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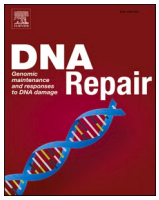

DONSON: Slding in 2 the limelight

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ABSTRACT

For over a decade, it has been known that yeast Sld2, Dpb11, GINS and Polε form the pre-loading complex (pre-LC), which is recruited to a CDC45-bound MCM2–7 complex by the Sld3/Sld7 heterodimer in a phospho-dependent manner. Whilst functional orthologs of Dbp11 (TOPBP1), Sld3 (TICRR) and Sld7 (MTBP) have been identified in metazoans, controversy has surrounded the identity of the Sld2 ortholog. It was originally proposed that the RECQ helicase, RECQL4, which is mutated in Rothmund-Thomson syndrome, represented the closest vertebrate ortholog of Sld2 due to a small region of sequence homology at its N-Terminus. However, there is no clear evidence that RECQL4 is required for CMG loading. Recently, new findings suggest that the functional ortholog of Sld2 is actually DONSON, a replication fork stability factor mutated in a range of neuro-developmental disorders characterised by microcephaly, short stature and limb abnormalities. These studies show that DONSON forms a complex with TOPBP1, GINS and Polε analogous to the pre-LC in yeast, which is required to position the GINS complex on the MCM complex and initiate DNA replication. Taken together with previously published functions for DONSON, these observations indicate that DONSON plays two roles in regulating DNA replication, one in promoting replication initiation and one in stabilising the fork during elongation. Combined, these findings may help to uncover why *DONSON* mutations are associated with such a wide range of clinical deficits.

1. Introduction

The first eight weeks of pregnancy represent a critical time during foetal development where DNA replication and cell division need to be perfectly coordinated. This period of rapid proliferation is essential for producing sufficient cell numbers required to maintain normal foetal growth. As such, it is highly sensitive to any genetic perturbations that disrupt the balance between cellular replication and division. Driven by the genetic revolution, the relative ease with which deleterious gene variants can be identified using high-throughput sequencing has revealed that inherited mutations in genes encoding components of the replication machinery give rise to a group of related growth deficiency syndromes, termed microcephalic primordial dwarfism (MPD) disorders. Typically, patients with MPD exhibit severe microcephaly (small head and brain) and pre- and post-natal growth retardation. However, depending on the mutated gene, these patients can display additional clinical deficits, such as microtia (small ears), absent or hypoplastic thumbs, absent or hypoplastic patellae (knee caps), craniosynostosis (premature fusion of the skull sutures), intellectual disability, genital abnormalities, cardiac defects and hearing loss. Meier-Gorlin Syndrome (MGS) represents the archetypal human MPD disorder associated with developmental abnormalities linked to aberrant DNA replication. To date, mutations in 13 different replication genes have been associated with causing MGS: *ORC1*, *ORC4*, *ORC6*, *CDT1*, *CDC6*, *GMNN*, *MCM3*, *MCM5*, *MCM7*, *CDC45*, *GINS2*, *GINS3* and most recently, *DONSON*. Since details relating to the clinical heterogeneity and underlying genetic causes of MGS can be found elsewhere, such as the review by Nielsen-Dandoroff et al. (2023) [1], this review will specifically focus on the recent discoveries concerning how DONSON functions during DNA replication and how this relates to the various diseases associated with mutations in this protein.

2. Identification of DONSON as a human disease gene associated with multiple neurodevelopmental disorders

humpty dumpty (*hd*), the drosophila ortholog of *DONSON*, was originally identified from a mutagenesis screen as an essential gene that causes a thin eggshell phenotype when mutated [2]. Analysis of *hd* mutant flies revealed animals with small brains caused by a reduction in DNA replication and increased DNA damage in the developing nervous system. Furthermore, it was reported that the levels of Hd protein peaked in S-phase cells and formed foci that colocalised with sites of

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Table 1
Clinical symptoms associated with syndromes caused by *DONSON* mutations. MD: microcephalic dwarfism; MIMIS: microcephaly micromelia syndrome; MISSLA: microcephaly, short stature and limb abnormalities; MGS: Meier-Gorlin syndrome; FFS: Femoral-facial syndrome; FA: Fanconi Anaemia; ID: intellectual disability; DD: developmental delay; na: not available; √ indicates that this clinical deficit was found in at least one patient with the associated disorder but does not indicate that this clinical feature is ubiquitously associated with the specific disorder. x indicates that this clinical deficit has not been documented in a patient with the associated disorder to date.

Syndrome	IUGR	Shortstature	microcephaly	Structuralbrainabnormalities	ID/DD	Microtia	Patellara/hypoplasia	Craniosynostosis	Micromelia/limbshortening	Clinodactyly, syndactyly, brachydactyly	Hypoplasiaofcarpal, metacarpalorphalangealbone	Jointcontracturesordislocation	Radial – raydefects	Hypoplasticorabsentthumbs	Absenceorpairsofribs	Dysmorphicfacialfeatures	Micrognathia	Skinhyper/hypopigmentation	Hypoplasticorabnormallungs	Respiratoryfailure	Gastrointestinalabnormalities	Genitourinarydefects	Cardiacanomalies	Neonatallethality	Hearingimpairment/loss
MD	√	√	√	x	x	x	√	x	x	√	√	√	√	√	x	x	x	x	x	x	x	x	x	x	x
MIMIS	√	√	√	√	na	x	na	√	√	√	√	x	√	√	√	√	√	x	√	√	x	√	√	√	na
MISSLA	√	√	√	√	na	√	na	x	√	√	x	x	√	√	x	√	x	x	x	x	x	√	x	x	x
MGS-like	√	√	√	x	√	√	√	x	x	√	x	√	x	x	x	√	x	√	x	x	na	√	x	x	√
FFS	√	√	x	na	√	x	na	x	x	√	x	x	x	x	x	√	x	√	x	x	x	x	x	x	√
FA-like	√	√	√	x	x	x	x	x	x	√	x	√	x	√	x	√	x	√	x	x	x	x	x	x	x
Seckel-like	√	√	√	√	√	√	na	x	x	x	x	x	x	x	x	√	x	x	x	x	x	x	x	x	x

ongoing DNA synthesis [2]. Combined, these observations lead to *hd* being designated a gene important for DNA replication. Using sequence alignment, orthologs of *hd* were identified in most eukaryotes from plants to humans, although interestingly not in yeast. In mammalian cells, the *hd* ortholog was named *DONSON* for 'downstream neighbour of SON', owing to its chromosomal proximity to the splicing factor gene, *SON*. However, at the time, the function of DONSON was unknown.

Insight into the function of human DONSON came from a genome-wide siRNA-based screen aimed at identifying genes that when knocked down gave rise to microscopically visible phenotypes that affected cell size and shape, mitotic progression, the actin cytoskeleton and cellular proliferation [3]. From this study, it was demonstrated that depletion of DONSON caused cells to arrest in S-phase with elevated levels of spontaneous DNA damage or in G2/M-phase with severe mitotic abnormalities. This suggested that DONSON is important for maintaining genome stability.

The first indication that DONSON was important for DNA replication and genome stability in humans came from the identification of biallelic deleterious variants in the *DONSON* gene using whole exome sequencing (WES) in a cohort of 29 patients with microcephalic dwarfism [4]. All affected patients displayed microcephaly and short stature, whereas other clinical deficits, such as clinodactyly (curved fingers), syndactyly (fused or webbed fingers), brachydactyly (short fingers or toes), absent or hypoplastic patellae, hypoplastic thumbs and radial-ray defects (absent of hypoplastic radius) were present in a minor proportion of patients (Table 1). Notably, despite the mutations being localised across the entire open reading frame of *DONSON*, most seem to destabilise the protein. However, since *DONSON* is an essential gene, all patient cells express some residual protein. As a consequence of this, it is likely that some of the phenotypic variability observed between patients is not only determined by the specific mutation and its localisation but also by how stable the mutant protein is. Interestingly, two patients within this cohort with a homozygous intronic splicing mutation (c.1047–9 A>G) in *DONSON* exhibited a severe form of microcephalic dwarfism, termed microcephaly-micromelia syndrome (MIMIS), which is characterised by marked microcephaly with distinctive craniofacial features, pronounced shortening of the limbs (micromelia), craniosynostosis, absent ribs, hypoplastic or absent thumbs, brain abnormalities and perinatal lethality due to respiratory failure (Table 1). Whilst this disease phenotype diverged significantly from the rest of the patients within this cohort, a subsequent study identified 26 patients with MIMIS from 9 families of Saskatchewan origin, all with the same homozygous intronic splicing mutation in *DONSON* [5]. This indicates that either the mutant protein produced arising from the mispliced *DONSON* transcript has a particularly deleterious impact on development or that additional genetic factors that exacerbate the developmental deficits associated with DONSON dysfunction are co-inherited with the c.1047–9 A>G splice mutation. However, as yet, no functional studies have been carried out to determine what impact these splice site mutations have on DONSON function.

To add further complexity to the clinical phenotype caused by *DONSON* mutations, several additional studies have identified pathogenic variants in *DONSON* in patients exhibiting a Fanconi Anaemia (FA)-like phenotype, a Seckel-like syndrome, a phenotype associated with microcephaly, short stature and limb abnormalities (MISSLA) and also Meier-Gorlin Syndrome (Table 1) [6,7,8,9]. This indicates that *DONSON* mutations cause a spectrum of related clinical deficits. Notably, one study identified a de novo mutation in *DONSON* in a patient with prominent micrognathia (small lower jaw), short stature and a hypoplastic femur and tibia, clinically diagnosed with Femoral-Facial syndrome (FFS) (Table 1) [8]. Whilst the pathogenicity of this dominant DONSON mutation has not been formally verified, this observation indicates that the *DONSON*-associated diseases may be inherited in both an autosomal recessive and autosomal dominant manner.

3. DONSON functions as a replication stress response protein

At the time that DONSON was identified as a disease gene, very little was known about its function. However, the association of *DONSON* mutations with clinical phenotypes overlapping with other known replisomopathies (diseases linked with mutations in replication genes) in conjunction with previous work carried out on *Hd* strongly suggested that DONSON played a role in regulating DNA replication and/or the replication stress response. Functional analysis of cells depleted of DONSON using siRNA highlighted the essential nature of DONSON for maintaining replisome integrity. Loss of DONSON resulted in a high level of spontaneous replication fork collapse that resulted in an extreme form of genome instability characterised by highly fragmented or completely pulverised chromosomes, which could be rescued by co-depletion of the structure-specific nucleases XPF or Mus81 [4]. This indicated that DONSON was likely a novel component of the replication machinery required to maintain the stability of elongating forks. In keeping with this, DONSON was not only found to be associated with core components of the replisome e.g. the MCM helicase, but was also shown to be required to efficiently activate the ATR-dependent replication stress response [4]. These findings are consistent with the identification of *DONSON* mutations in patients with clinical phenotypes resembling both MGS, which can be caused by *MCM* gene mutations, and ATR-Seckel syndrome.

In line with patient-associated mutations in *DONSON* being hypomorphic, functional analysis of patient-derived cell lines revealed them to have increased spontaneous replication fork collapse and chromosomal instability, albeit not as severe as that observed in DONSON depleted cells [4]. Taken together, these observations indicated that the clinical deficits exhibited by patients arise as a consequence of a failure to complete DNA replication in a timely manner during embryonal development, particularly within the developing nervous system. In accordance with this, *Donson* was found to be expressed very highly within the proliferation and differentiation zones of the dorsal and ventral telencephalon of the developing mouse brain. Moreover, conditional loss of *Donson* in the telencephalon using *Emx1*- and *Nkx2.1*-driven Cre recombinase induced high levels of apoptosis in replicating neural progenitor cells located within the neocortex [10].

Further insight into DONSON functions as a replication stress response protein came from a recent study demonstrating that DONSON functions in parallel with FANCM to promote traversal of the replisome past a DNA inter-strand cross-link (ICL) [11]. Interestingly, it was observed that DONSON was predominantly present as part of the replication machinery when it was located within regions of euchromatin, whereas in contrast, FANCM was principally found associated with replisomes present in heterochromatic regions. This important finding revealed for the first time that the composition of the replication machinery is not static and changes not only depending on the absence/presence of DNA damage but also according to replication timing, chromatin accessibility and/or transcriptional competency. In addition to this, whilst it is known that FANCM possesses translocase activity capable of bypassing an ICL by remodelling secondary DNA structures that arise at stalled forks [12,13,14], DONSON does not have any intrinsic enzymatic activity. This suggested that DONSON acts as a scaffold protein that facilitates the recruitment of proteins, such as translocases/helicases like SMARCAL1, ZRANB3 and HLTF, to the replisome depending on the presence of DNA damage or the context of the surrounding chromatin [15].

4. A newly identified role for DONSON in regulating replication initiation

To date, the role of DONSON in regulation of DNA replication was has only been studied in context to its ability to sustain replication under conditions of replication stress. However, it was very clear from the initial studies using patient-derived cell lines and siRNA-mediated depletion of DONSON, that the most prevalent replication phenotype observed in these cells occurred in the absence of exposure to any exogenous genotoxins [4]. Moreover, using a sequential immunoprecipitation approach, it was shown that DONSON exists in two different replisome complexes: one that was present in undamaged cells and contained all the replisome components and one that was only present following the induction of DNA ICLs, which lacked the GINS complex but still retained CDC45 and the MCM helicase. In contrast, the FANCM containing replisomes only occurred after induction of ICLs and lacked both the GINS complex and DONSON [11]. This indicated that DONSON has two roles, one to regulate unperturbed replication and the other to control replication in the presence of DNA damage. However, whether DONSON plays a role in removing the GINS complex from the damage-associated replisome or is required to recruit it back again once the replication machinery has bypassed the DNA lesion is unclear.

In *Saccharomyces cerevisiae*, the process of converting the pre-replication complex (pre-RC) into the CMG (CDC45-MCM-GINS) complex requires several scaffold proteins, such as Sld2, Sld3, Sld5 and Dpb11, and both S-phase CDK (Cyclin-dependent kinase) and DDK (Dbf4/Drf1-dependent Cdc7 kinase) activity [16]. Initially, DDK binds and phosphorylates MCM2/4/6 [17–22], which facilitates binding of a sub-complex of the replisome, comprised of Sld3-Sld7-CDC45. This binding is mediated by an interaction between Sld3 and MCM [23,24]. At the same time, S-phase CDKs phosphorylate both Sld2 and Sld3. Phosphorylated Sld2 forms a sub-complex with Dpb11, GINS and Polε (termed pre-loading complex or pre-LC), which is then recruited to phosphorylated Sld3 via an interaction with Dbp11 [25,26,27]. This forms an inactive pre-initiation complex (pre-IC), which is activated by the binding of additional factors, such as MCM10 [28,29].

Whilst considerable sequence divergence has occurred between yeast and mammalian cells, functional orthologs of Sld3, Sld7 and Dbp11 have been identified in higher eukaryotes as TICRR, MTBP and TOPBP1 respectively [30–33]. However, in contrast to yeast, the TICRR/MTBP heterodimer and TOPBP1 associate with chromatin independently of each other [34–37]. Despite this, the phosphorylation of TICRR by CDK is required to stabilise its interaction with the pre-RC via binding the BRCT1/2 domains of TOPBP1 [38]. Furthermore, both TICRR and MTBP have been reported to bind CDC45 and promote its association with the pre-RC [31,32,33,36,39].

Interestingly, no clear ortholog of Sld2 has been identified in higher eukaryotes. It has been suggested that this function may be carried out by the RECQ helicase, RECQL4, which has limited sequence homology at its N-terminus with Sld2 [40,41]. Yet there is no clear evidence that RECQL4 is required for CMG assembly. Rather, it has been demonstrated that RECQL4 is more important for Polα loading during replication initiation [42,43]. Combined, this indicates that RECQL4 does not represent the true functional ortholog of Sld2.

Recently, four independent studies have identified DONSON as being the missing functional, 'Sld2-like' component of the vertebrate pre-LC, although it does not share any amino acid sequence similarity with Sld2 [44–47]. Together, these studies indicate that DONSON scaffolds the formation of the pre-LC and facilitates delivery of the GINS complex to origins for CMG assembly. It has been proposed that a dimer of DONSON binds to and is recruited to the pre-RC by the BRCT3 domain of TOPBP1, where it is required to position the GINS complex on the MCM2–7 helicase via an interaction with MCM3 (Fig. 1A) [44–46]. This allows the interaction of the GINS complex with the replicative helicase to be stabilised through its association with the GINI motif and/or BRCT4/5 domains of TOPBP1. Interestingly, whilst contacts are made between the structured part of the DONSON homodimer and both MCM3 and the GINS complex, structural modelling suggests that the disordered N-terminus of DONSON also plays an important role in stabilising binding to both TOPBP1 and the GINS complex [45,46]. In addition to TOPBP1 and the GINS complex, the N-terminus of DONSON also binds to Polε. However, since disrupting the interaction of DONSON and Polε has little impact on CMG formation, this indicates that TOPBP1,

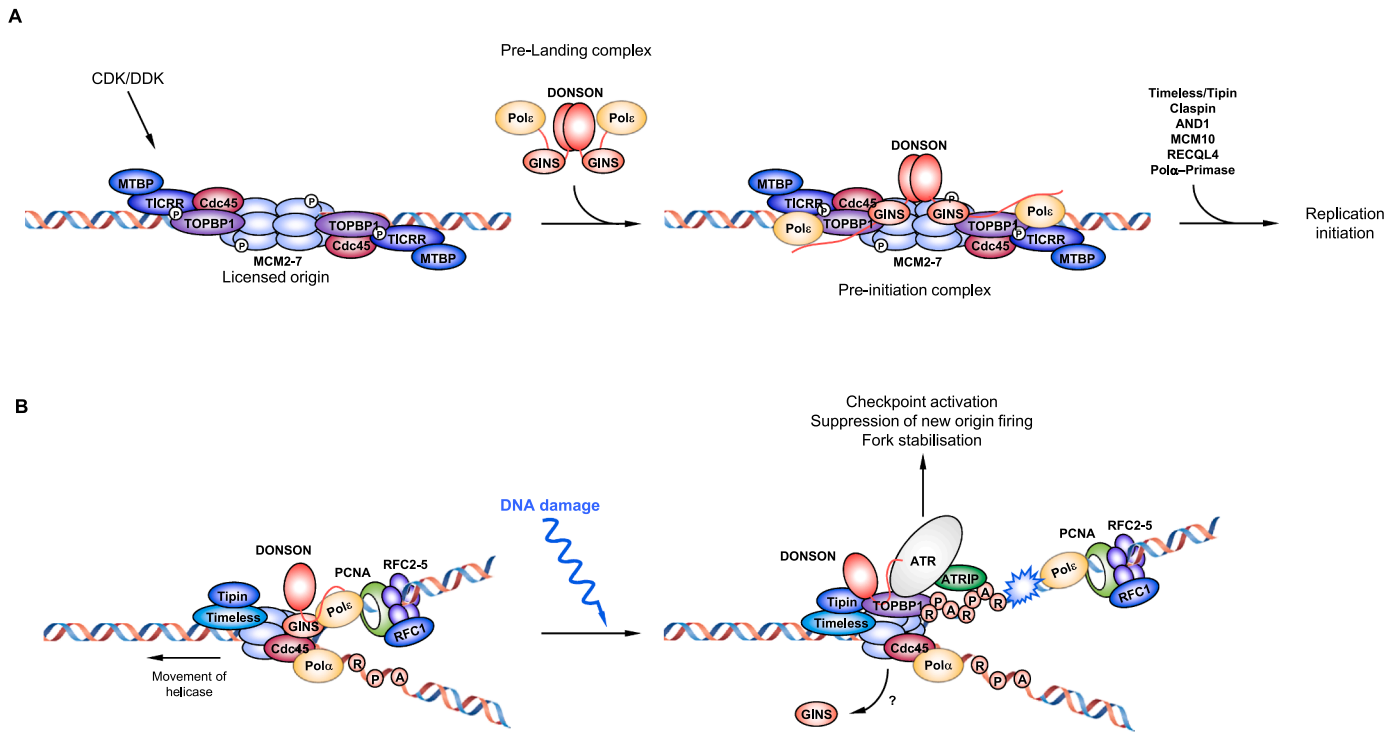

Fig. 1. A. Phosphorylation of the chromatin-bound MCM double hexamer complex and TICRR by Cyclin-dependent kinase (CDK)/Dbf4/Drf1-dependent Cdc7 kinase (DDK) facilitates the recruitment of TICRR-MTBP-CDC45 to licenced origins. The pre-landing complex (pre-LC) is composed of a DONSON dimer, the GINS complex and Polε⊡ The GINS complex and Polε bind to separate motifs within the disordered N-terminus of DONSON (depicted as a red line attached to the DONSON protein). The pre-LC is recruited to licenced origins by DONSON binding TOPBP1, potentially in a phospho-dependent manner, which is further stabilised by TOPBP1 also binding the GINS complex and TICRR. Additional proteins are then recruited to the pre-initiation complex, including the Timeless/Tipin heterodimer, Claspin, AND1, MCM10, RECQL4 and the Polα-Primase complex to initiation DNA replication. B. During replication elongation, DONSON travels with the replication machinery, either as a monomer or a dimer, potentially through its interaction with MCM3, GINS and/or Polε. In the presence of DNA damage, single-stranded DNA (ssDNA) generated by polymerase-helicase dissociation is coated by replication protein A (RPA). RPA-coated ssDNA triggers recruitment of the ATR/ATRIP complex, which is activated by binding the ATR-activating domain (AAD) of TOPBP1. Efficient activation of the ATR-dependent replication stress, also requires DONSON, Timeless/Tipin, Claspin, ETAA1, Rad17 and the Rad9-Rad1-Hus1 'PCNA-like' complex (some of which are not shown on the diagram for simplicity). In the presence of some types of DNA damage, it has been suggested that the GINS complex may dissociate from the CDC45-MCM-GINS (CMG) complex. Based on the role of DONSON as a component of the pre-LC, it is possible that it may play a role in recruiting GINS back to the CDC45-MCM complex once the DNA damage has been bypassed or repaired.

GINS and Polε binding to this region of DONSON are independent of one another. Consistent with this, GINS and Polε bind to two highly conserved motifs, 6-Pro-Gly-Tyr-8 and 78-Asn-Pro-Phe-80 respectively, within the hDONSON N-terminus separated by approximately 69 amino acids.

Notably, three of these studies also report that the depletion of DONSON disrupted not only GINS but also CDC45 recruitment to origins, whilst leaving the chromatin association of TICRR and MTBP unaffected [44,45,47]. It was suggested by one study that DONSON binds to CDC45 [44]. However, since the binding of CDC45 within the CMG complex is predominantly mediated by the MCM and GINS complexes [48,49], it is possible that cell extracts lacking DONSON fail to load CDC45 onto origins as a consequence of a reduced presence of the stabilising effects of the GINS complex.

The study by Xia *et al.* [46] identified DNSN-1 as the *C. elegans* ortholog of hDONSON and demonstrated that like its human counterpart, it is required for replication initiation. Importantly, cryo-EM analysis of DNSN-1 bound to the CMG/TIM-1/TIPN-1 complex confirmed the predictions made by AlphaFold-Multimer that DONSON functions as a dimer when bound to the CMG and makes critical interactions with MCM3 and components of the GINS complex [45,46]. Despite this, DNSN-1 is not required for CDC45 loading onto chromatin and is dispensable for replication fork elongation in contrast to the situation in *Xenopus* egg extracts and human cells [4,44,45,47]. This indicates that whilst the mechanism for loading GINS onto the MCM helicase during replication initiation is conserved among metazoans, some functions of DONSON have diverged during eukaryotic evolution.

5. Understanding the basis for DONSON-associated disease

This newly identified role for DONSON in facilitating the assembly of the CMG and initiating DNA replication helps to explain why some mutations in DONSON are associated with a clinical disease that mimics MGS, which is typically associated with mutations in components of the pre-RC and pre-IC. Despite this, there are still many clinical discrepancies between the different syndromes associated with defective replication initiation that are not explained by these findings. For example, given that DONSON is required to facilitate the loading of CDC45 and GINS, it is not clear why craniosynostosis is not observed in DONSON mutant patients, since this is the principal distinguishing feature between MGS patients with biallelic *CDC45* and *GINS2* mutations and other MGS patients with mutations in the *ORC* or *MCM* genes [50,51]. Furthermore, patients with biallelic *GINS1* and *POLE1* mutations develop NK deficiency and/or immunodeficiency, neither of which have been observed in DONSON patients [52–54]. Therefore, whilst Xenopus egg-extract replication system is a powerful technique with which to dissect the fine mechanistic details of how replication is regulated, it is sometimes difficult to extrapolate experimental observations derived from using this system into human disease models. Interestingly, one of the recent studies identifying a role for DONSON in initiating DNA replication also presented a mouse knockin model of a previously identified patient-associated mutation in DONSON, p.

Met446Thr (p.Met440Thr in mouse) [45]. Unexpectedly, the mouse exhibited developmental defects more consistent with MISSLA rather than an FA-like phenotype, which is displayed by the three affected patients [4]. Quite why this Donson mutation had a more severe impact on limb development in the mice as compared to the patients with the equivalent mutation is unknown. However, reassuringly cells from these mice displayed a reduction in replication fork speed, an increase in inter-origin distance and a reduced ability to load both GINS and CDC45 onto chromatin, all of which are consistent with a reduced ability to initiate DNA replication [45]. Importantly, cells from these mice also exhibited significant replication fork asymmetry and an inability to activate the ATR-dependent replication checkpoint. This is consistent with previous observations that DONSON is also critical for maintaining the stability of and restarting elongating forks that have stalled, which is thought to occur in part through its ability to facilitate the activation of the ATR-Chk1 pathway [4]. Although it is not clear whether this ability of DONSON is mediated by directly activating ATR or through its interaction with proteins known to be required for Chk1 activation e.g. TOPBP1, Claspin or Timeless/Tipin.

In contrast to the observations relating to the role of DONSON in regulating fork restart from human cells, it was reported by Hashimoto et al. [44] that whilst DONSON is essential for efficient activation of Chk1 in response to replication stress in Xenopus egg extract, it is dispensable for the restart of a stalled replication fork. In this case, it was shown that Topbp1, Claspin and Polε could be reloaded onto chromatin, which had been stripped of these factors using high salt following replication initiation and then inhibition, even in the absence of DONSON. Furthermore, Hashimoto et al. [44] demonstrated that the reloading of Topbp1, Claspin and Polε but not DONSON onto chromatin was sufficient to support replication fork restart once the inhibition of replication elongation was removed. Whilst these findings are somewhat contradictory with the situation in mammalian cells, it supports the idea that regulation of the replication machinery is different during initiation versus elongation/restart and that DONSON plays separate roles during these two processes. However, since most components of the replisome do not unload from the chromatin when a fork stalls, it is difficult to extrapolate observations made under these artificial conditions in Xenopus egg extract to the situation in cells when an ongoing fork encounters an obstacle or replication blocking lesion. Notably, it has been shown that during traversal of an ICL by a replication fork in mammalian cells, components of the GINS complex are unloaded from the replisome, which does not occur when replicating Xenopus chromatin was treated with high salt. In this situation, given the role for DONSON in loading the GINS complex onto chromatin, it seems plausible that it might have a similar role at stalled forks in reloading GINS once the ICL has been bypassed [11]. Similarly, it is also possible that DONSON may be involved in restarting a stalled fork by reloading Polε following the bypass of a DNA lesion by a trans-lesion synthesis (TLS) associated polymerase, such as Polη.

Whilst there is still much more to learn about this new replication protein, it evident from recent work that the controversy surrounding the identity of the mammalian ortholog of yeast Sld2 has been resolved and that DONSON, rather than RECQL4, is missing component of the pre-LC required to load the GINS complex onto licenced origins. However, some important additional questions remain unanswered, such as if the phosphorylation of DONSON by CDKs is required to facilitate its binding to TOPBP1 at licenced origins, in a manner similar to Sld2. This may be of particular importance to understanding genotype-phenotype relationships in patients since a relatively well conserved phosphorylation site (Ser-28) within the DONSON N-terminus, is altered to an arginine residue and coinherited with two other variants (c.1466 A>C [p.Lys489Thr] and c.786–33 A>G) as part of a common disease haplotype identified in several affected patients [4]. Although, it was previously shown that the p.Lys489Thr variant is pathogenic, it is possible that loss of this N-terminal phosphorylation site could have a modifying effect on the function of the mutant protein, which could alleviate or aggravate clinical presentation of the disease. Also, it is not clear how DONSON differentially regulates replication initiation versus elongation/restart and whether this is linked to changes in dimerization following separation of the two MCM hexamers (Fig. 1B). In this respect, structure of the DONSON dimer bound to the CMG has been recently solved [55]. This study demonstrated that DONSON dimerization is critical for replication initiation. Critically, this study also showed that tryptophan-228 lies at the dimer interface and when changed to leucine to mimic the dominant DONSON mutation associated with FFS [8], it specifically disrupts dimerization without compromising protein stability [55]. This indicates that DONSON mutations that just disrupt dimerization can be inherited in a dominant fashion and maybe associated with milder developmental defects. In contrast, those mutations that destabilise the DONSON protein are inherited in a recessive manner, compromise replication initiation/elongation and the ATR-dependent replication stress response and as such, are associated with more severe clinical deficits. Taken together, it is likely that defining how post-translational modifications regulate the binding of DONSON to itself and other components of the replisome will not only increase our knowledge about how DONSON functions to control unperturbed DNA replication and the response to damaged replication forks but it may also help us to understand how DONSON dysfunction causes such variable clinical disease.

CRediT authorship contribution statement

G.S.S. wrote and revised the manuscript.

Declaration of Competing Interest

The author declares that he does not have any competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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