drosophila* epithelial cells, and this process depends on the activation of the stress response JNK pathway [46]. Remarkably, the tumorigenic response of epithelial tissues to aneuploidy also depends on the activation of JNK. Thus, JNK is primarily used to remove aneuploid cells by apoptosis, but persistent activation of this pathway in aneuploid cells unable to activate the apoptotic program drives a tumorigenic transcriptional program. It is unlikely that this rapid tumorigenic response of the tissue is caused solely by the maintenance of chromosomes carrying certain oncogenes or loss of chromosomes carrying tumor suppressor genes. Quantitative proteomic analysis in aneuploid budding yeast and human cells indicate that changes in gene copy number are generally reflected in the amount of RNA and protein ([12,14], [76]). Most gene products interact with other cellular components in macromolecular complexes, and stoichiometric imbalances in such complexes might lead to fitness defects [77]. Analysis of gene expression data from aneuploid cells in several organisms, including yeast, plants, mice and humans, has revealed a consistent up-regulation of genes involved in the stress response [78]. This response is independent of the identity of the genes whose copy numbers are altered and appears to be a consequence of the stoichiometric imbalances caused by an unbalanced genomic state of aneuploid cells [79]. We propose that stoichiometric imbalances induce JNK activation and apoptosis in aneuploid *Drosophila* cells and that an unbalanced genomic state contributes to JNK-mediated tumorigenesis upon additional blockade of the apoptotic program. Perhaps the most accepted hypothesis regarding the role of aneuploidy—defined as a karyotype that is not a multiple of the haploid complement—in cancer development is that it is a source of mutability. Whether an unbalanced genomic state and the activation of the corresponding stress pathways also contribute to human cancer remains to be elucidated.

Acknowledgements

M.M. is an ICREA Research Professor and M.M.'s laboratory is funded by grants from the Spanish *Ministerio de Economía y Competitividad* (*BFU2010-21123* and Consolider CSD2007-00008) and the *Generalitat de Catalunya* (2005 SGR 00118).

References

[1] von Hansemann D. Ueber asymmetrische Zelltheilung in epithel Krebsen und deren biologische Bedeutung [On the asymmetrical cell division in epithelial cancers and its biological significance]. Virchow's Arch Path Anat 1890;119:299.
[2] Boveri T. Zur Frage der Entstehung Maligner Tumoren [Origin of malignant tumors]. Gustav Fisher, Jena 1914:1–64.
[3] Duijf PH, Schultz N, Benezra R. Cancer cells preferentially lose small chromosomes. Int J Cancer 2013;132:2316–26.
[4] Li M, Fang X, Baker DJ, Guo L, Gao X, Wei Z, et al. The ATM-p53 pathway suppresses aneuploidy-induced tumorigenesis. Proc Natl Acad Sci USA 2010;107:14188–93.
[5] Duijf PH, Benezra R. The cancer biology of whole-chromosome instability. Oncogene 2013;32:4727–36.
[6] Janssen A, Medema RH. Genetic instability: tipping the balance. Oncogene 2013;32:4459–70.
[7] Sotillo R, Schvartzman JM, Socci ND, Benezra R. Mad2-induced chromosome instability leads to lung tumour relapse after oncogene withdrawal. Nature 2010;464:436–40.
[8] Hanks S, Coleman K, Reid S, Plaja A, Firth H, Fitzpatrick D, et al. Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. Nat Genet 2004;36:1159–61.
[9] Fang X, Zhang P. Aneuploidy and tumorigenesis. Semin Cell Dev Biol 2011;22:595–601.
[10] Williams BR, Prabhu VR, Hunter KE, Glazier CM, Whittaker CA, Housman DE, et al. Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. Science 2008;322:703–9.
[11] Torres EM, Sokolsky T, Tucker CM, Chan LY, Boselli M, Dunham MJ, et al. Effects of aneuploidy on cellular physiology and cell division in haploid yeast. Science 2007;317:916–24.
[12] Stingele S, Stoehr G, Peplowska K, Cox J, Mann M, Storchova Z. Global analysis of genome, transcriptome and proteome reveals the response to aneuploidy in human cells. Mol Syst Biol 2012;8:608.
[13] Silk AD, Zasadil LM, Holland AJ, Vitre B, Cleveland DW, Weaver BA. Chromosome missegregation rate predicts whether aneuploidy will promote or suppress tumors. Proc Natl Acad Sci USA 2013;110:E4134–41.
[14] Torres EM, Dephoure N, Panneerselvam A, Tucker CM, Whittaker CA, Gygi SP, et al. Identification of aneuploidy-tolerating mutations. Cell 2010;143:71–83.
[15] Bridges CB. Triploid intersexes in *Drosophila melanogaster*. Science 1921;54:252–4.
[16] Patterson JT, Stone W, Bedichek S. The genetics of X-hyperploid females. Genetics 1935;20:259–79.
[17] Lindsley DL, Sandler L, Baker BS, Carpenter AT, Denell RE, Hall JC, et al. Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics 1972;71:157–84.
[18] Ripoll P. Effect of terminal aneuploidy on epidermal cell viability in *Drosophila melanogaster*. Genetics 1980;94:135–52.
[19] Laverty C, Lucci J, Akhtar A. The MSL complex: X chromosome and beyond. Curr Opin Genet Dev 2010;20:171–8.
[20] Larsson J, Chen JD, Rasheva V, Rasmuson-Lestander A, Pirrotta V. Painting of fourth, a chromosome-specific protein in Drosophila. Proc Natl Acad Sci USA 2001;98:6273–8.
[21] Johansson AM, Stenberg P, Bernhardsson C, Larsson J. Painting of fourth and chromosome-wide regulation of the 4th chromosome in *Drosophila melanogaster*. EMBO J 2007;26:2307–16.
[22] Belote JM, Lucchesi JC. Male-specific lethal mutations of *Drosophila melanogaster*. Genetics 1980;96:165–86.
[23] Vicoso B, Bachtrog D. Reversal of an ancient sex chromosome to an autosome in Drosophila. Nature 2013;499:332–5.
[24] Stenberg P, Lundberg LE, Johansson AM, Ryden P, Svensson MJ, Larsson J. Buffering of segmental and chromosomal aneuploidies in *Drosophila melanogaster*. PLoS Genet 2009;5:e1000465.
[25] Zhang Y, Malone JH, Powell SK, Periwal V, Spana E, Macalpine DM, et al. Expression in aneuploid Drosophila S2 cells. PLoS Biol 2010;8:e1000320.
[26] Devlin RH, Holm DG, Grigliatti TA. The influence of whole-arm trisomy on gene expression in Drosophila. Genetics 1988;118:87–101.
[27] Devlin RH, Holm DG, Grigliatti TA. Autosomal dosage compensation *Drosophila melanogaster* strains trisomic for the left arm of chromosome 2. Proc Natl Acad Sci USA 1982;79:1200–4.
[28] Malone JH, Cho DY, Mattiuzzo NR, Artieri CG, Jiang L, Dale RK, et al. Mediation of Drosophila autosomal dosage effects and compensation by network interactions. Genome Biol 2012;13:r28.
[29] Stark MB. An hereditary tumor in the fruit fly, Drosophila. J Cancer Res 1918;3:279–301.
[30] Stark MB. A benign tumor that is hereditary in Drosophila. Proc Natl Acad Sci USA 1919;5:573–80.
[31] Stark MB, Bridges CB. The linkage relations of a benign tumor in Drosophila. Genetics 1926;11:249–66.
[32] Stark MB. The origin of certain hereditary tumors in Drosophila. Am J Cancer 1937;31:253–67.
[33] Gateff E. Malignant neoplasms of genetic origin in *Drosophila melanogaster*. Science 1978;200:1448–59.
[34] Brumby AM, Richardson HE. Using *Drosophila melanogaster* to map human cancer pathways. Nat Rev Cancer 2005;5:626–39.

[35] Pastor-Pareja JC, Xu T. Dissecting social cell biology and tumors using Drosophila genetics. Annu Rev Genet 2013;47:51–74.
[36] Gonzalez C. *Drosophila melanogaster*: a model and a tool to investigate malignancy and identify new therapeutics. Nat Rev Cancer 2013;13:172–83.
[37] Ferres-Marco D, Gutierrez-Garcia I, Vallejo DM, Bolivar J, Gutierrez-Avino FJ, Dominguez M. Epigenetic silencers and Notch collaborate to promote malignant tumours by Rb silencing. Nature 2006;439:430–6.
[38] Pagliarini RA, Xu T. A genetic screen in Drosophila for metastatic behavior. Science 2003;302:1227–31.
[39] Brumby AM, Richardson HE. Scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. EMBO J 2003;22:5769–79.
[40] Uhlirova M, Jasper H, Bohmann D. Non-cell-autonomous induction of tissue overgrowth by JNK/Ras cooperation in a Drosophila tumor model. Proc Natl Acad Sci USA 2005;102:13123–8.
[41] Ohsawa S, Sato Y, Enomoto M, Nakamura M, Betsumiya A, Igaki T. Mitochondrial defect drives non-autonomous tumour progression through Hippo signalling in Drosophila. Nature 2012;490:547–51.
[42] Caussinus E, Gonzalez C. Induction of tumor growth by altered stem-cell asymmetric division in *Drosophila melanogaster*. Nat Genet 2005;37:1125–9.
[43] Betschinger J, Mechtler K, Knoblich JA. Asymmetric segregation of the tumor suppressor brat regulates self-renewal in Drosophila neural stem cells. Cell 2006;124:1241–53.
[44] Lee CY, Robinson KJ, Doe CQ. Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation. Nature 2006;439:594–8.
[45] Lee CY, Wilkinson BD, Siegrist SE, Wharton RP, Doe CQ. Brat is a Miranda cargo protein that promotes neuronal differentiation and inhibits neuroblast self-renewal. Dev Cell 2006;10:441–9.
[46] Dekanty A, Barrio L, Muzzopappa M, Auer H, Milan M. Aneuploidy-induced delaminating cells drive tumorigenesis in Drosophila epithelia. Proc Natl Acad Sci USA 2012;109:20549–54.
[47] Dekanty A, Milan M. Aneuploidy, cell delamination and tumorigenesis in Drosophila epithelia. Cell Cycle 2013;12:728–31.
[48] Uhlirova M, Bohmann D. JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumors in Drosophila. EMBO J 2006;25:5294–304.
[49] Fukasawa K. Centrosome amplification, chromosome instability and cancer development. Cancer Lett 2005;230:6–19.
[50] Basto R, Brunk K, Vinadogrova T, Peel N, Franz A, Khodjakov A, et al. Centrosome amplification can initiate tumorigenesis in flies. Cell 2008;133:1032–42.
[51] Castellanos E, Dominguez P, Gonzalez C. Centrosome dysfunction in Drosophila neural stem cells causes tumors that are not due to genome instability. Curr Biol 2008;18:1209–14.
[52] Bushman DM, Chun J. The genomically mosaic brain: aneuploidy and more in neural diversity and disease. Semin Cell Dev Biol 2013;24:357–69.
[53] Morais da Silva S, Moutinho-Santos T, Sunkel CE. A tumor suppressor role of the Bub3 spindle checkpoint protein after apoptosis inhibition. J Cell Biol 2013;201:385–93.
[54] Venkei Z, Przewloka MR, Glover DM. Drosophila Mis12 complex acts as a single functional unit essential for anaphase chromosome movement and a robust spindle assembly checkpoint. Genetics 2011;187:131–40.
[55] Muller HJ. The production of mutations by X-rays. Proc Natl Acad Sci USA 1928;14:714–26.
[56] Haynie JL, Bryant PJ. The effects of X-rays on the proliferation dynamics of cells in the imaginal wing discs of *Drosophila melanogaster*. Wilhelm Roux's Arch 1977;183:85–100.
[57] Brodsky MH, Nordstrom W, Tsang G, Kwan E, Rubin GM, Abrams JM. Drosophila p53 binds a damage response element at the reaper locus. Cell 2000;101:103–13.
[58] Peters M, DeLuca C, Hirao A, Stambolic V, Potter J, Zhou L, et al. Chk2 regulates irradiation-induced, p53-mediated apoptosis in Drosophila. Proc Natl Acad Sci USA 2002;99:11305–10.
[59] Perez-Garijo A, Martin FA, Morata G. Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in Drosophila. Development 2004;131:5591–8.
[60] Perez-Garijo A, Shlevkov E, Morata G. The role of Dpp and Wg in compensatory proliferation and in the formation of hyperplastic overgrowths caused by apoptotic cells in the Drosophila wing disc. Development 2009;136:1169–77.
[61] Warner SJ, Yashiro H, Longmore GD. The Cdc42/Par6/aPKC polarity complex regulates apoptosis-induced compensatory proliferation in epithelia. Curr Biol 2010;20:677–86.
[62] McEwen DG, Peifer M. Puckered, a Drosophila MAPK phosphatase, ensures cell viability by antagonizing JNK-induced apoptosis. Development 2005;132:3935–46.
[63] McNamee LM, Brodsky MH. p53-independent apoptosis limits DNA damage-induced aneuploidy. Genetics 2009;182:423–35.
[64] Wichmann A, Jaklevic B, Su TT. Ionizing radiation induces caspase-dependent but Chk2- and p53-independent cell death in *Drosophila melanogaster*. Proc Natl Acad Sci USA 2006;103:9952–7.
[65] Dekanty A, Barrio L, Milan M. Contributions of DNA repair, cell cycle checkpoints, and cell death to suppressing the DNA damage-induced tumorigenic behavior of Drosophila epithelial cells. Oncogene 2014, http://dx.doi.org/10.1038/onc.2014.42 [Epub ahead of print].
[66] Staeva-Vieira E, Yoo S, Lehmann R. An essential role of DmRad51/SpnA in DNA repair and meiotic checkpoint control. EMBO J 2003;22:5863–74.
[67] Kooistra R, Vreeken K, Zonneveld JB, de Jong A, Eeken JC, Osgood CJ, et al. The *Drosophila melanogaster* RAD54 homolog, DmRAD54, is involved in the repair of radiation damage and recombination. Mol Cell Biol 1997;17:6097–104.
[68] Romeijn RJ, Gorski MM, van Schie MA, Noordermeer JN, Mullenders LH, Ferro W, et al. Lig4 and rad54 are required for repair of DNA double-strand breaks induced by P-element excision in Drosophila. Genetics 2005;169:795–806.
[69] McVey M, Larocque JR, Adams MD, Sekelsky JJ. Formation of deletions during double-strand break repair in Drosophila *DmBlm* mutants occurs after strand invasion. Proc Natl Acad Sci USA 2004;101:15694–9.
[70] McVey M, Adams M, Staeva-Vieira E, Sekelsky JJ. Evidence for multiple cycles of strand invasion during repair of double-strand gaps in Drosophila. Genetics 2004;167:699–705.
[71] Garcia AM, Calder RB, Dolle ME, Lundell M, Kapahi P, Vijg J. Age- and temperature-dependent somatic mutation accumulation in *Drosophila melanogaster*. PLoS Genet 2010;6:e1000950.
[72] Salomon RN, Jackson FR. Tumors of testis and midgut in aging flies. Fly (Austin) 2008;2:265–8.
[73] Kusano K, Johnson-Schlitz DM, Engels WR. Sterility of Drosophila with mutations in the Bloom syndrome gene—complementation by Ku70. Science 2001;291:2600–2.
[74] Garcia AM, Salomon RN, Witsell A, Liepkalns J, Calder RB, Lee M, et al. Loss of the bloom syndrome helicase increases DNA ligase 4-independent genome rearrangements and tumorigenesis in aging Drosophila. Genome Biol 2011;12:R121.
[75] Clemente-Ruiz M, Muzzopappa M, Milán M. Tumor suppressor roles of CENP-E and Nsl1 in Drosophila epithelial tissues. Cell Cycle 2014;13(9.) [Epub ahead of print].
[76] Pavelka N, Rancati G, Zhu J, Bradford WD, Saraf A, Florens L, Sanderson BW, Hattem GL, Li R. Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. Nature 2010;468(7321):321–5.
[77] Birchler JA, Veitia RA. Gene balance hypothesis: connecting issues of dosage sensitivity across biological disciplines. Proc Natl Acad Sci U S A 2012;109(37):14746–53.
[78] Sheltzer JM, Torres EM, Dunham MJ, Amon A. Transcriptional consequences of aneuploidy. Proc Natl Acad Sci U S A 2012;109(31):12644–9.
[79] Tang YC, Amon A. Gene copy-number alterations: a cost-benefit analysis. Cell 2013;152(3):394–405.