Chapter 9

Germ Cell Apoptosis and DNA Damage Responses

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Abstract In the past 12 years, since the first description of *C. elegans* germ cell apoptosis, this area of research rapidly expanded. It became evident that multiple genetic pathways lead to the apoptotic demise of germ cells. We are only beginning to understand how these pathways that all require the CED-9/Bcl-2, Apaf-1/CED-4 and CED-3 caspase core apoptosis components are regulated. Physiological apoptosis, which likely accounts for the elimination of more than 50% of all germ cells, even in unperturbed conditions, is likely to be required to maintain tissue homeostasis. The best-studied pathways lead to DNA damage-induced germ cell apoptosis in response to a variety of genotoxic stimuli. This apoptosis appears to be regulated similar to DNA damage-induced apoptosis in the mouse germ line and converges on p53 family transcription factors. DNA damage response pathways not only lead to apoptosis induction, but also directly affect DNA repair, and a transient cell cycle arrest of mitotic germ cells. Finally, distinct pathways activate germ cell apoptosis in response to defects in meiotic recombination and meiotic chromosome pairing.

Keywords Apoptosis • DNA repair • DNA damage response • Meiosis checkpoints • Recombination

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9.1 Introduction

Apoptosis is a highly conserved mechanism to eliminate superfluous or damaged cells in multicellular eukaryotes. Apoptosis is remarkably prevalent in germ cells. It is estimated that out of ~7,000,000 germ cells occurring in the ovaries of 5 months old human embryos only ~300,000 remain at an age of 7 years, and less than a thousand oocytes are left in the years before ovarian senescence; for review, see Tilly (2001) and Morita and Tilly (1999). It thus appears that apoptosis is especially important during germ cell development. This is likely to be the case because germ cells and somatic cells are subjected to distinct selective pressures. Somatic cells are optimized to contribute to the fitness for one generation, whereas germ cells are optimized both for the maintenance of eternal proliferative potential and for maintaining pluripotency to allow for differentiation after fertilization. Surveillance mechanisms are thus likely to be extremely important in the germline to ensure quality control. Germ cell apoptosis might also be required to maintain germline tissue homeostasis. This is especially challenging given the enormous size of mature oocytes compared to other germ cells. The dichotomy between somatic cells and germ cells is most extreme in the worm life cycle. Somatic cells are entirely post-mitotic and invariantly derive from the fertilized zygote by a very limited number of cell cycles, rather than being continuously replenished by adult somatic stem cells as in most other animals. Thus, while somatic tissues may tolerate high levels of DNA damage or other environmental insults, germline-specific checkpoints might have evolved to guard germ cells from acquiring deleterious mutations that could be passed from one generation to the next.

We summarize our knowledge of germ cell apoptosis in the *C. elegans* germline. Since the first report on worm germ cell apoptosis in 1999 it became clear that multiple pathways are required (Gumienny et al. 1999). A basal level of germ cell apoptosis, termed physiological germ cell apoptosis, occurs in the absence of external stimuli and appears to be linked to maintaining tissue homeostasis (Gartner et al. 2000). Furthermore, germ cells are culled by genetically distinct checkpoints that monitor genome integrity, meiotic recombination, and meiotic chromosome synapsis (Gartner et al. 2000; Bhalla and Dernburg 2005). At present we know most about DNA damage response pathways leading to apoptosis. Finally, stresses such as starvation, heat shock, bacterial infection, or a high glucose diet also trigger excessive germ cell apoptosis (Salinas et al. 2006; Angelo and Van Gilst 2009; Aballay and Ausubel 2001; Choi 2011).

9.2 The Core Apoptosis Pathway in *C. elegans*

Seminal studies on *C. elegans* apoptosis occurring during the invariant development of the worm were important for our general understanding of apoptosis induction. EGL-1, CED-4, and CED-3 are conserved proteins required for the vast majority of the 131 cell deaths that occur during the invariant development; for review, see

Conradt and Xue (2005) and Conradt (2009). The sole worm Bcl2 family member, CED-9, is required to protect healthy cells from undergoing apoptosis. *ced-9* loss-of-function mutants lead to the induction of apoptosis in cells that are normally destined to survive, while a *ced-9* gain-of-function mutant was shown to be defective for apoptosis induction. In the worm, apoptosis in somatic tissues is thought to typically involve the transcriptional up-regulation of the BH3-only domain protein EGL-1 in cells destined to die. Combinations of transcriptional regulators have been found to be required for the transcriptional induction of *egl-1* in specific cells destined to die. EGL-1 antagonizes CED-9, which in turn antagonizes the activity of the pro-apoptotic CED-4, the Apaf1 homolog. According to the current model, mitochondrial CED-9 directly binds pro-apoptotic CED-4 to inhibit apoptosis induction. Upon transcriptional induction, EGL-1 binding to CED-9 leads to the disruption of the CED-9/CED-4 complex and to the release of CED-4 from mitochondria. This event leads to the oligomerization of CED-4 and to the ultimate activation of apoptosis induction through CED-3 caspase activation. However, more recent evidence suggests that there might not be a direct interaction of CED-9 and CED-4 in cells destined to survive, implying a more complex mode of regulation of CED-9 and CED-4 (Pourkarimi et al. 2012). In the worm, in contrast to germ cell apoptosis, somatic apoptosis appears to be part of a developmental process and neither occurs in adult animals nor in response to environmental stimuli.

9.3 *C. elegans* Germ Cell Apoptosis

When *C. elegans* germ cell apoptosis was first described in 1999 (Gumienny et al. 1999), it was observed that a steady-state level of ~1–5 apoptotic corpses occur, the number of which increases with the age of the worm. This apoptosis, termed physiological germ cell death apoptosis, occurs in the absence of any stress and was estimated to eliminate approximately 50% of all germ cells. Indeed this number, initially measured by the number of corpses observed at a given time in relation to the number of oocytes that are laid, might be even higher. More recent estimates of germ cell proliferation rates indicate that approximately 20 germ cells are produced every hour, while only ~3 oocytes are laid (Fox et al. 2011). Apoptosis only occurs in the female and not in the male germline and is thus only present in hermaphrodites (Gumienny et al. 1999; Gartner et al. 2000; Jaramillo-Lambert et al. 2010). Mutational analysis indicated that a female germline is required for apoptosis, while the gender of somatic tissues does not affect germ cell apoptosis competency. Within the germline, apoptosis is restricted to the late stage of pachytene cells, residing just distal of the loop of the germline, close to entering the diplotene stage of meiosis. During late-stage pachytene, meiotic cells have completed meiotic recombination and the majority of meiosis-induced DNA double-strand breaks are repaired (see Lui and Colaiácovo 2012, Chap. 6). With the exception of late stage, nearly mature oocytes, germ “cells” are not fully enclosed by a plasma membrane, leaving an opening to a shared cytoplasmic space at the centre of the germline called the rachis. The first

morphologically visible step of germ cell apoptosis is the complete cellularization of the affected cell (Gumienny et al. 1999). Such “cellularization” is likely to be needed to restrict full caspase activation to single apoptotic cells, thus preventing the spreading of apoptosis to neighbouring cells. However, this mechanism might not be perfect, as apoptotic germ cell corpses tend to occur in clusters. While the molecular mechanism of apoptotic corpse engulfment is the same as for somatic corpses, only sheath cells but not germ cells are able to engulf apoptotic cells. At present we do not know why apoptosis is restricted to late pachytene stage germ cells.

Due to the invariant nature of the somatic development of the worm, the extent of developmental apoptosis can be precisely measured (Schwartz 2007). As the germline is a dynamic tissue, the interpretation of apoptosis steady-state levels is more complicated. Factors such as the rates of apoptotic corpse-engulfment and germ cell proliferation, the precise age of the worm, and even the temperature have to be considered when reporting the number of germ cell corpses, ideally done as time course experiments. Also the possibility that bacterial contamination might trigger germ cell apoptosis has to be considered (Aballay and Ausubel 2001; Anton Gartner, unpublished observation). Methods for detecting germ cell corpses and for labelling them with GFP markers or specific dyes have been described (Gartner et al. 2004, 2008). Nevertheless, it is generally not possible to discriminate between small differences in the frequency of germ cell apoptosis.

9.4 Physiological Germ Cell Apoptosis

Germ cell apoptosis generally requires CED-3 and CED-4 and excessive apoptosis occurs in the absence of CED-9 (Fig. 9.1), but there are important genetic differences between physiological germ cell apoptosis and somatic cell death. Physiological cell death does not require EGL-1, and a *ced-9* gain-of-function mutant (*n1950*), which blocks somatic apoptosis, does not have any effect on the germline (Gumienny et al. 1999). Therefore, physiological germ cell death involves the induction of the core apoptosis pathway by an unknown mechanism. Indeed, it is not even clear whether physiological germ cell apoptosis is triggered by single or by multiple distinct genetic pathways. Several transcriptional regulators are known to generally affect the level of germ cell apoptosis by regulating the expression of core apoptosis proteins in the germline (Fig. 9.1). This regulation affects physiological apoptosis, and DNA damage-induced apoptosis, and thus likely affects all forms of germ cell death. *C. elegans* Pax 2/5/8 transcription factors, mutations of which cause excessive apoptosis, are required for efficient *ced-9* transcription in the germline and in somatic tissues (Park et al. 2006). Conversely, the *C. elegans* RB retinoblastoma susceptibility protein ortholog, LIN-35, mutants of which confer reduced germ cell apoptosis, is required for normal levels of germ cell apoptosis, likely by regulating the level of *ced-9* transcription (Schertel and Conradt 2007). In addition, worm subunits of the E2F transcription factor promote physiological germ cell apoptosis by increasing *ced-3* and *ced-4* transcription in germ cells (Schertel and Conradt 2007).

Fig.9.1 Physiological germ cell apoptosis

Finally, there is evidence that MAP kinase signalling might be required for physiological germ cell death (Gumienny et al. 1999). These studies are complicated by the fact that MAP kinase signalling is required for the transition to the proximal pachytene stage where apoptosis occurs (Lee et al. 2007). Thus, the absence of physiological germ cell apoptosis in MAP kinase mutants might be related to a developmental defect rather than the lack of apoptosis induction. However, excessive apoptosis occurs when mutants of the MAP kinase pathway are combined with a *ced-9* loss-of-function mutant (Gumienny et al. 1999). Therefore, MAP kinase signalling defective germlines show apoptosis competency and the lack of apoptosis in MAP kinase signalling defective mutants might be a genuine lack of physiological germ cell death. Consistent with this view, excessive apoptosis occurs when MAP kinase signalling is elevated in mutants of the *lip-1* MAP kinase phosphatase (at high temperature) or the *gla-3* RNA binding protein which can affect MAP kinase signalling (Kritikou et al. 2006). However, there is also emerging evidence that MAP kinase signalling might be needed to repress germ cell apoptosis. Germ cell nuclei accumulate in the temperature sensitive *mpk-1(ga111)* mutant, 24 h after shifting to the restrictive temperature of 25°C, and RNAi inactivation of putative MAP kinase substrates increases germ cell apoptosis observed in *mpk-1(ga111)* mutants but not in wild-type worms (Arur et al. 2011). Given that MAP kinase signalling is regulating multiple aspects of germline differentiation, it still remains to be established whether this pathway directly affects physiological germ cell apoptosis, or directs germ cells to stages that are more or less susceptible to pro-apoptotic signals (also see below for ionizing irradiation-induced apoptosis).

Inactivation of multiple proteins increases the level of germ cell apoptosis and it was argued that various RNA binding proteins, such as the RGG-box protein CAR-1,

the DEAD box helicase CGH-1, and the CPEB ortholog CPB-3, prevent excessive apoptosis induction (Boag et al. 2005). However, inactivation of these and other genes by mutation or RNAi leads to additional germline defects, such as an extended pachytene zone, reduced progeny, full or partial sterility, or a generally misshapen germline (Audhya et al. 2005; Green et al. 2011; Squirrell et al. 2006). Thus excessive apoptosis might be a secondary consequence of pleiotropic germ cell defects. In many cases, excessive germ cell apoptosis might be caused by triggering the DNA damage checkpoint in response to cell cycle perturbation (see below).

9.5 Why Do Cells Die by Physiological Germ Cell Apoptosis and Evolutionary Conservation

Why do the majority of female germ cells die by apoptosis? Physiological germ cell apoptosis is not required for general oogenesis, but the size and number of viable oocytes can be reduced when apoptosis is blocked (Gumienny et al. 1999). Smaller oocytes and a reduction in oocyte viability are especially prominent in old apoptosis-defective germlines (Andux and Ellis 2008). Using female animals generated by mutants blocking sperm generation, reduced oocyte size and increased lethality has been correlated. Such females can be mated at different stages to generate fertilized embryos, and it could be shown that oocyte quality declines with the age of the mother. It will be interesting to determine whether oocyte survival is increased in long-lived worm mutants. It is unlikely that under normal conditions a large proportion of germ cells are culled because they are genetically compromised or because the meiotic recombination or synapsis checkpoints are engaged as apoptosis is only slightly or not reduced in mutants affected by those checkpoints (see below) (Andux and Ellis 2008). However, a slightly increased incidence of male progeny, indicative of meiotic problems, is observed in old animals (Luo et al. 2010). At present the most attractive model proposed in the initial *C. elegans* germ cell apoptosis paper is that apoptotic germ cells might act as nurse cells to provide nutrients and cytoplasmic material for a limited number of oocytes. The finding that the quality of oocytes and size is decreased in old apoptosis-defective mutants supports this model. This occurs even though egg laying rates in apoptosis-defective mutants are not increased, a finding that, albeit not discussed in the literature, indicates that the rate of germ cell proliferation is reduced to compensate for the lack of apoptosis. Similarly oocyte quality declines in mutants where corpse engulfment is largely defective, consistent with a model that recycling apoptotic germ cells is important for the generation of fully grown, healthy oocytes (Andux and Ellis 2008). The use of oocytes as nurse cells appears to be evolutionarily conserved. In the fruit fly, egg chambers contain 16 interconnected cells (for review, see Buszczak and Cooley 2000). All but one of these cells serve as nurse cells and nutrients are transported to the single surviving oocyte while the remaining cells succumb to apoptosis. In mammals, developing oocytes are also interconnected and occur in cysts (Pepling 2006; Pepling and Spradling 1998, 2001). At around birth two thirds of oocytes die by

apoptosis and surviving oocytes form primordial follicles. Nevertheless, at least in *C. elegans*, physiological germ cell apoptosis might not solely be explained by dying germ cells acting as nurse cells. Consistent with the nurse cell hypothesis it has been observed that cytoplasmic streaming occurs in rachis, commencing from the mid-pachytene area to the last not yet fully cellularized oocyte (Wolke et al. 2007). However, the transport of nutrients also occurs from healthy pachytene stage cells. Thus, germ cells do not necessarily have to die to act as nurse cells, and death might have merely evolved to cull the majority of those ~20 germ cells that are being produced every hour in the proliferative zone of the germline to the mere ~3 oocytes that are eventually laid. It is unclear why this system is so wasteful, given that the same result could be obtained by merely reducing germ cell proliferation. The extent of apoptosis might correlate with the number of nurse cells that are needed to produce high-quality oocytes at a rate of ~3 per hour; under optimal growth conditions to produce as many progeny as fast as possible. Alternatively, apoptosis might be part of the mechanism that allows worms to quickly adapt to maintain germ cell homeostasis in response to environmental challenges. The observed increase of germ cell apoptosis upon starvation makes sense to focus limited resources to ensure the survival of some oocytes (which are post-pachytene and thus cannot undergo apoptosis) at the expense of culling pachytene cells (Salinas et al. 2006; Angelo and Van Gilst 2009). Since mitotic germ stem cells do not have the potential to die by apoptosis, down-regulation of germ cell proliferation would still waste the majority of later germ cells if limiting resources were equally distributed amongst developing oocytes. Indeed when L4 stage worms are starved, they appear to be able to cease germ cell net proliferation and preserve the viability of some of their fertilized eggs (Angelo and Van Gilst 2009). Apoptosis-defective worms kept under the same conditions have a larger germline, but the chance of embryo survival is reduced. All in all, it will be interesting to study the mechanisms that lead to germline homeostasis under normal and stressful conditions.

9.6 DNA Damage Checkpoint Signalling

The fact that germ cell apoptosis occurs, together with the notion that the germline is the only proliferative tissue in the worm, encouraged searching for DNA damage-induced apoptosis in the *C. elegans* germline (Gartner et al. 2000). Genome maintenance and the correct duplication of the genetic information are constantly challenged by the exposure to genotoxic agents, such as environmental toxins, or UV and ionizing irradiation. Equally, mistakes in DNA replication or mis-incorporation of nucleotides, oxidative stress or reactive metabolites lead to base modification and DNA lesions that have to be repaired. In the context of the germline, transposon activity, which results in transient DNA double-strand breakage, equally has to be dealt with. In response to DNA damage, cells activate DNA damage response pathways that will coordinate various DNA repair pathways best suited to repair specific lesions (for review, see Jackson and Bartek

2009). At the same time DNA damage response pathways lead to a transient cell cycle arrest, in order to allow for DNA repair before mutations might be fixed. Finally, when the level of DNA damage is excessive, cells might choose to permanently cease proliferation by entering the senescence program. Alternatively, cells might undergo apoptosis. This is thought to eliminate cells whose continued existence might become detrimental for the survival of the entire organism. Within a year of the initial observation of *C. elegans* germ cells apoptosis it was shown that germ cells can be induced to undergo apoptosis in response to genotoxic stress, and upon failure to undergo meiotic recombination (see below) (Gartner et al. 2000). Apoptosis is maximally induced by genotoxic agents that cause DNA double-strand breaks. Increased apoptosis is also observed with agents that primarily act by conferring base modifications such as EMS or ENU, or upon UV treatment that leads to the formation of tyrosine and thymidine dimers (Gartner et al. 2000; Derry et al. 2007; Stergiou et al. 2007). Similar to physiological cell death, DNA damage-induced apoptosis is restricted to late pachytene stage cells and requires the core apoptotic machinery formed by CED-3 and CED-4 proteins (Gartner et al. 2000). However, this type of inducible cell death also requires the BH3-only protein EGL-1 and the second worm BH3-only protein CED-13 contributes to DNA damage-induced germ cell apoptosis (Schumacher et al. 2005a). Like developmental apoptosis but in contrast to physiological germ cell death, DNA damage-induced apoptosis is blocked by the *ced-9 n1950* gain-of-function mutant (Gartner et al. 2000). At the same time a second DNA damage response, the transient cell cycle arrest of mitotically proliferating germ cells, was described (Gartner et al. 2000). This transient cell cycle arrest, which under typical conditions last from 10 to 20 h, leads to a distinct phenotype under DIC optics. Germ cells arrest cell proliferation, but continue to grow, thus leading to a mitotic germline where all cells are enlarged. At least in response to ionizing irradiation, cell cycle arrest largely occurs in the G2 cell cycle stage. Finally, the most important assay to score for DNA damage response and DNA repair pathway activity is to assess the relative survival of embryos, as well as the reduced numbers of oocytes laid that are generated from germlines treated with genotoxic agents (Gartner et al. 2000, 2004; Bailly et al. 2010).

9.7 Upstream DNA Damage Checkpoint Signalling in the *C. elegans* Germline

Various mutants differentially affect DNA damage responses. In core apoptosis pathway mutants, DNA damage-induced apoptosis but not any other damage responses is affected (Gartner et al. 2000). Other mutants, the most prominent example of which is the worm p53-like protein CEP-1 solely affects DNA damage-induced apoptosis (see below) (Derry et al. 2001; Schumacher et al. 2001). A third class of mutants are generally defective in both DNA damage-induced cell cycle arrest and apoptosis, and in most cases are also repair defective, as measured by

reduced progeny survival (Gartner et al. 2000). DNA repair mutants tend to show excessive apoptosis, likely because persistent DNA damage leads to constitutive checkpoint activation. In the worm, DNA damage response and repair pathways dealing with DNA double-strand breaks (DSBs) are best characterized. DSBs that are induced, for example by ionizing radiation (IR), are the most serious type of DNA lesion. However, DSBs are also generated during natural cellular programmes, such as in meiosis by the conserved topoisomerase II-like enzyme SPO-11 (Keeney et al. 1997). DSB repair in the worm is extensively covered by a recent review and also in a chapter of this book (Lui and Colaiácovo 2012, Chap. 6; Lemmens and Tijsterman 2011). Two major pathways repair DSBs; non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ is an error-prone DNA repair mechanism that is mostly restricted to the G1 cell cycle stage. It involves the ligation of the broken DNA ends and is mostly used during somatic development of the worm. HR predominates in the germline. In somatic cells and mitotically dividing germ cells, the sister chromatid is taken as a template. During meiosis, HR is a more tricky undertaking. Meiotic cells must differentiate between the homologous chromosome as a template to facilitate homologous meiotic recombination, as opposed to using the sister chromatid to facilitate DNA double-strand break repair (Couteau and Zetka 2011; Bickel et al. 2010; Adamo et al. 2008).

The two related protein kinases ATM and ATR are critically important regulators of DNA damage responses in cells. ATM is “directly” activated by double-strand breaks (for review, see Jackson and Bartek 2009). ATR-activation occurs when double-strand breaks are recessed to generate single-stranded DNA (ssDNA). ATR recruitment to ssDNA requires protein-A and ATRIP. In *C. elegans* DNA damage responses are mediated largely by ATL-1, the worm ATR homolog, while no ATRIP-like molecules have been described so far. In addition, the related ATM kinase has only a minor role in DNA damage signalling. CtIP, a protein related to yeast Sae2, acts in conjunction with the MRN (MRE11, RAD50, NBS1) complex to resect the double-strand breaks which yields 3' overhangs that can initiate homologous recombination. This processing event is crucial for the initiation of both repair and DNA damage signalling. It is not known whether the *C. elegans* CtIP, which is required for the repair of SPO-11 generated DNA double-strand breaks, has such a role in the worm (Penkner et al. 2007). The MRE-11 nuclease is required for ATL-1 loading (Garcia-Muse and Boulton 2005) but DNA damage checkpoint response defects have not been reported in the *mre-11* worm mutant (Chin and Villeneuve 2001). A DNA damage-specific clamp loader, comprised of Rad17 in a complex with the four smallest RFC (Replication Factor C) subunits, recruits a PCNA (proliferating cell nuclear antigen)-like complex referred to as “9-1-1” complex to the dsDNA–ssDNA transition at resected DNA ends. The heterotrimeric 9-1-1 complex, composed of RAD9, RAD1 and HUS1, is phosphorylated by ATR and is needed for full ATR-activation in yeast and vertebrate systems. In *C. elegans* the corresponding mutants are defective in triggering DNA damage checkpoint responses upon IR treatment (Gartner et al. 2000; Boulton et al. 2002; Hofmann et al. 2002; Boerckel et al. 2007). At the same time these genes are involved in telomere replication, possibly by being required for recruiting telomerase. Interestingly, it was recently

shown that sister chromatid cohesion is not only required for efficient repair of meiotic DSBs but that cohesin is also required for triggering DNA damage-induced germ cell apoptosis (Lightfoot et al. 2011). This function is likely to act very upstream in the DNA damage response cascade that leads to apoptosis induction, as HUS-1 is not appropriately localized to chromosomes upon treatment with ionizing irradiation (Lightfoot et al. 2011). ATM- and ATR-activation lead to the phosphorylation of downstream targets including Chk1 and Chk2, which initiates a secondary wave of phosphorylation events. In the worm *chk-1*, like *atl-1* is required for all checkpoint responses and for the maintenance of genome stability in unchallenged germlines (Garcia-Muse and Boulton 2005). In contrast, *chk-2* does not affect IR-dependent checkpoint responses, but is rather required for meiotic chromosome pairing (MacQueen and Villeneuve 2001). Interestingly, however *chk-2* is required for DNA damage-induced apoptosis in response to UV treatment (Stergiou et al. 2007).

C. elegans genetics was instrumental in defining new conserved genes generally affecting checkpoint responses or specifically being required for checkpoint-dependent apoptosis. *clk-2/rad-5* was initially found in *C. elegans* and shown to be involved in both the S-phase checkpoint (which senses stalled replication forks) and the IR-induced apoptosis as well as cell cycle arrest response (Ahmed et al. 2001). Two alleles affecting the same locus were separately isolated. *clk-2* alleles are temperature sensitive but display a dramatic defect in response to IR-induced damage as well as replication stress at the “permissive” temperature. At the restrictive temperature, *clk-2* mutations are sterile and embryonic lethal (Ahmed et al. 2001). In the embryo, a checkpoint-dependent delay in cell cycle timing occurs, indicating that *clk-2* might be required for the integrity of DNA replication (Moser et al. 2009). Genome instability also occurs in *clk-2* mutant germlines, but strong alleles lead to a late G2-like cell cycle arrest. Subsequent studies on mammalian and yeast CLK-2 orthologs, referred to as TEL2 in yeasts, established that CLK-2/TEL2 is required for the full activation of all PIKKs-type protein kinases, a class of protein kinases that includes ATM/*atm-1* and ATL-1, thus explaining checkpoint-signalling phenotypes (Hayashi et al. 2007; Kanoh and Yanagida 2007; Hurov et al. 2010; Takai et al. 2010; Kaizuka et al. 2010). The current model suggests that CLK-2/TEL2 might have a chaperon-like function required for the full activation of those kinases (Horejsi et al. 2010). It remains to be seen whether CLK-2/TEL2 has further functions, as the above model is not fully consistent with the *C. elegans* genetics data: During embryogenesis, depleting ATL-1 and CLK-2 reveals that they have opposing functions, in the germline the cell cycle arrest phenotype conferred by *atl-1* inactivation leads to excessive genome instability while this is not the case for the cell cycle arrest phenotype observed in *clk-2* null mutants (Moser et al. 2009).

DNA damage signalling is generally triggered during the early steps of DNA double-strand break repair. Signalling is mediated by ATM directly bound to DNA double-strand breaks and/or by ATR, which often enforces the initial signal. The full activation of ATR requires the initial nucleolytic processing of DNA double-strand breaks, leading to extended single-stranded DNA stretches, which ultimately act as the landing platform for ATR. Little is known about how the persistence of late-stage recombination events impacts on DNA damage checkpoint signalling and

Fig.9.2 DNA damage-induced germ cell apoptosis

apoptosis induction. An important indication that such signalling occurs came from the analysis of *C. elegans gen-1*, which likely acts as a Holliday Junction resolvase (Bailly et al. 2010). Holliday Junctions are four-way DNA structures that arise during recombinational repair between homologous chromosomes and which can be resolved by symmetrical cleavage (Ip et al. 2008; West 2009). *gen-1* was initially isolated based on an unbiased genetic screen for mutants required for DNA damage signalling and DNA double-strand break repair (Fig. 9.2). The repair defect associated with *gen-1* mutation can be explained by a defect in resolving Holliday Junction DNA double-strand breaks intermediates. However, the DNA damage signalling defect conferred by *gen-1* was surprising as the initial nucleolytic processing events thought to trigger checkpoint induction appeared to be normal in the mutant. Not only this, cytological and biochemical evidence indicates that the canonical ATM/ATR-dependent checkpoint pathways are activated in *gen-1* mutants. Thus GEN-1, besides its function in DNA repair, appears to act in a noncanonical DNA damage response signalling cascade. This pathway possibly senses the completion or the absence of Holliday Junction processing. It will be interesting to further dissect how GEN-1 affects DNA damage signalling. The biochemical activity of the mammalian GEN1 resolvase has been extensively characterized (Rass et al. 2010). At present no checkpoint function of mammalian GEN1 or yeast YEN1 was reported, and its

depletion was not linked to repair defect. This is likely due to redundancy, as GEN1 depletion in cells defective for Blooms syndrome helicase leads to increased genome instability (Wechsler et al. 2011).

9.8 Pathways Leading to DNA Damage-Induced Apoptosis

The pathways leading to DNA damage-induced apoptosis are surprisingly complex and are the subject of intense research. The idea that the *C. elegans* germline recapitulates apoptotic mechanisms found in mammalian cells was reinforced by the identification of a p53 homolog in the worm genome, *cep-1* (*C. elegans* p53-like 1) (Derry et al. 2001; Schumacher et al. 2001; for recent reviews, see Rutkowski et al. 2010). The identity of *cep-1* remained elusive despite the *C. elegans* genome sequence being known for several years, based on its low level of sequence conservation, which is essentially restricted to its DNA-binding domain. In mammals, the p53 family of transcription factors includes p63 and p73 and phylogenetic analysis that included many invertebrate species indicated that the radiation into those three groups occurred during vertebrate evolution (Rutkowski et al. 2010). However, it was recently shown that placozoans, which are very simple animals, and ticks have Mdm2-like proteins and p53 family members much more related to mammalian p53 (Lane et al. 2010a, b). Mammalian p53 acts as a transcriptional activator, which is essential for genome stability and the elimination of damaged cells by apoptosis. Human p53 or components of the p53 pathway are mutated in the majority of human cancers (for a recent review series, see Lane and Levine 2010). p63 and p73 have roles in stem cell proliferation, epithelial and neuronal differentiation and also have roles in the DNA damage response. p63 possibly encodes for the more ancient form of the p53 family and might be thus most related to *cep-1*. Besides structural considerations this notion is further supported by the more recent finding that p63 acts analogously to CEP-1 to specifically affect DNA damage-induced apoptosis in the female mammalian germline (Suh et al. 2006; Ou et al. 2007). The *C. elegans* p53-like protein CEP-1 turned out to be a direct transcriptional activator of *egl-1* and a second BH3-only protein called CED-13 (Schumacher et al. 2005a) (Fig. 9.2). *egl-1* and *ced-13* are the strongest *cep-1*-dependent genes induced by IR (Greiss et al. 2008a). EGL-1 induction plays a predominant role in DNA damage-induced apoptosis, while CED-13 appears to be important for UV-induced apoptosis (Schumacher et al. 2005a; Stergiou et al. 2007). Conceptually, the worm CEP-1 pathway acts analogously to the mammalian p53-dependent apoptosis induction of the BH3 proteins Puma and Noxa, which in turn leads to apoptosis induction analogous to EGL-1 by Bcl-2/CED-9 inactivation (Jeffers et al. 2003; Villunger et al. 2003; Shibue et al. 2003; Oda et al. 2000). The *cep-1* null mutant worms do not have any overt developmental or germline-specific phenotype but are fully defective in DNA damage-induced apoptosis. *cep-1* mutations do not affect DNA damage-induced cell cycle arrest and only show a slight reduction in progeny survival upon ionizing irradiation (Derry et al. 2001; Schumacher et al. 2001). Thus CEP-1 does not appear

to have a prominent DNA repair function in response to ionizing irradiation. Interestingly, CEP-1 seems to be required for both UV-induced cell cycle arrest and apoptosis (Derry et al. 2007; Stergiou et al. 2007). Like mammalian p53, CEP-1 is phosphorylated in response to ionizing irradiation but the importance of this has not been identified (Schumacher et al. 2005b; Gao et al. 2008). The identification of CEP-1 and the tremendous interest in the mammalian p53 family prompted searches for CEP-1 regulatory mechanisms. Below, some of these mechanisms are described but other reviews are more comprehensive and include all components identified (Gartner et al. 2008; Rutkowski et al. 2010). Studying CEP-1 regulation in an intact developmental system allows the addressing not only of how CEP-1 function is activated or inactivated, but also how apoptosis induction might be restricted to a certain germ cell compartment. Importantly, this developmental system also allowed the defining of a cell non-autonomous mechanism by which radiation-induced apoptosis is regulated (see below).

9.9 CEP-1 Regulation

During normal development, or in the absence of DNA damage, the activity of human p53 has to be kept at bay to prevent inappropriate apoptosis induction. The best-characterized negative regulator of p53 is MDM2, an E3 ubiquitin ligase required for p53 degradation. Mdm-2 knockout mice die by excessive apoptosis, but Mdm2, p53 double knockout mice survive as excessive apoptosis is blocked (de Oca et al. 1995; Jones et al. 1995). There does not appear to be an Mdm2 homolog in worms, but several mechanisms restrict CEP-1 activation. A screen for mutations hypersensitized for CEP-1-dependent ionizing irradiation-induced apoptosis (Fig. 9.2) revealed a hypermorphic mutation of *gld-1*, a gene required for many facets of germline development (Schumacher et al. 2005b; Francis et al. 1995a, b; Jones et al. 1996). GLD-1 acts primarily as a translational repressor by binding to the 3'UTR of its targeted genes, hundreds of which are known (Lee and Schedl 2001; Wright et al. 2011; Carmel et al. 2010). The *gld-1(op236)* mutant at the permissive temperature, unlike all other *gld-1* mutants that have been isolated previously, does not affect overall germline development or germ cell identity, and germ cell apoptosis in the absence of ionizing irradiation is nearly normal. Biochemical analysis indicated that *cep-1* mRNA is a target of GLD-1 and that *cep-1* binding to GLD-1(*op236*) is compromised. In contrast, other tested GLD-1 targets are still bound to this mutant protein. GLD-1 expression is highest in early and mid-pachytene, but starts to decline as cells pass through the late pachytene stage where apoptosis normally occurs. Conversely, CEP-1 protein is absent in early and mid-pachytene but gradually increases in late pachytene. In *op236* mutants, CEP-1 expression is higher and extends more distally. This does not increase *cep-1*-dependent apoptosis under unchallenged conditions but sensitizes germ cells towards DNA damage-induced apoptosis. It is likely that this mechanism helps to ensure that DNA damage-induced apoptosis does not occur earlier in mid-pachytene.

In this stage, meiotic recombination intermediates occurring naturally and in the presence of CEP-1 could trigger apoptosis induction. The same unbiased genetic screen uncovered another CEP-1 regulatory module. Increased activation of the *C. elegans* MPK ortholog MPK-1, resulting from either loss of the *lip-1* phosphatase or activation of *let-60* Ras, results in enhanced *cep-1*-dependent, DNA damage-induced apoptosis (Rutkowski et al. 2011) (Fig. 9.2). Conversely MPK-1 is required for DNA damage-induced germ cell apoptosis. MPK-1 appears to affect CEP-1 in several ways. MPK-1 signalling regulates the apoptotic competency of germ cells by restricting CEP-1 protein expression to cells in late pachytene. MPK-1 signalling regulates CEP-1 expression in part by regulating the levels of GLD-1, a translational repressor of CEP-1, but also via a GLD-1-independent mechanism. In addition, MPK-1 is phosphorylated and activated upon ionizing radiation in late pachytene germ cells, and MPK-1-dependent CEP-1 activation may directly lead to the activation of CEP-1 as MPK-1 and CEP-1 interact in a yeast two-hybrid assay. Thus, GLD-1 and MPK-1 signalling might act primarily by setting the responsiveness to DNA damage-induced apoptosis, and by restricting apoptosis induction to late-stage pachytene cells. However, MAP signalling is also activated in response to DNA damage and seems to play an additional, more direct, role in CEP-1 regulation. In *gld-1(op236)* mutants, as well as in mutant situations that lead to excessive MPK-1 signalling, ionizing irradiation-induced apoptosis is enhanced while no obvious DNA repair defects occur. Thus, it is likely that these factors directly impinge on CEP-1 regulation and expression rather than generally causing excessive apoptosis triggered by the failure to repair DNA lesions.

Other regulatory mechanisms have been shown to impact on CEP-1 transcriptional regulation. The protein arginine methyltransferase 5 (PRMT-5) acts as a negative regulator of the CEP-1 function by binding and methylating the conserved transcriptional co-factor CBP-1 (Yang et al. 2009) (Fig. 9.2). Mammalian p300/CBP-1 acts as transcriptional co-factor for p53. Worms carrying a deletion in the *prmt-5* gene display an enhanced apoptotic response upon treatment with IR that fully depends on *cep-1*. In addition, PRMT-5 physically interacts with CEP-1 and CBP-1 in an in vitro pull-down assay as well as in an expression system in mammalian cells, and importantly can directly methylate CBP-1. These studies suggest that CEP-1 function as a transcriptional factor requires the co-factor CBP-1, which may be negatively regulated by the methyltransferase activity of PMRT-5. Given the absence of MDM2 it appears that other ubiquitin E3 ligases might regulate CEP-1 turnover. Skp1/cullin/F-box (SCF) ubiquitin ligases are activated by neddylation of the cullin subunit. The cullin 3 SCF^{FSN-1} complex is required to dampen the apoptosis response, and likely acts in the same pathway as neddylation (Gao et al. 2008). In addition, the level of CEP-1 is significantly increased in *fsn-1* mutants, suggesting that the E3 ubiquitin ligase complex SCF^{FSN-1} negatively regulates CEP-1 activity in *C. elegans* germline, possibly by the degradation of CEP-1 (Fig. 9.2). Intriguingly, the human FSN-1 homolog FBXO45 promotes the degradation of p73 in tissue culture-based experiments (Peschiaroli et al. 2009) (Fig. 9.2). Nevertheless, at present it is not clear whether the genetic interaction of SCF^{FSN-1} and *cep-1* is direct. We know little about CEP-1 post-translational modifications. It will be

important to identify the modifications and to determine their importance in knock-in experiments, which should be possible using transposon excision-based gene replacement technologies (Frokjaer-Jensen et al. 2008).

9.10 Pathways Parallel to CEP-1

Uncovering the CEP-1 pathway provided a simple model for DNA damage-induced apoptosis: DNA damage, or recombination intermediates would be sensed and signalling would lead to CEP-1 transcriptional activation. This in turn would lead to the transcriptional induction of EGL-1 and CED-13, which in turn would trigger the core apoptosis pathway by the direct binding to CED-9. In theory such a model is sufficient to explain apoptosis induction in the germline. It therefore came as a surprise that mutations exist where DNA damage-induced apoptosis is largely abrogated, while CEP-1-dependent transcription still occurs (Fig. 9.2). Studying such factors is a tricky undertaking as care must be taken not to mistake reduced germ cell proliferation, or a reduced number of apoptosis competent cells with a genuine defect in apoptosis induction; however, such controls are possible.

The first report of such a situation was based on the analysis of SIR-2.1, a histone deacetylase which is homologous to human SIRT1 (Greiss et al. 2008b) (Fig. 9.2). Worms deleted for the *sir-2.1* locus display a complete defect in apoptosis response upon IR treatment. While germ cell proliferation in *sir-2.1* mutants is slightly reduced, apoptosis competency is not affected. For instance, the basal level of physiological germ cell death is not affected; more apoptosis occurs in mutants that are thought to increase physiological germ cell apoptosis, and most importantly the depletion of *ced-9* leads to excessive apoptosis induction to a degree comparable to wild type. Thus, in *sir-2.1* mutants, DNA damage-induced apoptosis seems to be genuinely affected, and the reduction in apoptosis induction is almost as strong as in *cep-1* mutants, where *egl-1* transcription is not induced. In summary, these data suggest that SIR-2.1 acts in parallel or downstream to CEP-1. A likely target of SIR-2.1 might be the EGL-1 protein, which is only required for DNA damage-induced germ cell apoptosis. Equally CED-9 could be affected. The *ced-9 n1950* gain-of-function allele does not affect physiological germ cell death, while DNA damage-induced apoptosis is compromised; thus, there are likely differential modes of CED-9 regulation. Given that CED-4 and CED-3 are required for all forms of germ cell apoptosis, at the first glance they appear as less likely targets unless their regulation is much more complicated than anticipated. We do not know how SIR-2.1 acts mechanistically, but there are intriguing cytological correlations, possibly hinting at CED-4. SIR-2.1 translocates from the nucleus to the cytoplasm during early apoptosis. This translocation occurs very early in apoptosis where the nuclear envelope still appears to be intact. In addition such translocation, which correlates with CED-4 accumulation at the nuclear periphery, also occurs in apoptosis execution defective *ced-3* germ cells. These findings indicate that such translocation might have a functional role rather than being a mere consequence of

apoptosis induction. During translocation, SIR-2.1 appears to transiently co-localize with CED-4 at the nuclear periphery. In a separate study, the HECT-domain E3 ligase EEL-1, which is homologous to human Huwe1/ARF-BP1/Mule, was shown to genetically behave like SIR-2.1, as does KRI-1 the ortholog of human *KRIT1/CCM1*, a gene frequently mutated in the neurovascular disease cerebral cavernous malformation (for cell non-autonomous apoptosis regulation, see below) (Ross et al. 2011; Ito et al. 2010) (Fig. 9.2). We do not know whether these genes act in the same genetic pathway or what their molecular function is. Intriguingly, the human EEL-1 homolog Mule was found to regulate levels of the mammalian anti-apoptotic Mcl1, a Bcl2-family protein. Unfortunately, we do not know whether EEL-1 directly binds to CED-9 or EGL-1.

In the damage response field, ATR and ATM are considered to be the most upstream signalling factors that affect all DNA damage responses including apoptosis. This assumption is likely to be an oversimplification, especially when it comes to apoptosis induction (Deng et al. 2008). Ceramide is a central molecule in sphingolipid metabolism and critical for plasma membrane integrity. The role of ceramides in apoptosis induction has been controversial in mammalian cells (Kolesnick and Hannun 1999; Perry and Hannun 1998; Hofmann and Dixit 1998, 1999). The cellular level of ceramide increases after treatment with diverse apoptotic stimuli such as IR, UV light or TNF. Blocking ceramide synthesis reduced apoptosis in various human cell lines. Some reports suggest that ceramides can form channels in mitochondrial outer membranes and promote the release of pro-apoptotic factors, an obligate step in mammalian apoptosis execution. In worms, mutations blocking ceramide biogenesis completely abrogate IR-induced apoptosis and this defect appears to be rescued by micro-injecting long-chain ceramides into the worm gonad, and evidence was provided that ceramide might accumulate upon apoptosis induction (Deng et al. 2008) (Fig. 9.2). Importantly, blocking ceramide does not affect *egl-1* and *ced-13* as is the case for the aforementioned factors and, like SIR-2.1, was shown not to affect developmental and physiological germ cell apoptosis.

It will be interesting to see whether these and other factors affecting DNA damage-induced apoptosis induction independent of CEP-1 act in a single pathway. This is probably unlikely. However, even “linear” DNA damage response pathways might be more complicated. For instance, as mentioned above, GEN-1 is also required for all DNA damage checkpoint responses including apoptosis, but *egl-1* induction is not compromised (Bailly et al. 2010). A further question that arises is why multiple pathways are needed to allow for full apoptosis induction? It might be possible that this is a fail-safe mechanism to ensure that germ cells are not inappropriately eliminated. Such a mechanism makes sense, as DNA lesions occur, even in the absence of exogenous DNA damaging agents. Furthermore, programmed DNA breaks and repair intermediates occur during meiosis, as discussed below. Why nature would be so careful about not losing cells by apoptosis, even when under normal conditions the majority of cells are eliminated by apoptosis remains a mystery.

Fig. 9.3 Cell non-autonomous regulation of germ cell apoptosis

9.11 Apoptosis Induction by Cell Non-Autonomous Mechanism

Although the worm germline provides a powerful model system to investigate stress-induced apoptosis, most of the studies have focused on cell-autonomous response to cellular stress, assuming that pro-apoptotic stimuli and their effects occur in the same cell. This is not necessarily always the case and the worm provides a model where stress responses can be analyzed in a developmental context. The Hengartner lab has recently reported a cell non-autonomous way of regulating germ cells apoptosis showing that hypoxia perceived in neuronal cells blocks cell death induction in the germline (Sendoel et al. 2010). The cellular response to hypoxia is highly conserved and one of the critical factors is the transcriptional activator HIF-1. Under normoxic conditions, HIF-1 is prolyl-hydroxylated and this modification is required for its binding to VHL, the substrate-binding subunit of an E3 ligase that mediates HIF-1 degradation (Fig. 9.3). Upon exposure to low oxygen pressure, HIF-1 is dehydroxylated and becomes resistant to VHL-mediated degradation. HIF-1 stabilization induces the expression of various hypoxia-specific genes. The authors show that hypoxia is a robust repressor of DNA damage-induced apoptosis. Thus, worms grown under low oxygen conditions do not trigger germ cell apoptosis after treatment with IR. Low oxygen leads to HIF-1 stabilization, and HIF-1 stabilization also occurs in *vhl-1* mutants even under normoxic conditions (Fig. 9.3). A *vhl-1* mutant mimics HIF-1 activation normally occurring in hypoxic conditions. Scanning through HIF target genes, the authors found that a mutation of the *tyr-2* tyrosinase restores IR-dependent apoptosis (Fig. 9.3). The surprise came when the authors looked for the localization of TYR-2. *tyr-2* is expressed in epidermis/hypodermis and uterine muscle cells under normoxic conditions but hypoxia induces *tyr-2* expression only in the amphid sensory neurons, located in the head of the worm. Laser-based ablation of these two neurons completely restores the ability of germ cells to respond to IR under low oxygen condition, reinforcing the idea that

the signal somehow transits between these two compartments. Finally, the ectopic expression of *tyr-2* under the control of a germline-specific promoter impedes DNA damage-induced apoptosis in germ cells, suggesting that either TYR-2 itself or a product of TYR-2 activity carries a signal from neuronal to germ cells that inhibits the DNA damage checkpoint pathway under low oxygen conditions. It is of note that KRI-1, described previously, was also shown to affect apoptosis by acting in somatic tissues (Ito et al. 2010). It will be interesting to see where it acts and whether it relates to the hypoxia-sensing pathway. Also the worm retinoblastoma-like gene *lin-35*, discussed in the physiological apoptosis section and which is also required for *egl-1* independent apoptosis induction upon IR function, is required in both the somatic gonad and the germline to promote constitutive germ cell apoptosis (Schertel and Conradt 2007). We can only speculate why worms would want to block apoptosis under hypoxic conditions. As an organism found in soil and rotten fruits, it is not unlikely that they encounter a low oxygen environment.

9.12 Meiotic Recombination and Chromosome Synapsis Checkpoints

During meiosis (see Lui and Colaiácovo 2012, Chap. 6), gametes are produced by two sequential cell divisions that first separate homologous chromosomes (meiosis I) and then sister chromatids (meiosis II). During prophase of meiosis I, homologous chromosomes align (Fig. 9.4, left panels) and undergo meiotic recombination. Recombination initiation is triggered by SPO-11-generated DNA double-strand breaks and is followed by the formation of the synaptonemal complex, a proteinaceous structure which tightly links paired homologs and blocks excessive cross-over recombination (Fig. 9.4, right panels). In the worm this cross-over interference is “perfect” and generally only a single cross-over recombination event occurs on each chromosome. During meiotic prophase the integrity of both meiotic recombination and meiotic chromosome synapsis is monitored by checkpoints, and such checkpoint activation leads to a cell cycle arrest phenotype in yeast and apoptosis in animals. In most organisms meiotic recombination and chromosome pairing are genetically linked. In contrast, in the fly and the worm, homologous chromosome pairing occurs in the absence of meiotic recombination, thus allowing these two events to be separately studied.

9.13 Meiotic Recombination Checkpoint and Template Preference

The *C. elegans* recombination checkpoint was uncovered in worms by inactivating genes required for homologous recombination such as *rad-51*, which leads to a high level of apoptosis (Gartner et al. 2000). This cell death is induced by unprocessed

Fig. 9.4 Meiotic recombination and pairing checkpoints. Diagram adapted from Meier and Gartner (2006). Chromosomes are indicated in *black*. The *left panel* show early transition zone stage chromosomes. The corresponding *right panels* show corresponding mid-pachytene chromosomes. Pairing centres are indicated in *yellow*, HIM-8 is indicated in *green*. The localization of PCH-2 was not reported. Presynaptic, synapsis-independent pairing is indicated by *dotted blue lines*. Synapsis and its directionality is indicated by *blue arrows*. The *dotted blue arrow* in the three lower panels indicates no or residual synapsis. DNA double-strand breaks marked by RAD-51 foci are depicted in *red*. *Yellow* and *red* arrows indicate the triggering of the synapsis and recombination checkpoint, respectively

recombination intermediates as excessive apoptosis induction is blocked in *spo-11* mutations. Since then, mutations in multiple genes required for homologous recombination have been shown to trigger this checkpoint. At present, the meiotic recombination and the DNA double-strand break-induced checkpoint pathway cannot be genetically separated. In both cases checkpoint genes like *mrt-2* and *clk-2*, and importantly also *cep-1* p53-like are required for apoptosis induction (Gartner et al. 2000). The recombination checkpoint is also activated when individual meiotic chromosomes completely fail to pair. This occurs when both pairing centres (PCs), which are *cis*-elements needed to initiate chromosome pairing and to stabilize synapsis, are deleted or when the proteins that bind to those specific PCs and mediate pairing, like HIM-8 for the X-chromosome PC, are missing (Bhalla and Dernburg 2005) (Fig. 9.4, third and forth panel). The recombination checkpoint is also

triggered when chromosomes globally fail to synapse, as it is the case for *syp-1* or *syp-2* synaptonemal complex mutants (see below, Fig. 9.4, lowest panels). The recombination checkpoint can be triggered, even when only a single meiotic chromosome fails to pair (Colaiacono et al. 2003; Alpi et al. 2003; Mets and Meyer 2009). In those pairing defective mutants apoptosis is thought to be triggered by the excessive SPO-11-generated double-strand breaks, which in the absence of a homologous chromosome fail to be repaired (Fig. 9.4). Male germ cells contain an unpaired sex chromosome (XO). Why does this not lead to apoptosis induction? In males germ cell apoptosis generally does not occur, albeit molecular markers such as the accumulation of ATR-1 on DNA double-strand breaks or CHK-1 phosphorylation occur when autosomes (but not sex chromosomes) fail to pair (Jaramillo-Lambert et al. 2010). XO germ cells can also be analyzed in female germ lines, taking advantage of *fem-3* sex determination mutants. In such a context pairing defects of autosomes leads to apoptosis induction, while the unpaired X-chromosome does not lead to recombination checkpoint triggered apoptosis. The unpaired X-chromosome is transcriptionally repressed and bears the histone H3K9m2 methylation mark (Checchi and Engebrecht 2011), a signature generally associated with a transcriptionally repressed state. When the corresponding methyltransferase Met-2, or two other methyltransferases, MES-2 or MET-1 are depleted a single unpaired X-chromosome triggers apoptosis. H3K9m2 methylation thus blocks checkpoint activation triggered by unrepaired X-chromosomes.

SPO-11 induces more than one DNA double-strand break per chromosome, and these excessive breaks are repaired by gene conversion events initially by taking the homologous chromosome as a template (see below). In late pachytene, both the homologous chromosome and the sister chromatid can be used for repair. The former mode of HR is required for meiotic cross-over recombination and gene conversion. Both *C. elegans* *brc-1*, the worm homolog of the mammalian breast and ovarian cancer susceptibility gene BRCA1, and the structural maintenance of chromosomes (SMC) proteins SMC-5 and SMC-6 were shown to be specifically required for meiotic sister-chromatid recombination (Bickel et al. 2010; Adamo et al. 2008). In the corresponding mutants, no overt defect in meiotic cross-over recombination can be observed and chiasmata occur as in wild type. In contrast, homolog-independent repair defects caused by the failure to use the sister chromatid as template can be detected in *smc-5/6* and *brc-1* mutants. A chromosome fragmentation phenotype arises in those mutants when inter-homolog repair is blocked.

There appears to be a template switch occurring during late pachytene. In wild-type *spo-11*-dependent RAD-51 foci are generated in the transition zone and are generally repaired in the early and mid-pachytene stages. In pairing defective mutants, foci stay, but appear to be repaired in the very late pachytene stage (independent of non-homologous end-joining), likely by sister chromatid templated repair (Adamo et al. 2008; Colaiacono et al. 2003; Alpi et al. 2003). It is intriguing that germ cell apoptosis induction also affects these very late-stage pachytene cells. It will be interesting to further address if this switch in HR repair templates is linked to apoptosis induction. In summary, it is possible that DNA double-strand breaks that fail to be repaired by cross-over recombination or homolog templated gene

conversation, can be repaired from the sister chromatid. If this fails, apoptosis appears to be the last resort to prevent passing on a DNA lesion to the next generation. Interestingly, the failure to cull affected pachytene cells upon recombination checkpoint activation, which can be conferred by *cep-1* or by *ced-4* apoptosis-defective mutants, leads to an increased rate of meiotic chromosome missegregation (Bhalla and Dernburg 2005; Jaramillo-Lambert et al. 2010; Jaramillo-Lambert and Engbrecht 2010). Thus, it appears possible that chromosomes that fail to properly repair excessive SPO-11-generated double-strand breaks are preferentially culled. It appears that repair from the sister chromatid is the preferred mode of repair for exogenously inflicted DNA double-strand breakage (Couteau and Zetka 2011). A recent report showed that synapsed late-stage pachytene chromosomes transiently desynapse upon treatment with ionizing irradiation. This separation, which correlates with the deacetylation on H2A Lysine 5 in the affected chromosomal domains, is thought to ensure repair by the sister-chromatid (Couteau and Zetka 2011).

9.14 The Meiotic Pairing Checkpoint

The landmark study describing the *C. elegans* synapsis checkpoint used the X-chromosome pairing centre, which is deleted by the *meDf2* deficiency (Bhalla and Dernburg 2005) (Fig. 9.4, second panel from top). X-chromosome pairing is perturbed but not eliminated in strains hemizygous for the X-chromosome PC in early meiotic prophase. This initial pairing defect reduces the frequency of synapsis to approximately 45%. Worms, hemizygous for *meDf2*, show the same excessive level of germ cell apoptosis as *meDf2* X-chromosome PC homozygous mutants that trigger the recombination checkpoint. However, the excessive apoptosis in the PC hemizygous mutant is not *spo-11* or *cep-1* dependent, and apoptosis induction rather requires the *C. elegans* homolog of Pch2p, a yeast protein previously implicated in meiotic checkpoint control (San-Segundo and Roeder 1999, 2000). As it is the case for germ cells that activate the recombination checkpoint, germ cells in pairing defective mutants are preferentially eliminated: In *meDf2*/+ hermaphrodites, 60% of meiotic nuclei exhibit unsynapsed X-chromosomes, but only ~6% of their self-progeny are males. The low percentage of males, which is indicative of sex chromosome missegregation, can only be explained if cells carrying unsynapsed chromosomes are preferentially culled by apoptosis. Consistent with this notion, more males occur in *ced-4* and *pch-2* mutants. The authors argue that a PC not engaged in chromosome pairing is required to trigger the synapsis checkpoint as the synapsis checkpoint is not engaged in PC homozygous *him-8* mutants. Conversely, if DNA double-strand break intermediates indeed trigger the DNA damage checkpoint, why is the DNA damage checkpoint not activated in PC hemizygotes as opposed to PC or *him-8* homozygote mutants? The argument is that the failure of DNA damage checkpoint activation could be due to the repression of the DNA damage checkpoint once the pairing checkpoint is activated. Alternatively, the reduced double-strand breaks in

PC hemizygous mutants might not be sufficient to trigger the DNA recombination checkpoint. Consistent with this hypothesis, the meiotic DNA damage and pairing checkpoint can be activated simultaneously in *syp-1* and *syp-2* mutants (Fig. 9.4, lowest panels). In these mutants, synapsis independent chromosome pairing occurs but synaptonemal complex formation is globally abrogated on all chromosomes. In *C. elegans*, *pch-2* has no reported roles in regulating unperturbed apoptosis. In budding yeast and mice, where *PCH2* was reported to affect the pachytene checkpoint that monitors both recombination of chromosome pairing, *PCH2* has recently shown to also affect multiple functions occurring during unperturbed meiosis in yeast and mice (Roig et al. 2010; Zanders et al. 2011; Zanders and Alani 2009; Joshi et al. 2009). Mutant phenotypes include alternations in the rate and timing of meiotic recombination, alteration in the level of cross-over interference, in the level of meiotic chromosome condensation, and in the extend of synapsis. It will be interesting to learn how *PCH-2* triggers germ cell apoptosis and to elucidate the interplay between pairing and recombination checkpoints.

9.15 Concluding Remarks

Male germ cells fully activate the recombination checkpoint cascade, and CEP-1-dependent *egl-1* transcription is induced (Jaramillo-Lambert et al. 2010). While all apoptosis proteins are expressed in the male, the CED-3 caspase is not induced. Thus it appears that in males checkpoint induced repair might be very efficient and possibly compensates for failure to cull damaged cells. Why would apoptosis induction occur in the female and not in the male germline? Why is nature so wasteful to trigger such extensive levels of germ cell death? Why is germ cell apoptosis regulation so intricate? Why can only a subset of germ cell undergo apoptosis even though cell death proteins appear to be expressed throughout the germline? We do not have answers to those questions. Furthermore, we are only beginning to understand how apoptosis, DNA damage, recombination and pairing checkpoints are controlled. It is difficult to address these questions in the context of germline development in mammals. The worm system will continue to provide important insights. It will be interesting to directly assess mutagenesis rates in male and female germlines, and to establish to what extent they depend on the ability to induce apoptosis.

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