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Safeguarding genetic information in *Drosophila*

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Abstract

Eukaryotic cells employ a plethora of conserved proteins and mechanisms to ensure genome integrity. In metazoa, these mechanisms must operate in the context of organism development. This mini-review highlights two emerging features of DNA damage responses in *Drosophila*: a crosstalk between DNA damage responses and components of the spindle assembly checkpoint, and increasing evidence for the effect of DNA damage on the developmental program at multiple points during the *Drosophila* life cycle.

Introduction

Generally speaking, eukaryotic cells ensure genetic integrity through successive cellular generations by following four basic rules: (1) that each genomic DNA molecule is copied precisely once and only once during S phase, (2) that chromosome duplication (S phase) alternates with chromosome segregation (M phase), (3) that during M phase, duplicated chromosomes are equally divided between daughter cells, and (4) that cells respond appropriately to eliminate damaged DNA, by pausing proliferation to fix the damage or by dying (Fig. 1a). Rule 1 is enforced by mechanisms that ensure that origins of DNA replication are competent to initiate only once per S phase. Mechanisms that enforce rule 2 include the DNA replication checkpoint that blocks mitosis in response to incompletely replicated DNA and those that allow origin licensing only when cells have successfully completed mitosis. Mechanisms that enforce rule 3 comprise the spindle assembly checkpoint (SAC) that monitors the attachment of sister chromatids to the spindle and allows chromosome segregation only when bipolar attachments have been achieved. Mechanisms that enforce rule 4 are collectively referred to as the DNA damage response (DDR) and include cell cycle arrest by checkpoints, activation of DNA repair, and apoptosis.

The following sections summarize the mechanistic understanding of these rules in *Drosophila*. These sections are meant to serve as a portal for other articles that discuss each rule in more detail and to provide context for more detailed discussions of two interesting features of *Drosophila* DDR: a crosstalk between the DNA and spindle checkpoints and the link between genome surveillance mechanisms and organism development.

Rule 1. Replicate once and only once per S phase

To preserve genetic information through the cell cycle, each genomic DNA sequence must be replicated once and only once per S phase. Studies in yeast and vertebrate systems inform us on how this control is exerted at the level of the initiation of DNA replication.

Chromosomal origins of DNA replication are competent to initiate only once per S phase, thus preventing re-replication of any locus within each S phase. Initiation requires the assembly of multi-protein complexes at the origins. The Origin Recognition Complex (ORC) remains bound at potential origins through the cell cycle but recruit additional factors to form the pre-Replication Complex (pre-RC) as cells exit mitosis. Besides ORC, key components of the pre-RC include the MCM2-7 helicase complex, Cdc6, and Cdt1 (Li and Jin 2011; Tanaka and Araki 2010; Truong and Wu 2011). The latter two are needed to load the MCMs. As cells transit through G1, pre-RC recruits additional proteins such as CDC45 to form the pre-initiation complex (pre-IC). Once initiated, pre-RC disassembles and is prevented from reassembly within the same S phase. Multiple levels of control that prevent pre-RC reassembly in other systems also operate in *Drosophila*. For example, *Drosophila* Cdt1, encoded by *double park* (*dup*; see Table 1 for the names of *Drosophila* genes and their orthologs in other organisms), is degraded as cells transit G1/S and is further restrained through direct inhibition by Geminin protein that is present through S, G2, and early M phases (Quinn et al. 2001; Thomer et al. 2004; Whittaker et al. 2000).

Studies in *Drosophila* add another dimension, how rules are broken to meet developmental needs. A clear exception to the rule of replicating once per S phase is seen in the case of chorion gene amplification in follicle cells that surround the oocyte. These cells are charged with extensive synthesis of a small number of proteins needed to make the eggshell and do so by selectively amplifying specific chromosomal loci while the rest of the genome remains unreplicated. Amplification of chorion loci occurs by repeated firing of specific origins within a single S phase (Tower 2004). Amplifying loci retain ORC, Dup (Cdt1), and MCM proteins, and recruitment of an ORC subunit, ORC2, to these loci requires transcription factors, E2F1/Dp (Bosco et al. 2001; Claycomb et al. 2002; Royzman et al. 1999). *Drosophila* homolog of oncoprotein Myb is also found at chorion loci, and Myb binding may be necessary for amplification as well (Beall et al. 2002). Once initiated, progression of replication forks at one chorion locus, ACE3 on chromosome III, is limited to about 50 kb in each direction (Park et al. 2007). Interestingly, limitation of fork movement requires a G1 cyclin, cyclin E. Localized recruitment of ORC via association with transcription factors and changes in cyclin/cdk activity are thus thought to play a role in breaking rule 1 in this case.

Studies, mostly from yeast and *Xenopus*, have revealed another level of regulation that ensures that replication, once initiated, goes to completion, thereby obeying the rule to “replicate once.” Preserving and protecting replication forks from DNA damage, disassembly, or stalling require proteins that function in the DNA damage checkpoint (for review, see Branzei and Foiani 2010; Zegerman and Diffley 2009). Corresponding mechanisms in *Drosophila* remain to be investigated.

Rule 2. Alternate S and M phases

The failure to alternate S and M phases would lead to polyploidy (from successive S phases) or aneuploidy (from successive M phases). S and M alternate during normal proliferation because only cells that have completed S phase can undergo mitosis, and only cells that have completed mitosis can undergo S phase. A DNA replication checkpoint becomes activated once replication initiates and prevents mitosis until after S phase is complete (Smits et al. 2010). This mode of regulation is essential for the success of *Drosophila* embryogenesis. *Drosophila* embryogenesis begins with 13 rapid synchronous nuclear divisions that occur in a common cytoplasm (a syncytium) and rely on maternally supplied gene products. Interphase in syncytial cycles consists only of S phase and no detectable gap phases. Indeed, DNA replication has been shown to maintain interphase length by delaying the onset of mitosis (McClelland et al. 2009; Shermoen et al. 2011). This regulation requires DNA checkpoint kinases ATR (encoded by *mei-41*) and Chk1 (encoded by *grapes*). In embryos

lacking *mei-41* or *grp* gene products, nuclei enter mitosis ahead of schedule. Because interphase comprises S phase, nuclei in *mei-41* and *grp* mutants are thought to enter mitosis with incompletely replicated DNA. The presence of γ -H2Av on mitotic chromosomes in *grp*-deficient embryos supports this interpretation (Takada et al. 2007). The ultimate consequence of premature mitotic entry is chromosome segregation failure and embryonic death.

Another set of mechanisms ensures that only cells that have completed mitosis are competent to enter S phase. Generally speaking, eukaryotic cells achieve this by coupling the building of pre-RCs to protein degradation that occurs during mitosis (Li and Jin 2011; Tanaka and Araki 2010; Truong and Wu 2011). Pre-RC formation is prevented by mitotic cyclin/cdk activity and by Geminin homologs. These activities are present during S, G2 and early M phases. Mitotic cyclins and Geminin are targeted for Ubiquitin-mediated degradation as cells exit mitosis. In fact, degradation of mitotic cyclins is a prerequisite for successful exit from M. Mitotic cyclin/cdks phosphorylate and inhibit ORC and MCM subunits as well as CDC6 whereas Geminin binds and inhibits Cdt1, which is needed to load MCMs. Thus, only cells that have successfully degraded mitotic cyclins, along with Geminin, can build pre-RCs and have the ability to enter S phase.

In *Drosophila* embryos, association between MCMs and chromosomes occurs in anaphase, when mitotic cyclin/cdk activity is declining. Of three *Drosophila* mitotic cyclins, A, B, and B3, cyclin A (complexed to Cdk1) may be the most relevant to inhibition of pre-RC formation; a non-degradable mutant of cyclin A but not B blocks MCM–chromosome association (B3 was not tested) (Su and O’Farrell 1997). Knocking down cyclin A, but not cyclin B, in *Drosophila* SD2 cells also led to complete re-replication of the genome (Mihaylov et al. 2002). Prevention of re-replication by cyclin A/cdk1 appears to be further reaching than regulation by Geminin in *Drosophila*. Depletion of Geminin in *Drosophila* Kc cells by RNAi increased DNA content but produced less than complete re-replication of the genome (Ding and MacAlpine 2010). Re-replication in Kc cells accompanies re-loading of MCMs and preferentially occurs in per-centric heterochromatin. Co-depletion of cyclin A, in contrast, led to complete re-replication, much like in SD2 cells. How re-replication in heterochromatin escapes regulation by cyclin A in these cells remains to be investigated.

A blatant disregard for the rule to alternate S and M phases is seen in endoreplicating cells of *Drosophila* larvae. *Drosophila* larvae are composed of diploid and polyploid cells. Diploid cells differentiate into adult structures during metamorphosis whereas most polyploid cells are histolysed during metamorphosis to serve a nutritive role. A rare exception occurs in some polyploid cells of the larval rectum that return to mitotic cycles during pupal growth (Fox et al. 2010). These increase in number via error-prone mitosis during metamorphosis and contribute to adult rectum.

Polyploidy in larvae is achieved by successive rounds of S phase without intervening mitosis in endoreplication cycles. The number of rounds of endoreplication larval cells undergo depends on the tissue, with cells of the salivary glands, for example, reaching more than 1,000C and cells of the fat body reaching about 200C (Nordman et al. 2010).

Endoreplication cycles break not one but two rules. First, S phase does not alternate with M; in fact, there are no mitoses. The erasure of mitotic program occurs by the absence of expression of mitotic cyclins. Furthermore, G1 cyclin/cdk activity provided by cyclin E/cdk2 oscillates. Pre-RCs are built when cdk activity is low, and replication initiates when cdk activity is high (Lilly and Duronio 2005), thereby producing successive rounds of S phase. Second, during each S phase, not all DNA sequences are replicated. There is selective under-replication of certain loci in both heterochromatic and euchromatic regions. Under-

replication is an active, regulated process; mutants in cyclin E or Suppressor of Under-Replication (SuUR) undergo a more complete S phase (Lilly and Spradling 1996; Makunin et al. 2002; Pindyurin et al. 2008). SuUR shares regions of amino acid similarity with SNF2/SWI2 chromatin remodeling proteins and binds to Heterochromatin Protein 1 (HP1). Comparative genomics analysis of salivary gland, fat body, and mid-gut cells reveals under-replication of common loci as well as tissue-specific loci (Nordman et al. 2010). While under-replication is associated with reduced transcription and repressive chromatin marks, regions with active transcription are also known to be under-replicated, and regions with silent transcription can also escape under-replication. The role of under-replication remains to be determined; SuUR mutants are viable and fertile (Belyaeva et al. 1998).

Rule 3. Segregate chromosomes equally into daughter cells in mitosis

Equal segregation of duplicated chromosomes into daughter cells during mitosis requires that sister chromatids of each chromosome achieve a bipolar attachment to the spindle. Incorrect attachments trigger the SAC and prevent the onset of anaphase. A detailed description of proteins that associate with the kinetochore and participate in the SAC in *Drosophila* can be found in recent reviews (Karess 2005; Orr et al. 2010). The key proteins of SAC are the homologs of yeast Mad, Bub, and Mps1. In addition, in metazoa, an RZZ complex made of Rod, Zw-10, Zwilch, and possibly other proteins also functions in SAC; for example, *Drosophila* Rod and Zw-10 are required for the recruitment of Mad2 to the kinetochore (Buffin et al. 2005).

Crosstalk between DNA checkpoints and SAC proteins

Earlier analysis of *dup* (Cdt1) mutants suggested a crosstalk between DNA defects and SAC. Cells in *dup* mutant embryos do not complete S phase and delay entry into mitosis via activation of the DNA replication checkpoint. But once these cells enter mitosis, they experience yet another delay before anaphase (hence the name “*double park*”) (Whittaker et al. 2000). The second delay requires *mei-41* (ATR) as well as BubR1. BubR1 is a SAC kinase that localizes to unattached kinetochores and prevents anaphase onset by inhibiting the APC, an E3 Ub-ligase complex that targets mitotic cyclins for degradation. The requirement for BubR1 was thought to be indirect; unreplicated centromeres would preclude bipolar attachment and thus trigger SAC (Garner et al. 2001). More recent studies in larval neuroblasts also found a role for BubR1 in responding to DNA breaks, but indicated a more direct role (Royou et al. 2010, 2005). In these cells, double-strand breaks caused by a sequence-specific endonuclease, I-CreI, also triggered a delay in anaphase onset. The delay requires Grapes (Chk1) and Polo kinase, whose homologs have roles in multiple stages during the cell cycle. Interestingly, BubR1 is not necessary to delay anaphase onset in this context but serves a different function. I-CreI cuts within centric heterochromatin to create acentric chromosome fragments. Surprisingly, extensive and persistent presence of acentric fragments did not alter organism survival. Further analysis revealed that acentric chromosome fragments remain attached to their centric counterparts by DNA tethers and segregate correctly for the most part (Fig. 1b). The DNA tethers are coated with BubR1 and Polo. BubR1/Polo-coated DNA tethers also help maintain chromosome fragments that result from X-ray-induced cuts in euchromatin.

BubR1 also plays a direct role in responding to DNA double-strand breaks at uncoated telomeres. Unlike in other eukaryotes, *Drosophila* telomeres are not made of simple repeated DNA sequences but are composed of retro-transposon sequences that are maintained by transposition in a telomerase-independent manner. Moreover, chromosome termini that lack retro-transposons can still recruit telomere proteins and remain stable through cellular generations. Despite these differences, assembly of protein complexes, protection of telomeres, and prevention of telomere fusion in *Drosophila* and other

eukaryotes depend on conserved proteins such as ATM and MRN complex (Mre11, Rad50, and Nbs1) (reviewed in Cenci 2009; Cenci et al. 2005). In addition, *Drosophila* telomeres also recruit HP1, ORC-Associated Protein (HOAP). In mutants in HOAP (encoded by *caravaggio*), telomeres in larval neuroblasts are uncapped and trigger DDR (Cenci et al. 2003). In these cells, BubR1 localizes to telomeres and contributes to delaying anaphase onset (Musaro et al. 2008).

The role of SAC proteins in response to telomere defects may be conserved in other eukaryotes. Mad2 is required for cell cycle arrest of a yeast mutant with elevated single-stranded DNA in sub-telomeric regions (Maringele and Lydall 2002). And in mouse epithelial cells overexpressing TRF1, telomeres are shorter and show localization of BubR1 and Mad2 (Munoz et al. 2009). The significance of telomeric localization of SAC proteins in the latter situation remains to be investigated.

Rule 4. Fix the damage or die (unless you are an endoreplicating cell)

DNA double-strand breaks (DSBs) activate responses that include cell cycle arrest by checkpoints, activation of DNA repair and apoptosis, and are collectively referred to as DDR (for recent reviews, see Cook 2009; Smith et al. 2010; Smits et al. 2010; Zegerman and Diffley 2009). DDR in eukaryotes is facilitated by protein complexes that sense the damage (e.g., MRN and 9–1–1 complexes), protein kinases that transduce the signal (e.g., ATM, ATR, Chk1, and Chk2), and effectors (e.g., p53, CDC25) that become modified and, in turn, produce changes in the proteome and the transcriptome within the cell. One hallmark of DDR is the phosphorylation of the tail of histone variant H2AX (H2Av in *Drosophila*). This modification, referred to as γ -H2AX or γ -H2Av, can be detected with an antibody and serves as a ready assay for successful sensing of DNA damage.

The core components of DDR are conserved in *Drosophila* and, where examined, perform similar or related functions (reviewed in Su 2006). In addition, a recent genome-wide RNAi screen in *Drosophila* cell culture has identified new genes needed for G2/M checkpoint in response to DNA damage, *mus101* (encoding an ortholog of mammalian TOBP1) and *mus312* (encoding an ortholog of mammalian BTBD12 that provides resolvase activity during DSB repair) (Kondo and Perrimon 2011). Mus101 was one of several members of the replication pre-initiation complex identified in the screen, suggesting that pre-IC has an unexpected role in DNA damage response at G2/M. Studies in *Drosophila* have added to our knowledge of DDR by illustrating how conserved DDR functions can be utilized in cell, tissue, and developmental stage-specific manner to safeguard the genome yet allow plasticity in regulation inherent to metazoan development.

As described in a preceding section, nuclei in *Drosophila* embryos depleted of ATR (Mei-41) or Chk1 (Grp) enter mitosis without completing DNA replication. In these and in embryos in which nuclei enter mitosis with DSBs, a Chk2-dependent checkpoint becomes activated and targets the centrosome (Sibon et al. 2000; Takada et al. 2003). This results in dispersal of γ -Tubulin Ring Complex from the centrosome, failure to segregate chromosomes, and return to interphase without completion of karyokinesis. Aneuploid nuclei thus formed are eliminated from the monolayer of nuclei that will contribute to subsequent development. This response could selectively eliminate damaged nuclei while leaving intact ones alone and could be a syncytial equivalent of apoptosis.

DNA damage induced by ionizing radiation or genetic manipulations elicit canonical DDR responses in the diploid cells of the larvae that include cell cycle arrest, DNA repair, and apoptosis (e.g., Brodsky et al. 2004; McNamee and Brodsky 2009; Oikemus et al. 2004; Wichmann et al. 2006). In contrast, endoreplicating cells of the larvae do not show a full spectrum of DDR seen in diploid cells (Mehrotra et al. 2008). Chromosomes during normal

endoreplication contain under-replicated regions and show γ -H2Av staining. Overexpression of Dup (Cdt1) in diploid cells also results in incomplete re-replication of the genome and γ -H2Av staining. Overexpression of Dup in endoreplicating cells increases the γ -H2Av further. While the presence of γ -H2Av is indicative of damage recognition in both diploid and endoreplicating cells, diploid cells undergo apoptosis, but endoreplicating cells do not. Indeed, a chromosomal locus that contains pro-apoptotic genes *hid*, *rpr*, and *skl* becomes transcriptionally active in diploid cells but not in endoreplicating cells in larvae that are overexpressing Dup.

Developmental regulation in response to loss of genetic integrity

In addition to triggering DDR, DNA double-strand breaks can also arrest development. What might be the purpose of such a response? Inhibition of development could serve a quality control purpose, for example by removing defective eggs before they contribute to the population. This may be the population equivalent of programmed cell death that removes damaged cells from a multi-cellular organism. Another benefit could be that it is important to pause development, specifically the expression of developmentally relevant genes, until after damage has been fixed or the defective cell has been removed by apoptosis and replaced through compensatory proliferation. Whatever the reason, there is a clear link between DNA damage and development. The link is seen at multiple stages during the fly life: oogenesis, embryogenesis, and larval–pupal transition. Furthermore, Chk2 has emerged as a mediator of developmental control in response to DNA damage as illustrated by the following examples.

Meiotic recombination during oocyte development is initiated by chromosomal double-strand breaks. This accompanies the activation of p53 as seen by reporter activity (Lu et al. 2010). Failure to complete recombination results in persistent DSBs that trigger the meiotic checkpoint through *mei-41* (ATR) and *mnk* (Chk2) (Abdu et al. 2002; Ghabrial and Schupbach 1999), as well as persistent p53 activation (Lu et al. 2010). Initiation of meiotic checkpoint is distinct from the pachytene checkpoint that monitors the structure of chromosome axes and may function to promote an optimal number of crossovers (Joyce and McKim 2009). One downstream effect of meiotic checkpoint is the inhibition of accumulation of *gurken*, which encodes a TGF- α homolog. The failure to accumulate Gurken disrupts dorsal–ventral patterning of the egg and results in female sterility. Meiotic checkpoint also responds to DNA damage in mutants in rasiRNA pathways that suffer elevated transposition (Chen et al. 2007; Klattenhoff et al. 2007). A recent study identified the involvement of yet another kinase and another outcome of meiotic checkpoint. Reorganization of the oocyte nucleus that normally occurs is disrupted in mutants that fail to complete recombination, and this response requires NHK-1 kinase (Lancaster et al. 2010).

After the 13th syncytial cycle, the *Drosophila* embryo undergoes maternal-to-zygotic transition (MZT) that shares many key features with vertebrate mid-blastula transition. These include the lengthening of interphase and the onset of zygotic transcription. In embryos that lack maternally supplied products of *mei-41* (ATR) and *grapes* (Chk1), nuclei fail to lengthen interphase and enter mitosis prematurely. *mei-41* or *grp*-depleted embryos also fail to undergo MZT and lack zygotic transcription. The latter defects are the result of a Chk2-dependent checkpoint; mutation in *mnk* can restore zygotic transcription to *mei-41* and *grp* mutants (Fogarty et al. 1997; Sibon et al. 1999, 1997; Takada et al. 2007).

The connection between DNA DSBs and the developmental program is also seen in embryos after MZT. Genome-wide gene expression analysis shows that exposure to ionizing radiation results in a decrease in mRNA encoding developmentally relevant proteins such as *Drosophila* Wnt8 homolog and Tll, a nuclear hormone receptor with a role in neural development (Brodsky et al. 2004). The reduction in transcripts of most genes thus

identified was dependent on Chk2, again implicating this kinase in stalling development in response to damaged DNA.

Tissue damage by ionizing radiation or genetic manipulation in the larvae delays the onset of pupariation. This response is exacerbated in *mei-41* (ATR) mutants (Jaklevic and Su 2004) and is achieved by retinoic acid-dependent delay in the production of molting hormone ecdysone (Halme et al. 2010). The delay is thought to allow tissue regeneration before metamorphosis.

Why study safeguards of genetic integrity in *Drosophila*?

With multiple experimental systems to choose from, one should ask what the advantages are of studying mechanisms that protect genetic integrity in *Drosophila*. Five reasons are given here, but there may be more. First, in addition to cell-autonomous responses, *Drosophila* can be used to study systemic responses to DNA damage. For example, localized UV irradiation of the larval epidermis results in changes in the number of circulating hemocytes, activation of JAK/STAT signaling in the rest of the larval body, and transient inhibition of insulin-like growth factor signaling (Karpac et al. 2011). These responses appear to be important for larval survival after UV exposure. Second, *Drosophila* is an ideal system to study how cellular responses to DNA defects may be coordinated with tissue differentiation, regeneration, and development. Several examples described in this mini-review illustrate this advantage. Third, *Drosophila* is an ideal system to study the regulation and mis-regulation that leads to gene amplification and altered ploidy. Both phenomena are relevant to normal metazoan development and to disease. Fourth, *Drosophila* is already an established model for the study of chromatin structure and epigenetics. Therefore, it is ideally suited for current and future analyses of the role of epigenetics in regulation of DNA replication, DNA repair, and chromosome segregation. For example, a recent study shows that DSBs in heterochromatin initiate DDR and DNA repair but then are moved outside of the heterochromatin domain for the completion of repair (Chiolo et al. 2011). In another example, genome-wide chromatin modifications have been mapped and correlated with the timing of replication and protein association, setting the stage for functional studies (Eaton et al. 2010, 2011). The last two studies and another that has revealed additional loci of gene amplification (Nordman et al. 2010) help illustrate the final advantage of *Drosophila*, which is the feasibility of whole-genome analyses that hold much promise.

There are firm examples of findings in *Drosophila* that foreshadow what would be seen in mammalian systems. Two examples are provided here. *Drosophila* cyclin E was found to promote the loading of MCMs onto chromatin immediately before S phase in endoreplicating cells of larval salivary glands (Su and O'Farrell 1998). This came as a surprise because cyclin/cdks typically inhibit MCM loading. Analysis of cyclin E knock-out mice and cells several years later revealed a similar situation (Geng et al. 2003; Parisi et al. 2003). Mouse embryo fibroblasts that lack cyclin E fail to load MCMs onto chromatin at the G0 to G1 transition, paralleling the result in *Drosophila*. Remarkably, mice lacking both cyclin E1 and cyclin E2 die during embryogenesis due to the loss of placental tissue; mutant embryos could be rescued to birth by replacing mutant placental cells with wild type. Further analysis showed that cyclin E is essential for endoreplication cycles of trophoblasts, progenitors of the placenta, further illustrating the parallel between *Drosophila* and mice. In another example, dying cells in the larval imaginal disk epithelium were found to stimulate proliferation of the neighbors by signaling through JNK, Wg (Wnt), Dpp, and Hh (Fan and Bergmann 2008; Martin et al. 2009; Ryoo et al. 2004). A similar phenomenon has now been seen in a mammalian system, wherein apoptotic cells signal to the neighbors to stimulate proliferation although a different signaling pathway than *Drosophila* (Li et al. 2010). We predict that there will be many more examples of how cells behave in a multi-cellular and developmental context in *Drosophila* that will be applicable to mammalian systems.

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Fig. 1.

a The chromosome cycle and four rules that govern it to safeguard genetic integrity. During a canonical cell cycle, each molecule of chromosomal DNA is copied once and only once per S phase (rule 1). Ongoing S phase inhibits the start of M (2a). Likewise, cells in mitosis cannot initiate S until after protein degradation at exit from M (2b). Thus, S and M phases are mutually exclusive, and initiation of one requires the completion of the other. This ensures that S and M phases alternate (rule 2). G1 and G2 are in *parentheses* to indicate the fact that some cell cycles lack gap phases. The Spindle Assembly Checkpoint (SAC) ensures equal chromosome segregation in mitosis (rule 3) by inhibiting anaphase onset until all sister chromatids have achieved bipolar attachment. Cells respond to damaged DNA by arresting the cell cycle, activating DNA repair, or undergoing apoptosis (rule 4). In *Drosophila* (responses in *green*), gene amplification violates rule 1, while endoreplication cycles violate rule 2. Responses to DNA damage include the disruption of development at multiple points in the life cycle. **b** Crosstalk between DNA damage and SAC in *Drosophila* includes the delay in metaphase–anaphase transition in response to uncapped telomeres that operates

through SAC proteins and the role of BubR1 in the formation of DNA tethers that help segregate acentric chromosome fragments

Table 1

Drosophila genes and their orthologs in other eukaryotes

Gene name	Protein name (if different)	Molecular function in <i>Drosophila</i>	Cellular process involved in	Vertebrate ortholog
<i>double park (dup)</i>		MCM loading	Initiation of DNA replication	CDT1
<i>mei-41</i>		Protein kinase (predicted)	DNA checkpoints	ATR
<i>grapes (grp)</i>		Protein kinase (predicted)	DNA checkpoints	Chk1
SuUR		?	Prevents complete replication of the genome during endoreplication	?
<i>caravaggio</i>	HOAP	Binds HP1 and ORC	Protein recruitment at telomeres, telomere capping	?
<i>his2Av</i>	H2Av	DNA binding (histone variant)	DDR	H2AX
<i>mus101</i>		?	G2/M checkpoint	TOPBP1
<i>mus312</i>		Binds to proteins involved in DSB repair	Meiotic recombination, G2/M checkpoint	BTBD12
<i>hid, rpr, skl</i>		Bind IAP (Inhibitor of Apoptosis Protein)	Caspase activation and apoptosis	Smac/DIABLO
<i>mnk</i>		Protein kinase (predicted)	DNA checkpoints	Chk2
<i>gurken (grk)</i>		Growth factor, receptor binding	Oocyte patterning	TGF- α
<i>ballchen, nhk-1</i>	NHK-1	Protein kinase	Meiotic checkpoint	VRK-1

Genes with obvious orthologs and functional information (e.g., cyclin A) have been omitted