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Review

C. elegans: A model of Fanconi anemia and ICL repair

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ABSTRACT

Fanconi anemia (FA) is a severe recessive disorder with a wide range of clinical manifestations [M. Levitus, H. Joenje, J.P. de Winter, The Fanconi anemia pathway of genomic maintenance, Cell Oncol. 28 (2006) 3–29]. In humans, 13 complementation groups have been identified to underlie FA: A, B, C, D1, D2, E, F, G, I, J, L, M, and N [W. Wang, Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins, Nat. Rev. Genet. 8 (2007) 735–748]. Cells defective for any of these genes display chromosomal aberrations and sensitivity to DNA interstrand cross-links (ICLs). It has therefore been suggested that the 13 FA proteins constitute a pathway for the repair of ICLs, and that a deficiency in this repair process causes genomic instability leading to the different clinical phenotypes. However, the exact nature of this repair pathway, or even whether all 13 FA proteins are involved at some stage of a linear repair process, remains to be defined. Undoubtedly, the recent identification and characterisation of FA homologues in model organisms, such as *Caenorhabditis elegans*, will help facilitate an understanding of the function of the FA proteins by providing new analytical tools. To date, sequence homologues of five FA genes have been identified in *C. elegans*. Three of these homologues have been confirmed: *brc-2* (*FANCD1*/*BRCA2*), *fcd-2* (*FANCD2*), and *dog-1* (*FANCJ*/*BRIP1*); and two remain to be characterised: W02D3.10 (*FANCI*) and *drh-3* (*FANCM*). Here we review how the nematode can be used to study FA-associated DNA repair, focusing on what is known about the ICL repair genes in *C. elegans* and which important questions remain for the field.

Contents

1. Introduction

Fanconi anemia (FA) is a rare syndrome associated with various congenital abnormalities, bone marrow failure and a predisposition to early onset leukaemia and solid tumours [1]. Cells lacking FA pathway components commonly exhibit chromosomal instability and sensitivity to interstrand cross-linking agents, such as mitomycin C, cisplatin and diepoxybutane [1,3,4]. Thus, the FA pathway is thought to be involved in the repair of DNA damage that interferes with replication, such as interstrand cross-links (ICLs). In humans, the FA pathway includes at least 13 components, defined by genetic deficiencies in FA patients: FANCA, B, C, D1/BRCA2, D2, E, F, G/XRCC9, I, J/BRIP1/BACH1, L, M/Hef and N/PALB2 (reviewed in

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[1,5,6]). FANCA, B, C, E, F, G, L and M form the FA core complex, which is required for monoubiquitylation of FANCD2 and FANCI and their subsequent recruitment to chromatin in response to DNA damage [1,7]. The other pathway components BRCA2, FANCJ and FANCN appear to function in downstream repair roles, as they are not required for the monoubiquitylation of FANCD2–FANCI [1,5]. It has been suggested that the FA pathway may help to promote repair through translesion synthesis and/or homologous recombination [8–10]. However, the mechanism by which this is achieved is not known and this is one of the greatest caveats to the understanding of FA. Five of the FA proteins, BRCA2, FANCD2, FANCI, FANCJ and FANCM, have known or candidate homologues in the model organism *Caenorhabditis elegans* [11–14]. To date, the components of the FA core complex have not been identified in the nematode, although it is possible that homology searches have failed due to a lack of sequence conservation. Alternatively, the factors currently identified in the nematode may represent the minimum requirement for a functional FA repair pathway, which exists in lower organisms. Therefore, *C. elegans* could represent a simplified model for the FA pathway. This review aims to discuss some of the techniques, advantages and disadvantages of the nematode as a model for studying FA repair, detailing the current understanding of the conserved FA genes as a basis to raise pertinent questions related to the fields of FA and ICL repair.

2. Why the worm: approaches, advantages and constraints of the worm as a model for the FA repair pathway

C. elegans is one of the most well-characterised model organisms. The worm eats bacteria and can be cultured without difficulty on small petri plates; thus, it is inexpensive and easy to maintain, and requires little space. The nematode takes just 3 days to develop from egg to fertile adult (Fig. 1A and B), and exists as both hermaphrodites and males, making genetic manipulation relatively quick and easy. Isogenic populations of animals can be maintained over time by serially transferring the self-fertilizing hermaphrodites. Furthermore, each adult produces 300 progeny, providing large populations for experiments. As the nematode has been used as a model organism since the 1970s, there are numerous resources available to aid in research, and many methods for working with this organism have already been established. The *C. elegans* genome was the first genome of a multicellular organism to be completely sequenced (*C. elegans* Sequence Consortium, Science, 1998). There are many knock-out mutants available from the *Caenorhabditis* Genetics Centre, the National Bioresource Project of Japan and the Insertional Mutagenesis Project. Libraries of RNAi constructs for knock-down of genes by the RNAi feeding method are available for a large portion of the genome [15]. In addition, strains can be constructed containing a gene or promoter of interest fused to a green fluorescent protein (GFP) sequence, and this allows visualization of the expression pattern of the gene (Fig. 1C; [16,17]). Useful strains, including mutant or GFP-containing strains can be frozen for decades, allowing stocks of strains to be stored for the long term without accummulating further mutations. Although the adult animal has just 959 cells, the cell lineages have been described in detail and the fates of every cell are known. In addition, the nematode is a model that allows the study of DNA repair in the context of a multicellular organism; this is a perspective that cannot be achieved in simpler models such as yeast or in cell culture.

C. elegans as a model system has been key to understanding several important biological pathways. For instance, the mechanism for RNA interference was first elucidated in the nematode [18,19]. Research in *C. elegans* has also been instrumental in understanding the genetic basis of programmed cell death and the core components of the apoptotic machinery [20,21]. *C. elegans* is particularly useful for the study of DNA repair because many of the repair proteins and pathways present in human cells are conserved in the nematode. For example, DNA damage checkpoint genes such as *atl-1*/ATR, *chk-2*/CHK2 and *clk-2*/HCLK2 have been described in the nematode [22–25]. Furthermore, the *BRCA1* and *BRCA2* genes have orthologs in *C. elegans*, known as *brc-1* and *brc-2*; both human genes are implicated in familial breast cancer, while BRCA2/FANCD1 is mutated in a subset of FA patients [26,27]. Multiple repair helicases are conserved in *C. elegans*, including *dog-1/FANCJ*, *him-6/BLM*, *wrn-1/WRN*, *Y50D7A.2/XPD* and *rcq-5/RECQ5*, among others [14,28–30]; www.wormbase.org). Pathways including homologous recombination repair, non-homologous end-joining, mismatch repair, nucleotide excision repair and ICL repair are conserved mechanisms for DNA repair in *C. elegans* [11,29]. Given the history, simplicity and tools available for working with the nematode, it is likely that research using *C. elegans* as a model will continue to make substantial contributions to the DNA repair field, amongst others.

The *C. elegans* germline is frequently the focus for studying repair in the nematode, as its structure provides a unique visual tool (Fig. 2A). The germline is organized in a temporal-spatial manner from distal to proximal regions such that nuclei can be observed as they progress from mitosis into and through the various stages of meiosis. The germline begins at the distal tip, where nuclei undergo mitosis in the mitotic zone, which acts as the stem cell compartment for the germline. Beyond the mitotic zone, nuclei progress into the transition zone, where meiosis begins, and nuclei in the leptotene stage of meiosis I can be observed to have a characteristic crescent shape. In this stage, homolog pairing and synapsis begin so that homologous chromosomes are held together by the synaptonemal complex. Meiotic DNA double strand breaks (DSBs) are formed through the action of the SPO-11 endonuclease as nuclei progress into the pachytene stage of meiosis. In this region, staining with anti-RAD-51 antibody can be used to visualize meiotic DSBs. After meiotic recombination is completed, and following diplotene and diakinesis, the highly condensed DNA can be observed as six bivalents that correspond to the homologous chromosome pairs held together by chiasma (Fig. 2A).

Damaged DNA results in visible changes in the *C. elegans* germline. For example, detection of DNA damage in the mitotic nuclei by the S-phase checkpoint results in cell cycle arrest, which provides time for repair before replication is allowed to proceed [22,24,31]. S-phase arrest results in visibly enlarged nuclei and a reduced number of nuclei in the mitotic zone (Fig. 2B; [22,24,31]). A low level of physiological apoptosis occurs in late pachytene at the bend region of the gonad in the absence of damage; in addition, damaged DNA can also lead to elevated apoptosis (Fig. 2B; [22,24,31]). As well, fragmented chromosomes, chromatin bridges and other types of damage can also be easily observed throughout the germline with DAPI staining, allowing for the evaluation of defects in strains carrying mutant alleles of DNA repair genes (Fig. 2B). The study of mutant phenotypes can also be informative with respect to gene function. For example, hermaphrodies have two X chromosomes, while males have a single X and normally arise at a frequency of about 1 in 1000 through stoichastic loss of the second X chromosome during meiotic divisions. The High incidence of males (Him) phenotype is caused by more frequent loss of the X chromosome and can be indicative of defects in chromosome segregation.

DNA damage sensitivity is most easily assessed in the nematode through the treatment of young adult animals with the agent of interest. For chemically induced DNA damage, such as nitrogen mustard, cisplatin and camptothecin, the nematodes can be soaked in a buffer solution containing the chemical agent along with bacteria as a food source. Treatment is followed by a 24-h period that allows for the progression of damaged nuclei through the germline. The eggs laid by drug-exposed young adults are then collected