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DOI:
10.1099/jgv.0.001918

JOURNAL OF
GENERAL VIROLOGY

REVIEW
Grand, *Journal of General Virology* 2023;104:001918
DOI 10.1099/jgv.0.001918

MICROBIOLOGY SOCIETY

OPEN ACCESS

SARS-CoV-2 and the DNA damage response

Roger J. Grand*

Abstract

The recent coronavirus disease 2019 (COVID-19) pandemic was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 is characterized by respiratory distress, multiorgan dysfunction and, in some cases, death. The virus is also responsible for post-COVID-19 condition (commonly referred to as 'long COVID'). SARS-CoV-2 is a single-stranded, positive-sense RNA virus with a genome of approximately 30 kb, which encodes 26 proteins. It has been reported to affect multiple pathways in infected cells, resulting, in many cases, in the induction of a 'cytokine storm' and cellular senescence. Perhaps because it is an RNA virus, replicating largely in the cytoplasm, the effect of SARS-Cov-2 on genome stability and DNA damage responses (DDRs) has received relatively little attention. However, it is now becoming clear that the virus causes damage to cellular DNA, as shown by the presence of micronuclei, DNA repair foci and increased comet tails in infected cells. This review considers recent evidence indicating how SARS-CoV-2 causes genome instability, deregulates the cell cycle and targets specific components of DDR pathways. The significance of the virus's ability to cause cellular senescence is also considered, as are the implications of genome instability for patients suffering from long COVID.

INTRODUCTION

By mid-October 2023 over three-quarters of a billion cases of coronavirus disease 2019 (COVID-19) had been reported, with almost seven million deaths, worldwide [WHO Coronavirus (COVID-19) Dashboard]. Approximately half of COVID-19 survivors, regardless of hospitalization status, still experience a range of symptoms after 4 to 6 months, which has been termed 'long COVID' [1–7]. Prolonged symptoms include immune dysregulation, vascular and circulatory problems, respiratory and gastrointestinal system problems, neurological problems and general fatigue, with symptoms resembling myalgic encephalaomyelitis/chronic fatigue syndrome (ME/CFS) (reviewed, for example, in [7]). COVID-19 is caused by infection with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [8–10]. SARS-CoV-2, like other coronaviruses, is a member of the order Nidovirales and the family *Coronaviridae*. It is closely related to other betacoronaviruses – severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), with approximately 79 and 50% homology, respectively, at the nucleotide level [8, 11].

SARS-CoV-2 has a single-stranded, positive-sense RNA genome of approximately 30 kb. Following infection, two open reading frames, ORF1a and ORF1b, encompassing approximately two-thirds of the viral genome at the 5′ end, are translated into two polyproteins (pp1a and pp1ab), while four structural proteins (spike, envelope, membrane and nucleocapsid; S, E, M, N, respectively) and a number of accessory proteins (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b and ORF14) are encoded by the 3′ third of the genome [12–17]. The polyproteins are cleaved by viral proteases, encoded by Nsp3 and Nsp5, to give 16 non-structural proteins (Nsp1–16) [18–21].

Infection with SARS-CoV-2 leads to dysregulation of many pathways in the host cell, most notably those involved with the immune response and inflammation, oxidative stress, RNA metabolism, homeostasis, cell cycle regulation, senescence, autophagy and apoptosis. Up to now, relatively little attention had been paid to the relationship between the virus and genome stability and the DNA damage response (DDR). However, it is now becoming clear that SARS-CoV-2 causes damage to the host cell DNA and has a complex interaction with the cellular DDR. In this review I have attempted to summarize published data relevant to this relationship and have gone on to consider what the implications might be for patients suffering from long COVID. Because

Received 01 September 2023; Accepted 27 October 2023; Published 10 November 2023
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Keywords: SARS-CoV-2; COVID-19; DNA repair; DNA damage response; senescence.
Abbreviations: COVID-19, coronavirus disease 2019; Nsp, non-structural protein; ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

almost all the information discussed here has been published recently, and may be preliminary in a few instances, in some cases there are inconsistencies and divergent views, which will need further evaluation for verification in future; however, it is hoped that the case has been made that the DDR is an important target for SARS-CoV-2.

VIRUSES AND THE DNA DAMAGE RESPONSE

The cellular DDR comprises a series of complex, sometimes overlapping, pathways that can detect and repair different forms of damage to the cellular genome. At least seven pathways can be distinguished. For example, double-strand breaks (DSBs), arising as a result of exogenous or endogenous factors, are repaired either by non-homologous end joining (NHEJ) or homologous recombination (HR) [22–28]. HR is an error-free repair mechanism, occurring during the S and G2 phases of the cell cycle, which relies on the presence of the sister chromatid as a repair template. NHEJ occurs throughout the cell cycle and may, very rarely, introduce errors into the repaired DNA. Mismatch repair (MMR) is primarily for the repair of base–base mismatches and insertion/deletion mispairs generated during DNA replication and recombination [29–32]. The base excision repair (BER) pathway repairs most endogenous base lesions and abnormal bases in the genome and is also involved in repair of DNA single-strand breaks [33, 34]. Nucleotide excision repair (NER) is the main pathway used by mammals to remove bulky DNA adducts, which disrupt the structure of the DNA double helix, such as those formed by UV radiation [35, 36]. Inter-strand crosslink repair (ICL) fixes crosslinks formed between the two DNA strands. Inter-strand crosslinks are repaired by the Fanconi anaemia (FA) pathway, whereas intra-strand crosslinks are repaired by the NER pathway [37–39]. There is also a direct repair pathway that is dependent on the ability of O^6-methylguanine-DNA methyltransferase (MGMT) to remove alkyl groups from the O^6 position on guanine [40, 41]. These pathways, in the context of viral infection, have been described in more detail in several reviews over the past decade (for example [42–44]).

All DNA viruses seem to impinge on one or more of these repair pathways during infection, either activating or antagonizing them [42, 45–48]. Initial infection by DNA viruses generally results in activation of phosphatidylinositol 3-kinase-related kinases (PIKKs), resulting in phosphorylation of downstream targets such as γH2AX and the Chk1 and Chk2 kinases. This activation may be due to the presence of viral DNA and/or expression of viral proteins that affect host cell processes such as transcription [47, 48]. The viruses then go on to utilize or inactivate, depending on the virus in question, differing components of the repair pathways to facilitate viral replication. Thus, for example, some viruses, such as adenovirus (HAdV) and herpes simplex virus 1 (HSV1), inactivate double-strand break repair by targeted degradation of MRE11, BLM and DNA Ligase IV (by HAdVs, depending on the type) or RNF8 and DNA-PKcs, (by HSV1) [49–52]. On the other hand, most gammaherpesviruses require ATM for lytic replication [53–57], as do polyoma viruses [58, 59]. Regardless of whether a DNA virus activates or antagonizes the DDR they all recruit damage response proteins to their viral replication centres (VRCs) (see, for example, [55, 60–65]). It is assumed that their function there is to facilitate the production and integrity of viral DNA, although direct evidence for this is limited.

Our knowledge of the relationship of RNA viruses to the DDR pathways is much more limited (reviewed in [43]). The mode of replication of RNA viruses is governed largely by the configuration of their genomes. Coronaviruses, such as SARS-CoV-2, have a positive-sense, single-stranded genome that serves as mRNA and is translated in the cytoplasm largely into a polyprotein before its cleavage, giving rise to the replicase–transcriptase complex. This complex synthesizes a negative-strand RNA that acts as a template for transcription of genomic RNA and multiple subgenomic mRNAs in the infected cell. Membrane-associated VRCs, forming in the cytoplasm and containing viral proteins, are sites for the production of new viral RNA [66, 67]. (For a detailed description of the replication of RNA viruses in general see, for example, various chapters in [68]).

As most RNA viruses largely replicate in the cytoplasm, it might be expected that they have relatively little interaction with the cellular DDR. However, this is not the case and representative viruses from many families have been shown to cause genome instability and activation of DDR pathways (reviewed in [43]). For example, Chikungunya and Sindbis viruses – members of the family *Togaviridae* that, like SARS-CoV-2 and other coronaviruses, replicate in the cytoplasm – activate ATR and PARP1 pathways following infection [69, 70]. Other RNA viruses, such as human immunodeficiency virus-1 (HIV-1) (a member of the family *Retroviridae*) and influenza A virus (IAV) (a member of the family *Orthomyxoviridae*), replicate partially in the nucleus and so might be expected to be more likely to cause DNA damage. HIV-1 causes double-strand breaks as a result of binding of its VPR protein to chromatin [71]. Vpr expression also activates ATM and leads to the formation of repair foci [72]. IAV infection leads to DNA damage and formation of γH2AX repair foci [73, 74]. Thus, it appears that the conformation of the viral genome, positive or negative strand, or even location of replication, has little influence over whether an RNA virus species impacts on the host cell DDR. The explosion of scientific interest in coronaviruses, and SARS-CoV-2 in particular, triggered by the COVID-19 pandemic, has meant that our understanding of the relationship of this RNA virus to the DDR has increased appreciably. In this review I discuss these latest observations on the mechanisms by which SARS-CoV-2 can induce DNA damage and activate or inhibit DNA repair pathways. I also consider the implications of this for the long Covid syndrome. Previous reviews have also examined the relationship between SARS-CoV-2 and the DDR [75, 76]. In the latter case, emphasis has been placed on the host immune system.

SARS-COV-2

SARS-CoV-2, like SARS-CoV, gains entry to the host cell through association with cellular attachment factors, such as glycans and integrins, and binding of the spike (S) glycoprotein to angiotensin-converting enzyme 2 (ACE2) [77–81]. S is cleaved to give the functionally distinct S1 and S2 subunits by a cellular protease [79, 82]. The incorporation of the basic cleavage site (PRRAR) at the S1/S2 boundary facilitates the cleavage reaction, resulting in enhanced infection [82]. An additional cleavage site is present in S2 [79]. S1 contains the receptor-binding domain, whereas the S2 transmembrane domain mediates fusion of the viral and cellular membranes, leading to syncytial formation. Cleavage of the S protein, predominantly by transmembrane protease serine 2 (TMPRSS2), is necessary for fusion and the viral entry to occur [79, 81, 82].

The viral genomic RNA is then released into the host cell cytoplasm, initiating viral gene expression (reviewed, for example, in [12]). Initially, the SARS-CoV-2 genome is copied to produce a full-length negative version that is a template for the production of new positive-sense genomic RNA, which can be translated into proteins or used as genomes in progeny viruses. As with other members of the order Nidovirales, transcription is discontinuous, such that a set of 3′ to 5′ co-terminal sub-genomic RNAs are produced. These are used to synthesize negative-strand RNAs, which are then used as templates to synthesize positive-sense mRNAs, which, in turn, are translated to give structural and accessory proteins [12, 83–86].

As mentioned above, ORF1a and ORF1b are translated from the genome, giving rise to the polyproteins pp1a and pp1ab [83, 84]. Cleavage of these by viral cysteine proteases, located in Nsp3 and Nsp5, produces Nsps 1–11 from pp1a and Nsps 12–16 from pp1ab [12–14, 87–89]. Rapid proteolytic release of Nsp1 leads to inhibition of translation of certain host cell proteins [90–92]. Nsps 2–16 form the viral replication/transcription complex (RTC), with Nsps 12–16 providing the enzymes necessary for RNA synthesis, modification and proofreading. The remaining Nsps (2–11) appear to play slightly subsidiary roles, supporting replication (reviewed in [12, 93, 94]). Nsp 12 is the RNA-dependent RNA polymerase (RdRP), and Np7 and Nsp 8 act as co-factors [95, 96]. Nsp7 and Nsp 8 have been suggested to possess primase activity [97]. Nsp14 has proofreading capability and has both 3′–5′ exonuclease and guanine N7 methyltransferase activity [98–100]. The N7 methyltransferase activity is required for 5′ capping of viral RNAs, which protects them from host anti-viral defences [95–98]. Nsp10 associates with Nsp14 and enhances its exonuclease activity [98–100]. Nsp13 is an RNA helicase and is essential for transcription and translation [101, 102]. Nsp16 is a 2′-O-methyltransferase, whilst Nsp15 has endoribonuclease activity [103–106] (these and other functions, with additional relevant references, are summarized in Table 1).

The SARS-CoV-2 membrane protein (M) is the most abundant structural protein and is important for virus assembly and determining virus morphology and membrane budding [107–111]. M protein localizes in the endoplasmic reticulum Golgi intermediate compartment (ERGIC) and is essential for the recruitment of other viral proteins (see Table 1 for a summary of the subcellular distribution of SARS-CoV-2 proteins). It binds to the nucleocapsid (N) protein and together they can form virus-like particles [107]. Although N protein has a structural function, it is also essential for transcription and replication [112]. Through binding to the viral RNA genome, it is involved in the creation of the ribonucleoprotein complexes that regulate replication and viral RNA synthesis. Binding of viral RNA to N protein causes liquid–liquid phase separation; this enhances the interaction of RNA with the RdRP complex, favouring viral replication [112–116].

SARS-CoV-2 spike trimeric glycoprotein (S) is located on the surface of the viral membrane and is responsible for binding to the host cell ACE-2 receptor, as mentioned above [79–82, 117–119]. The envelope (E) protein is an integral membrane protein of low molecular weight (75 aa in SARS-CoV-2) (reviewed in [120, 121]). It is present at low concentration in virus particles but is abundant in infected cells. E protein interacts with the membrane protein (M), helping to maintain the structure of the virus particle. E protein is present inside the virus envelope. In addition, it polymerizes to form a cation-selective ion channel (also termed a viroporin), which is localized in the Golgi, endoplasmic reticulum and ERGIC of the infected cell.

A further set of proteins is encoded on the SARS-CoV-2 genome. These are the accessory proteins, which do not appear to be essential for viral replication but are important for virus–host cell interactions associated with virulence (reviewed in [14, 93, 122–124]) (Table 1). The SARS-CoV-2 accessory proteins ORF6, ORF3b, ORF7a, ORF7b, ORF8, ORF9b and ORF10 are considered to be interferon (IFN) antagonists and play a role in SARS-CoV-2 pathogenesis (reviewed in [125–127]).

CELL CYCLE REGULATION BY SARS-COV-2

Following damage to their DNA, eukaryotic cells adopt a number of responses. These include co-ordination of cell cycle progression with DNA repair, as well as chromatin remodelling and transcriptional regulation or cell death. Arrest or delay of cell cycle progression provides time for DNA repair, and this is controlled by a network of signalling pathways that are able to initiate various cell cycle checkpoints (reviewed, for example, in [128, 129]). Simplistically, different checkpoints are activated, depending on where in the cell cycle damage occurs. For example, to prevent entry into S phase with damaged DNA, cells in G1 activate the checkpoint kinases ATM/ATR and Chk1/Chk2. Similarly, the G2/M checkpoint prevents segregation of chromosomes that have incurred unrepaired damage during G2. Depending on which checkpoint is activated or the type of DNA damage, different repair pathways will be activated.

Table 1. SARS-CoV-2 proteins, summary of their subcellular localization and their function. A very brief description of the function is given, together with appropriate references

SARS-CoV-2 protein	Tentative subcellular location	Functions	References
Nsp1	Cytoplasmic	Induces host cell RNA cleavage, host shut off, inhibits host RNA translation and IFN response. Inhibits STAT1 phosphorylation	[90–92, 253–255]
Nsp2	Cytoplasmic	Impairs IFN production, represses mRNA translation	[256, 257]
Nsp3	Cytoplasmic	Protease activity (polyprotein processing). Involved in generation of DMVs with Nsp4. Interacts with N	[87, 258–260]
Nsp4	ER, cytoplasmic	DMV formation with Nsp3	[260]
Nsp5	Cytoplasmic	Protease activity – $3CL^{pro}$ and M^{pro} (polyprotein processing). Counteracts RIG-I MAVS signalling and IFN signalling	[20, 21, 88, 89, 261]
Nsp6	Golgi, ER	DMV formation. Replication organelle with Nsp3, Nsp4. ER membrane zippering	[260, 262]
Nsp7	Cytoplasmic	Accessory protein for RNA polymerase. Inhibits IFN production by targeting RIG-I	[96, 263–265]
Nsp8	Cytoplasmic	Primase. Accessory protein for RNA polymerase. Interacts with MDA-5	[96, 97, 263, 264, 266]
Nsp9	Cytoplasmic, nucleus	RNA binding protein. Associates with Nsp12. Recruits protein for 5′ capping. Binds nuclear pore complex proteins	[267–269]
Nsp10	Cytoplasmic, present in vesicular structures	Co-factor for Nsp14 and Nsp16 methyl transferases. Component of exonuclease complex	[98, 99, 105, 270, 271]
Nsp11	Cytoplasmic	Short peptide of unknown function	[272]
Nsp12	Cytoplasmic, Golgi	RNA-dependent RNA polymerase – associates with Nsp7 and Nsp8. Also complex with Nsp14 and Nsp16	[93, 94, 263, 264, 267, 273]
Nsp13	Cytoplasmic	RNA helicase, 5′ triphosphatase. IFN antagonist	[101, 102, 264, 274, 275]
Nsp14	Nucleus	3′–5′ exoribonuclease, N7-methyl transferase. Interacts with RdRP complex. IFN antagonist	[98, 99, 105, 274, 276, 277]
Nsp15	Diffuse; cytoplasmic, Golgi	Endoribonuclease. IFN antagonist	[103, 104, 274, 278]
Nsp16	Endosomes, cytoplasmic	2′-O-methyltransferase. Protects from MDA5 immune response	[105, 106, 279, 280]
Envelope protein (E)	ER, Golgi	Virion structure. Activates TLR2 pathway	[120, 121, 281]
Membrane protein (M)	ER, Golgi, endosomal, cytoplasmic	Virion structure. IFN antagonist. Binds PCNA and N protein	[107–109]
Nucleocapsid (N)	Diffuse cytoplasmic	Houses viral genome, virus particle release. Counteracts RIG-I signalling	[107, 113–115, 282, 283]
Spike protein (S)	ER, plasma membrane, cytoplasmic	Viral entry, binds host cell receptor	[77–82, 117, 118, 284, 285]
ORF3a	ER, Golgi, endosomal	Viroporin, also promotes virus release. Induces apoptosis. Suppresses INF signalling	[125, 126, 286–292]
ORF3b	ER, Golgi, endosomal	IFN antagonist	[293]
ORF6	ER, nuclear membrane, Golgi, membrane vesicles, cytoplasm	IFN antagonist	[109, 126, 294–296]
ORF7a	ER, Golgi	IFN antagonist, inhibits IRSE promoter activity	[126, 287, 297–299]
ORF7b	ER, Golgi, cytoplasmic	IFN antagonist, inhibits IRSE promoter activity	[126, 297]
ORF8	ER, Golgi, also secreted	IFN antagonist. Interacts with MHC-I and mediates its degradation	[126, 288, 300–303]

Continued

Table 1. Continued

SARS-CoV-2 protein	Tentative subcellular location	Functions	References
ORF9b	Diffuse, mitochondrial membrane	Compromising the mitochondria, binds TOM70. Affects IFN synthesis. Antagonizes IFN signalling. Interacts with host cell organelles	[126, 304–309]
ORF9c	Nuclear membrane, cytoplasmic	Affects lipid remodelling and ER stress response. Interacts with host cell organelles	[126, 310]
ORF10	ER, Golgi, cytoplasmic	Inhibits IFN signalling by inhibiting MAVS expression	[126, 311]

DMV, Double membrane vesicle.; RdRP, RNA dependent RNA polymerase.

Most viruses take control of the cell cycle of the infected cell to produce an environment conducive to viral replication (reviewed, for example, in [130]). For example, adenoviruses cause progression into a 'pseudo-S phase' by the action of E1A proteins that bind RB family members, de-repressing E2F transcription factors, and activating genes required for cell cycling (reviewed in [131, 132]). Similarly, lytic replication of Epstein–Barr virus (EBV) and Kaposi's sarcoma-associated virus (KSHV) leads to S phase accumulation and activation of S phase-specific DNA repair pathways [133, 134]. RNA viruses are also well known to regulate the cell cycle following infection; for example, Rift Valley fever virus (RVFV), a negative-strand RNA virus of the family *Bunyaviridae*, encodes a non-structural (NS) protein that causes S phase arrest by activating the ATM-Chk2 pathway [135]. Several SARS-CoV-2 proteins are involved in the viral regulation of the cell cycle. As will be discussed in detail below, ORF6 and Nsp13 separately cause downregulation of Chk1, leading to cell cycle arrest in S phase [136]. Similarly, SARS-CoV-2 Nsp12 binds to the p50 component of DNA polymerase delta (pol δ) [137]. This interaction, together with a likely association between Nsp13 and the p125 subunit of DNA pol δ, plays a synergistic role in the induction of S phase arrest following co-expression of SARS-CoV-2 Nsp12 and Nsp13 [137]. It has previously been shown that infection of cells with the coronavirus infectious bronchitis virus (IBV) activates the S phase checkpoint and induces cell cycle arrest in S and G2/M phases, partially through the interaction of Nsp13 with the p125 catalytic subunit of pol δ [138]. Contrasting evidence, however, has indicated that the SARS-CoV-2 N protein can cause arrest of kidney cells in G1 by interaction with Smad3, enhancing TGF-β/Smad3 signalling [139]. In further studies it was demonstrated that quercetin can block the interaction, which leads to an inhibition of Smad3 signalling and p21- and p16-dependent G1 cell cycle arrest and cell death *in vivo* and *in vitro* [139, 140]. In studies of SARS-CoV-2, expression of N protein has been shown to cause a cell cycle block in G1/S [141], whereas the SARS-CoV protein blocks S phase progression [142]. In the latter report it was observed that decreased S phase gene expression and decreased phosphorylation of a CDK2 substrate occurred in SARS-CoV-infected cells, which is perhaps more physiologically relevant [142]. However, several other investigations have shown that SARS-CoV 3a, 3b and 7a proteins, as well as murine coronavirus infection, cause G0/G1 arrest, and this is required for viral replication (reviewed in [143]).

In a large screen of protein phosphorylation following SARS-CoV-2 infection, kinases whose activity was predicted to be downregulated included several cell cycle kinases (CDK1/2/5 and AURKA) and cell growth-related signalling pathway kinases (PRKACA, AKT1/2, MAPK1/3 and PIM1) [144]. By comparison of public phosphoproteomics datasets, it was concluded that SARS-CoV-2 infection induced a cell cycle block at the S/G2 transition, with relatively few cells in G0, G1 or M [144]. Notably, the observed increase in activity of MAPKp38 is consistent with a cell cycle block [145]. Also, CDK2 T^{14}/Y^{15} phosphorylation increased after SARS-CoV-2 infection; this is normally linked to inhibition of progression from G2 phase to M [144, 146, 147]. The presence of the infected cells in S/G2 suggests that any DSBs that occurred would be repaired by homologous recombination, and it is notable that this pathway is activated during SARS-CoV-2 infection [136].

In a wide-ranging survey of transcriptome data obtained following SARS-CoV-2, infection, it was noted that some cell cycle genes were upregulated. These included aurora kinase B (*AURKB*), p21 (*CDKN1A*) and cyclin B1 (*CCNB1*). A limited number of genes were downregulated, such as p57 (CDKN1C) [148]. Overall, it is difficult to reconcile all these results, but it seems reasonable to conclude that SARS-CoV-2 induces infected cells into S phase, facilitating replication.

Because of its multiple roles in cell cycle regulation, DDR, and the induction of apoptosis and autophagy, p53 is a common target for infecting DNA viruses such as adenovirus, human papilloma virus (HPV) and EBV (reviewed in [42, 46]). It can also be a target for RNA viruses; for example, p53 is mislocalized to the cytoplasm by the NS2 protein during hepatitis C virus (HCV) infection [149]. However, only limited information is available on the relationship between SARS-CoV-2 and p53 (reviewed in [150]). It has been shown that the viral protease Nsp5 can act as a repressor of the p53 signalling pathway and that p53 can restrict SARS-CoV-2 production [151]. However, these experiments were carried out in HEK293T cells, where p53 transcriptional activity is compromised. The N protein from another coronavirus [porcine epidemic diarrhoea virus (PEDV)] can induce cell cycle arrest in S phase by direct interaction with p53 in the nucleus [152]. This association has the effect of maintaining high levels of p53, activating the p53-DREAM pathway. Whether such an interaction occurs in SARS-CoV-2-infected cells has not yet been shown. A further relevant interaction has been reported for SARS-CoV Nsp3, which associates with, and stabilizes, RCHY1 (ring finger and CHY zinc finger containing 1, also known as Pirh2) [153]. This has the effect of increasing RCHY1-mediated p53 degradation, presumably affecting cell cycle progression and the DDR [153]. RCHY1 also ubiquitylates the translesion DNA polymerase eta, inhibiting its DNA damage bypass activity, as well as several other significant substrates, such as Chk2, p73 and HDAC2 [154–157]. It has not been unequivocally demonstrated that SARS-CoV-2 Nsp3 binds RCHY1, but the interaction has been shown in a mass spectrometric screen [158] (see below).

SARS-COV-2 AND DDR

In general, the relationship between viruses and DNA damage and repair can be divided into two aspects. Firstly, damage inflicted on the host cell genome, either directly through viral proteins or indirectly, for example, through the generation of reactive oxygen species (ROS) or the generation of DNA replication stress. Secondly, by affecting host cell DNA damage repair pathways; this can also lead to increased DNA damage as repair to 'normally occurring' DNA lesions is compromised. It is clear that SARS-CoV-2 has the potential to induce DNA damage, as evidenced by the presence of numerous micronuclei in syncytia formed in cells expressing S protein and ACE2, as well as activation of the cGAS–STING pathway [159]. Similarly, it has been observed that the DNA of COVID-19 patients shows increased damage compared to healthy controls, as determined by comet assay [136, 160]. Furthermore, in these studies there was a positive correlation between severity of disease, extent of damage and oxidative stress.

Host cell DNA damage resulting from SARS-CoV-2 infection

Oxidative stress arises as a result of differences between the rate of ROS production and accumulation and the concentration of cellular antioxidants. All coronaviruses affect the cellular redox machinery in favour of viral replication. This also has the effect of increasing inflammation, apoptosis and cellular damage in the host (reviewed in [161, 162]). This is particularly marked during SARS-CoV, MERS-CoV and SARS-CoV-2 infection. Redox mechanisms play a role in control of coronavirus entry into mammalian cells. The ACE2 receptor and its ligand have been shown to be important for the induction of redox stress. Ang II, the ACE2 ligand, activates NADPH oxidase, increasing ROS production [162]; this is offset by ACE2, which converts Ang II to angiotensin 1–7, causing a reduction in ROS [161, 162]. Infection of cells with coronaviruses leads to reduced levels of ACE2 receptors and an increase in Ang II binding to ACE1, which causes an increase in ROS due to activation of NADPH oxidase [161, 162]. It has also been shown that S protein expression causes oxidative stress in endothelial cells, caused by activation of NOX2 [163]. Increased ROS and oxidative stress also increases the affinity of the spike protein for the human ACE2 receptor [164, 165].

Coronaviruses can induce production of mitochondrial ROS. The SARS-CoV Nsp3a protein activates the NLRP3 inflammasome, causing redistribution to the perinuclear space [166]. This activation is caused by increased mitochondrial ROS. Mitochondrial ROS regulates several pathways, which facilitate coronavirus replication in the cytoplasm. For example, it induces mitochondrial permeability transition pores, regulates endoplasmic reticulum stress and the unfolded protein response, and regulates mitophagy, which, in turn, contributes to coronavirus replication (reviewed in [161]). Most of these effects have been observed for SARS-CoV, but it seems reasonable to assume that similar effects would be observed with SARS-CoV-2.

SARS-CoV-2 can downregulate host cell antioxidant pathways, aiding replication. Specifically, it has been shown that NRF2-induced genes, such as heme oxygenase 1 (HO-1) and NAD(P)H quinone oxydoreducatse 1 (NqO1), and superoxidase dismutase (SOD), are suppressed in SARS-CoV-2-infected cells and that the NRF2 pathway inhibits SARS-CoV-2 replication, in a way quite distinct from the host inflammatory response [161, 167]. The viral Nsp14 protein interacts with the catalytic domain of the NAD-dependent deacetylase sirtuin 1 (SIRT1) and inhibits its ability to activate the NRF2/HMOX1 pathway [168]. SARS-CoV-2 also upregulates oxidative stress genes such as myeloperoxidase, calprotectin, sestrin, thioredoxin and sulfiredoxin-1 [169].

Oxidative stress induces multiple forms of DNA damage, such as single- and double-strand breaks, base and sugar oxidation products and DNA–protein crosslinks [170]. Oxidized guanine species (8-oxo-G) are usually formed by the interaction of ROS with the guanine base in nucleic acids and are taken as a measure of ROS activity. In a study to assess ROS in COVID-19 patients directly, 8-oxo-G levels were measured in cohorts of COVID-19 patients and non-infected healthy controls [171]. Increased levels of oxidized guanine were generally observed in COVID-19 patients with much higher levels in those with more severe disease. In a second study, perhaps surprisingly, similar levels of 8-oxo-G were observed in peripheral blood mononuclear cells (PBMCs) from healthy and SARS-CoV-2-infected individuals [172]. However, elevated levels of some, but not all, base excision repair proteins (for example, POLD1, MPG, ligase 1 and FEN1) and double-strand break repair proteins (for example, Chk1, RAD50 and RAD51) were reported in COVID-19 patients [172]. In addition, it was noted that more highly regulated DDR pathway protein expression was correlated with severity of disease.

Another possible source of DNA damage in SARS-CoV-2-infected cells could be the interaction of Nsp13 with DNA polymerase δ. It has been shown that the IBV protein Nsp13, which only differs from the SARS-CoV-2 orthologue in a single amino acid, binds to DNA polymerase δ p125, causing DNA replication fork stress, ATR activation, cell cycle arrest and DNA damage [138].

Inhibition of the ATR kinase by chemical inhibitors or siRNA-mediated knockdown reduced the IBV-induced ATR signalling and inhibited IBV replication [138]. It is likely that SARS-CoV-2 Nsp13 would have similar effects. Indeed, in further studies it has been shown that Nsp12 from IBV, SARS-CoV and SARS-CoV-2 interacts with the p50 regulatory subunit of pol δ [137]. This is considered to enhance the effect of Nsp13 in the induction of cell cycle arrest, as mentioned above [137].

It is also worth noting that the relatively high level of ROS could affect the viral genome as well (discussed in [173]). The effects of single amino acid substitutions in the SARS-CoV-2 S protein have been of great clinical significance during the COVID-19 pandemic. It is possible that some of these mutations could have arisen because of host cell oxidative species.

SARS-CoV-2 and the DDR pathways

As mentioned above, probably all DNA viruses impinge on the cellular DDR [42, 44–46]. It is now becoming apparent that the same is the case for many RNA viruses [43]. SARS-CoV-2 falls into that category in that viral proteins have been shown to associate with components of the DDR pathways (reviewed in [75, 76, 174]) and the virus can affect various DDR pathways such as those based on ATR and ATM signalling [136, 175].

Association of SARS-CoV-2 proteins with DDR components

There have been several large-scale studies to identify the SARS-CoV-2 interactome, based on co-immunoprecipitation, yeast 2 hybrid and biotin–streptavidin based-protocols (see, for example, [158, 176–184]); in some cases, published data have been consolidated into a resource summarizing reported interactions (for example, [185]). There are appreciable variations in the binding proteins reported in the different studies but similar major groups of proteins from specific cellular pathways have been identified as significant targets for the virus. For example, host factors involved in mRNA synthesis, nuclear export, translation and stability associating with the viral N protein have been reported [158, 176, 177, 180, 181]; similarly, proteins involved in membrane trafficking have been shown to bind to Nsp7 and M as well as other viral proteins [158, 176, 177, 179]. In most reports DNA repair and damage pathways have not been considered to be a major target for interaction with SARS-CoV-2 proteins. However, many well-characterized DDR components have been identified in association with SARS-CoV-2 proteins, although it is notable that there is very limited overlap between the different studies (summarized in Table 2).

In studies using the BioID interactome approach (which may have the disadvantage that it does not necessarily identify interacting proteins, but only those that are proximal), very large numbers of targets for each SARS-CoV-2 bait have been recorded and these include multiple DDR proteins, many associating with several SARS-CoV-2 proteins [158, 179]. For example, in the study by Samavarchi-Tehrani and colleagues, FANCI was considered to be present proximal to Nsps 1, 2, 5, 7,8, 9, 10, 13, 15 and 16, ORFs 3b, 6, 7b, 9b and 14 and the M and N proteins [179]. (Proteins identified in that study are not listed in Table 2 in order to keep it within manageable proportions.) This may be contrasted with other studies that identified FANCI interacting with Nsp3 and 7, M protein and ORF7a [158, 181, 183]. This was also the case for BRCA1, BRCA2, ERCC5, ERCC6L, XRCC5 and XRCC6, where a large number of associated SARS-CoV-2 proteins were identified for each [179]. However, this may be contrasted with other DDR proteins that appeared to be proximal to far fewer associates. For FANCA, only Nsp7 and Nsp10 were identified; for FANCM Nsp7 and 9 and ORFs 6 and 14 were proximal, and ligase III was reported to be proximal to Nsps 1, 5, 6, 10 and 15 and ORFs7b and 14 [179]. Just considering the studies summarized in Table 2, it is notable that very few of the interactors were identified in more than one study. For example, DNMT1 co-immunoprecipitates with SARS-CoV-2 ORF8 and yet was not reported as proximal to any SARS-CoV-2 protein in the studies by Laurent and colleagues and Samavarchi-Tehrani and colleagues [158, 176, 179]. Therefore, without further detailed mechanistic investigation it is difficult to know how much significance to give to these observations. For example, Laurent and colleagues and Samavarchi-Tehrani and colleagues each report interaction between Nsp7 and at least 10 DDR proteins, yet none of the other studies listed report any (Table 2). Furthermore, the BioID approach generates very large numbers of proximal targets, identified for each SARS-CoV-2 bait, sometimes exceeding a thousand [158, 179]. In that case it is not surprising that DDR proteins are included in potential binding partners.

In previous investigations it has been shown that the coronavirus infectious bronchitis virus induces cell cycle arrest by induction of an ATR-dependent DDR [138]. Additionally, the IBV Nsp13 interacts with DNA polymerase δ (POLD1), leading to DNA replication stress in IBV-infected cells [138]. Similarly, in a subsequent study it was shown that SARS-CoV-2 POLD1 probably associates with Nsp13 [137]. In the SARS-CoV-2 interactome screens summarized in Table 2, interaction of POLD1 has been reported for Nsp7 and Nsp10 but not for Nsp13 [158, 181]. However, in the BioID screen by Samavarchi-Tehrani and colleagues, POLD1 was found to be proximal to Nsps 5, 7, 8, 9, 10, 13, 15, and 16, as well as ORFs 3b, 6, 9b and 14 [179]. DNA polymerase δ is responsible for the 5′–3′ DNA polymerase and 3′–5′ exonuclease activity of polymerase δ. POLD1 has a role in several DNA repair pathways, such as mismatch repair (MMR), translesion synthesis (TLS), base excision repair (BER), nucleotide excision repair (NER) and double-strand break (DSB) repair [186].

Effects of SARS-CoV-2 on the cellular DDR

Obviously, further investigation is required before we can say that the reported interactions with cellular DDR components are biologically significant, either for the virus or the infected host cell. However, it is now clear that infection of mammalian cells by

Table 2. DNA damage response pathway components identified as interacting with, or being in close proximity to, SARS-CoV-2 proteins by mass spectrometric analysis. A very brief description of the role of the DDR protein is given, together with appropriate references

SARS-CoV-2 protein	Associated cellular DDR protein	DDR protein function	Reference
Nsp1	RUVBL1	AAA+ATPase. DSB repair	[178]
	RUVBL2	AAA+ATPase. DSB repair	[178]
	PRIM1	DNA primase. DNA replication	[176, 177, 183]
	PRIM2	DNA primase. DNA replication	[176, 177, 183]
	POLA1	DNA polymerase alpha1. DNA replication	[158, 176–178]
	POLA2	DNA polymerase alpha2. DNA replication	[176, 177, 180]
	Timeless	Involved in replication stress and genome stability	[180]
	Ku70 (XRCC6)	DNA PK component. NHEJ double-strand break repair	[183]
	Ku80 (XRCC5)	DNA PK component. NHEJ double-strand break repair	[183]
Nsp2	Bub1B	Protein kinase. Role in mitotic checkpoint	[158]
Nsp3	Bub1B	Protein kinase. Role in mitotic checkpoint	[158]
	RCHY1	E3 ubiquitin ligase. Regulates p53 levels among others	[158]
	RINT1	RAD50 interactor 1. Regulates cell cycle and telomere length	[158]
Nsp3+Nsp8	FANCI	Fanconi anaemia component I. Role in DSB repair and repair of inter-strand crosslinks	[181]
Nsp4	POLDIP2	DNA polymerase delta interacting protein 2. Role in DNA replication	[158]
	RAD23A	A role in nucleotide excision repair of bulky lesions and oxidative DNA damage	[181]
	RINT1	RAD50 interactor 1. Regulates cell cycle and telomere length	[158]
	XPC	Nucleotide excision repair of bulky lesions and oxidative DNA damage	[181]
Nsp5	ERCC3	Nucleotide excision repair of bulky lesions and oxidative DNA damage	[176]
	FANCD2	Fanconi anaemia component D2. Role in DSB repair and repair of inter-strand crosslinks	[178]
	p53	Transcription factor, involved in cell cycle checkpoints following DNA damage	[178]
	RAD9A	Component of the 9-1-1 cell cycle checkpoint response complex	[178]
Nsp7	BRCA2	Roles in homologous recombination, G2 checkpoint control, protection of stalled replication forks	[158]
	FANCA	Fanconi anaemia component A. Role in DSB repair and repair of inter-strand crosslinks	[158]
	FANCI	Fanconi anaemia component I. Role in DSB repair and repair of inter-strand crosslinks	[158]
	Bub1	Protein kinase. Role in mitotic checkpoint	[158]
	Bub1B	Protein kinase. Role in mitotic checkpoint	[158]
	POLD1	Catalytic subunit of DNA polymerase delta. Involved in DNA replication	[178]
	POLE	Catalytic subunit of DNA polymerase epsilon. Involved in DNA replication	[158]
	Rad54B	Involved in homologous recombination	[158]
	cdc25c	Phosphatase involved in cell cycle regulation	[158]
	claspin	Adaptor protein facilitating activation of Chk1, involved in replication fork stability	[158]
	Tipin	Timeless interacting protein, involved in replication stress	[158]

Continued

Table 2. Continued

SARS-CoV-2 protein	Associated cellular DDR protein	DDR protein function	Reference
	BAP1	BRCA1-associated protein 1. Role in DNA damage repair and cell cycle regulation	[158]
Nsp9	RCHY1	E3 ubiquitin ligase. Regulates p53 levels among others	[176]
	POLDIP3	DNA polymerase delta interacting protein 3. Role in DNA replication	[158]
Nsp10	POLD1	Catalytic subunit of DNA polymerase delta. Involved in DNA replication	[181]
Nsp12	FANCA	Fanconi anaemia component A. Role in DSB repair and repair of inter-strand crosslinks	[158]
Nsp14	MRE11	Exo-and endonuclease, component of the MRN complex, involved in DSB repair	[176]
	RAD50	Component of the MRN complex, involved in DSB repair	[176]
	RINT1	RAD50 interactor 1. Regulates cell cycle and telomere length	[158]
	p53	Transcription factor, involved in cell cycle checkpoints following DNA damage	[176]
	UBR5	Ubiquitin E3ligase. Regulates DNA topoisomerase II-binding protein (TopBP1) and Rnf168 in the DNA damage response	[176]
Nsp15	BLM	RecQ helicase with a role in homologous recombination	[181]
Nsp16	ERCC6L	Excision repair 6-like protein, a DNA translocase involved in the mitotic spindle	[158]
	FANCA	Fanconi anaemia component A. Role in DSB repair and repair of inter-strand crosslinks	[158]
	RINT1	RAD50 interactor 1. Regulates cell cycle and telomere length	[184]
ORF3a	SUN2	Chromosome reassembly factor	[180, 183]
	RAD23B	A role in nucleotide excision repair of bulky lesions and oxidative DNA damage	[158]
ORF3b	RAD23B	A role in nucleotide excision repair of bulky lesions and oxidative DNA damage	[158]
ORF7a	FANCI	Fanconi anaemia component I. Role in DSB repair and repair of inter-strand crosslinks	[158, 183]
	ATR	Kinase central to the DNA damage response, involved in sensing replication stress	[181]
	RAD23B	A role in nucleotide excision repair of bulky lesions and oxidative DNA damage	[158]
ORF7b	RINT1	RAD50 interactor 1. Regulates cell cycle and telomere length	[158]
ORF8	TP53RK	p53 regulating kinase	[158]
	DNMT1	DNA methyltransferase 1. Multiple functions	[177]
	POLDIP3	DNA polymerase delta interacting protein 3. Role in DNA replication	[158]
ORF9b	BRIP1	BRCA1 interacting protein1. A RecQ helicase, also known as FANCJ. Involved in homologous recombination and DNA replication stress	[178]
	hnRNPUL2	RNA-binding protein involved in homologous recombination with the MRN complex	[180]
	RAD21	Cohesin complex component. Involved in post-replicative DNA repair	[180]
	H2AX	Histone, which is central to genome stability through its role in the signalling DNA damage. Involved in the assembly of repair foci	[180]
ORF14	FANCI	Fanconi anaemia component I. Role in DSB repair and repair of inter-strand crosslinks	[158]
	RAD23B	A role in nucleotide excision repair of bulky lesions and oxidative DNA damage	[158]
	TP53RK	p53 regulating kinase	[158]
	POLDIP3	DNA polymerase delta interacting protein 3. Role in DNA replication	[158]

Continued

Table 2. Continued

SARS-CoV-2 protein	Associated cellular DDR protein	DDR protein function	Reference
Envelope (E) protein	RINT1	RAD50 interactor 1. Regulates cell cycle and telomere length	[158]
	POLDIP3	DNA polymerase delta interacting protein 3. Role in DNA replication	[158]
	ZC3H18	Binds the cap-binding complex on capped RNAs	[176, 180]
Membrane (M) protein	FANCI	Fanconi anaemia component I. Role in DSB repair and repair of inter-strand crosslinks	[176, 183]
	Ligase 3	Binds to XRCC1 and is involved in base excision repair	[180]
	Timeless	Involved in replication stress and genome stability	[165]
	RINT1	RAD50 interactor 1. Regulates cell cycle and telomere length	[158]
Nucleocapsid (N) protein	hnRNPUL2	RNA-binding protein involved in homologous recombination with the MRN complex	[181]
	ERCC6L	Excision repair 6-like protein, a DNA translocase involved in the mitotic spindle	[158]
	RAD23B	A role in nucleotide excision repair of bulky lesions and oxidative DNA damage	[184]
Spike (S) protein	RINT1	RAD50 interactor 1. Regulates cell cycle and telomere length	[158]

SARS-CoV-2 results in damage to the host cell DNA. Several studies have demonstrated this directly by comet assays and/or the presence of DNA repair foci. For example, DNA fragmentation, as measured by increased comet tail moment and the presence of micronuclei, was seen in SARS-CoV-2-infected Huh7 cells [136] and in patient cells [160]. Similarly, γH2AX foci were observed in the same cells and in SARS-CoV-2-infected lung cells from cynomolgus macaques and epithelial and endothelial lung cells from COVID-19 patients, indicative of DNA damage [136, 187, 188]. Senescence, associated with DNA damage, has also been reported following SARS-CoV-2 infection (discussed below).

As noted above, in a large proteomic screen the effects of SARS-CoV-2 on the phosphorylation of viral and host cell proteins was examined [144]. Seven viral proteins were substrates for cellular kinases at a total of 49 sites. Based on the amino acid sequences that were phosphorylated, casein kinase II (CK2), cyclin-dependent kinase (CDK), cdc2-like kinase (CLK) and protein kinase C (PKC) were highly activated. A single site probably phosphorylated by ATM or ATR was identified [144]. However, it was suggested that most of the phosphorylation sites did not play a functional role in viral replication, although the single site in Nsp9, sites in the S protein and sites towards the N-terminal region of protein N were close to proposed protein–protein interfaces [144].

On examination of cellular pathways, phosphorylation of proteins involved in double-strand break repair and single-strand DNA binding, among many others, were notably increased upon SARS-CoV-2 infection [144]. Additionally, heterochromatin and various chromosomal regions were increasingly phosphorylated. Considering more specific complexes involved in the DDR, BRCA1–HDAC1–HDAC2, BRAF53–BRCA2 and TIP5–DNMT–HDAC1 complexes were highly phosphorylated after infection. Other DDR complexes phosphorylated to a lesser extent were those comprising MGC1–DNA–PKcs–Ku, MDC1–H2AFX–TP53BP1, MDC1–p53BP1–SMC1, MDC1–MRE11–RAD50–NBS1, DNA–PK–Ku and DNA–PK–Artemis. Moreover, a complex comprising MDC1–MRN–ATM–FANCD2 was highly phosphorylated in the initial stage of SARS-CoV-2 infection but to a lesser extent later. These events are indicative of activation of double-strand break repair pathways. Overall, the activities of ~97 kinases were affected over a 24 h infection time course; the most highly activated were components of the p38 pathway, including p38ɣ (MAPK12), CK2 (CSNK2A1/2) and Ca2+/calmodulin-dependent protein kinase (CAMK2G). The activities of a large number of others was reduced, including CDK1, 2 and 5 and AKT 1 and 2 [144].

Of the 332 host proteins identified by Gordon and colleagues as interactors with viral components, 40 were significantly differentially phosphorylated following SARS-CoV-2 infection [144, 176]. Most of these had no direct involvement in the DDR; however, the phosphorylation of DNA polymerase α 2, which associates with SARS-CoV-2 Nsp1, is markedly reduced (on T^{127} and T^{130}) during infection, whereas the phosphorylation of DNMT1, which binds to ORF8, is increased (on S^{714}) [144]. ZC3H18 has been identified as an E protein-associated component of the DDR. Its phosphorylation on S^{868} decreases during infection, whereas phosphorylation on T^{611} initially decreases and then increases. ZC3H18 is a DNA-binding protein that promotes BRCA1 transcription, although the effects of its phosphorylation are not clear [189].

In a small pilot study, looking specifically at DDR pathways, it was observed that infection of Vero E6 cells with SARS-CoV-2 causes activation of the ATR pathway, seen as increased phosphorylation of Chk1 on S^{280} and H2AX on S^{139} [175]. In a more detailed investigation, it was observed that an ATR inhibitor, berzosertib, showed potent antiviral activity against SARS-CoV-2 in multiple cell types and blocked replication at the post-entry step [190]. Inhibition of viral replication was accompanied by a marked reduction of Chk1 phosphorylation on S^{345} [190].

Other studies have also indicated activation of DDR signalling following SARS-CoV-2 infection [136]. Over a 48 h time course, ATM and DNA-PKcs were phosphorylated on S^{1981} and S^{2056}, respectively. Activation of ATM led to increased phosphorylation of KAP1 (S^{824}) and H2AX (S^{139}) but not Chk2 (T^{68}). Furthermore, increased numbers of KAP1 S^{824}, RPA $S^{4/8}$ and γH2AX foci were seen in SARS-CoV-2-infected cells [136]. There was no significant phosphorylation of Chk1 (S^{317}), but there was a marked decrease in the level of the protein detected at a later time after infection. There was also a reduction in expression of RRM2, which is normally controlled by Chk1, resulting in decreased levels of dNTPs, probably contributing to an observed reduction in S phase progression [136]. In an attempt to determine which SARS-CoV-2 proteins were responsible for Chk1 depletion, individual genes were expressed. ORF6 associates with nuclear pores, preventing the import of Chk1, leading to its accumulation in the cytoplasm and subsequent degradation by the proteasome [136]. Nsp13 also contributes to degradation of Chk1 by causing its co-localization in autophagosomes and degradation through autophagy [136]. Nsp13 had been previously reported to target TBK1 for degradation by autophagy through direct interaction and association with p62 in SARS-CoV-2-infected cells [191, 192].

Significantly, SARS-CoV-2 N protein inhibits NHEJ [136]. This appears to be through binding to dilncRNA, which inhibits the interaction with 53BP1 and its recruitment to repair foci. It has previously been reported that dilncRNA, generated at DSBs, is responsible for liquid–liquid phase separation (LLPS) of 53BP1 [193, 194]. It has been observed that 53BP1 does not readily locate to DNA repair foci in SARS-CoV-2-infected cells or following transfection of N [136]. Earlier reports had indicated that N protein condenses with RNA by LLPS and that it associates with stress granules that form through LLPS [195, 196].

Interestingly, SARS-CoV-2 has been linked to various members of the PARP [poly (ADP)-ribose polymerase] family and PARP inhibitors, such as stenoparib or olaparib, have been suggested as anti-COVID-19 agents [197–199]. However, it appears that the PARP enzymes induced by SARS-CoV-2 are MARylating PARPs (PARP7, PARP 10, PARP 12 and PARP14), which add a single ADP-ribose rather than PARPs involved in DNA repair pathways, which are generally responsible for the addition of branched or linear chains of ADP-ribose moieties [198, 200, 201]. Increased expression of the MARylating PARPs following SARS-Cov-2 infection primarily impacts on NAD metabolism, with, for example, increased levels of NAMPT (nicotinamide phosphoribosyltransferase) [198]. Thus, NAD^+ levels were found to be reduced following increased expression of MARylating PARPs, as has been shown to be the case with PARP1 [198, 202]. The implications of these observations for any genome instability caused by SARS-CoV-2 infection are not clear at present. However, it has been demonstrated that PARP inhibitors can have anti-SARS-CoV-2 effects, although this is largely through direct inhibition of viral proteins, such as the main protease Mpro (Nsp5) [197, 203].

SARS-CoV-2 and telomeres

In early studies, it was concluded that the severity of COVID-19 increased with age, with older patients having higher mortality (see, for example, [204–206]). Therefore, molecular pathways underlying aging, such as telomere shortening, were thought to be important in determining the severity of the disease. Indeed, it has been reported that patients with a larger proportion of short telomeres developed more severe COVID-19 symptoms [207, 208]. However, this is not a view that is universally held and other reports have indicated that decreased telomere length is not necessarily linked to increased severity of COVID-19 (for example, [209, 210]).

The production of lymphocytes is closely linked to telomere length. Shorter telomeres due to aging can induce slow proliferation of lymphocytes. To achieve a requisite immune response to SARS-CoV-2 infection, generation and maintenance of T-cells is essential. Marked reduction of the T-cell count, lymphopenia, has been found to be a characteristic of severe COVID-19. In older patients infected with SARS-CoV-2, PBMC telomeres have been observed to be relatively short and this was linked to low lymphocyte counts. T-cell proliferative capacity is telomere length-dependent, and telomeres shorten with age. These observations may account for the vulnerability of older patients to COVID-19 [211]. In other studies, it has been shown that short blood leukocyte telomere lengths, as a marker of age, can contribute to the development of post-COVID-19 symptoms in the lungs of some patients [212].

Interestingly, expression of the ACE2 receptor increases with telomere shortening in mice and in cultured human cells [213]. Regulation is at the transcriptional level with the ACE2 promoter being DDR-dependent. Thus, ATM inhibition or inhibition of the telomeric DDR leads to increased ACE2 level. It has been concluded that during aging there is increased telomere shortening, increased DDR activity and increased ACE2 expression, resulting in more severe levels of SARS-CoV-2 infection [213]. Importantly, ATM and ATR activities are required for localization of telomerase to telomeres and telomere elongation [214]. It might be supposed in the case of prolonged infection, SARS-CoV-2 could cause alteration in telomere length by affecting PIKKs.

Senescence induced by SARS-CoV-2

Simplistically, two major forms of senescence can be delineated. Replicative senescence is associated with the shortening of telomeres with increasing age, whereas stress-induced senescence is a response to a wide variety of factors, including DNA damage and infection by viruses such as SARS-Cov-2, irrespective of telomere length [215–217]. It has now been shown that SARS-CoV-2 can induce virus-induced-senescence (VIS) by a number of different mechanisms (reviewed in [217, 218]). Many virus types, including single- and double-stranded DNA and RNA viruses can cause VIS, which is generally very similar to oncogene-induced senescence (reviewed in [217]). Infection with SARS-CoV-2 has been shown to be responsible for senescence-associated secretory phenotype (SASP), which involves secretion of pro-inflammatory cytokines such as IL-1β, IL-6 and Il-8 and the presence of γH2AX foci, which is probably due to the presence of ROS [219]. Reduction in ROS in *in vitro* models reduced DNA damage and senescence-associated β-galactosidase activity. Shedding of mitochondrial outer membranes in response to stress caused by infection could also contribute to DNA damage caused by the virus [220–222]. VIS is also marked by increased expression of the cell cycle inhibitors p16 and p21 and the inhibition of expression of cell cycle promoters, such as E2F targets and SASP [217]. (SASP is characterized by secretion of extracellular modifying factors, such as pro-inflammatory factors, ECM-degrading proteins and pro-coagulatory factors, amongst others [217]).

LONG COVID AND DNA DAMAGE

Long COVID [also referred to as 'post-COVID condition', 'post-COVID syndrome' and 'post-acute sequelae of COVID-19' (PASC)] is a prolonged, often severe condition with diverse symptoms occurring after SARS-CoV-2 infection (reviewed in [7, 223–227]). Symptoms include fatigue, anosmia and dysgeusia, persistent respiratory problems, such as shortness of breath, periodic cognitive difficulties, cardiovascular difficulties, such as chest pain and arrhythmia, gastrointestinal symptoms, rheumatic and dermatological problems, and neurological and psychiatric symptoms (reviewed, for example, in [224]). Because of the multiple, diverse symptoms associated with long COVID, explanations of its causes have been many and varied. For example, it has been suggested that it is due to residual virus, immune dysregulation, possibly due to reactivation of a latent herpesvirus, more general effects of SARS-CoV-2 on the host microbiota, autoimmunity and/or residual dysfunctional signalling (reviewed in [7]). Of particular interest from the point of view of long-term damage to the DNA and the induction of genome instability is the possibility that there is residual or perhaps latent virus remaining in the infected patient, ostensibly after recovery. It is now clear that components of SARS-CoV-2 are relatively common after the disappearance of symptoms. Two forms of persistence can be recognized [223, 227]. Firstly, the presence of persistent infectious SARS-CoV-2, capable of replicating. It has been suggested that persistent viral reservoirs could be present in patients, even though they test negative by PCR [228]. SARS-CoV-2 RNA was shown to be present at multiple sites within the bodies of a cohort of patients who had died from COVID-19 [229]. Virus could be isolated from approximately half of the tissues tested; in one case over 7 months since diagnosis [229]. Other studies have shown SARS-CoV-2 in sites such as the intestine, respiratory tract and lymph nodes [230–232]. The second form of persistence would be the retention of viral RNA and/or protein at specific sites. For example, SARS-CoV-2 N protein was detected in intestinal epithelium 4 months after COVID-19 diagnosis in a third of patients in one study. Viral RNA was detected in half of those who were positive for N protein, and all tested negative by PCR for the virus [233]. SARS-CoV-2 RNA has been detected in the kidney, respiratory tract and blood 2 months after infection [234–238]. In a further study, SARS-CoV-2 N protein was also observed at several sites up to 6 months after testing negative by PCR in a small cohort of patients [239].

Further evidence suggests that reverse-transcribed SARS-CoV-2 RNA can be integrated into host cell DNA with subsequent transcription of the integrated sequences [240]. These observations were made primarily on cells in culture, but it was also noted that in some patient-derived samples viral sequences were transcribed from integrated DNA copies of viral sequences, although these small fragments were not capable of producing infectious virus [240]. It should, however, be noted that these suggestions are controversial and have been contradicted in other reports [241, 242].

It is tempting to compare the effects of SARS-CoV-2 infection with those of HCV, another positive-sense, single-stranded RNA virus. HCV establishes chronic or persistent low-level infection in the liver, and this is a major contributory factor (with HBV) to hepatitis and hepatocellular carcinoma (HCC). Chronic HCV infection causes oxidative stress and high levels of ROS, as well as stimulating the production of NO via activation of inducible NO synthase (iNOS) [243–250]. The effects of these high levels of NO and ROS probably lead to the accumulation of DNA damage and the genetic abnormalities observed in HCV-infected cells; this can drive the progression of HCV-associated malignancies [246, 247, 249]. As well as inducing DNA damage, HCV proteins can directly impinge on the cellular DDR, generally reducing its effectiveness (reviewed in [43, 251]).

Chronic infection by HCV can initiate a non-specific immune-mediated inflammation, seen as hepatitis, which is linked to oxidative stress. As discussed above, SARS-CoV-2 infection is closely associated with inflammation and the generation of ROS. If the virus were to persist in a comparable fashion to HCV it is possible that it could also have serious deleterious consequences for the infected tissue and possibly cause DNA damage and genomic instability. Time will tell if this is the case.

CONCLUSIONS

It is less than 4 years since the initial outbreak of infection in Wuhan by a previously unknown virus. Yet our knowledge of many facets of SARS-CoV-2's life cycle, replication and effects on the infected host cell is considerable, although there are aspects of SARS-CoV-2 biology that have not, up to now, been examined in detail. One of these is the relationship between the virus and the cellular DDR, although it is now becoming clear that some of these pathways are a target for the virus, although perhaps in a minor way.

Importantly, as well as impinging on DDR pathways, SARS-CoV-2 causes damage to cellular DNA, generally through the generation of ROS, which can cause double- and single-strand breaks, intra- and inter-strand crosslinks, and non-bulky and bulky base modifications. ROS are generated at several points during viral infection, generally through activation of multiple inflammatory pathways, as outlined above. These effects are amplified by the ability of the virus to downregulate host cell antioxidant pathways, aiding replication and possible upregulation of pro-oxidant genes.

Although there have been several large-scale studies examining the interactome for SARS-CoV-2 proteins, the results have tended to be somewhat contradictory (see above). However, components of different DDR pathways have been identified as binding partners for viral proteins, such as the association of POLD1 with Nsp7 and Nsp10 [158, 181], the association of MRE11 and RAD50 with Nsp14 [161], and the association of POLA1 and POLA2 with Nsp1 [158, 176–178, 180, 181]. Most of the reported interactions are summarized in Table 2. Whether they are all significant, or even occur *in vivo*, is open to question, although it seems reasonable to assume that these data establish that DDR pathways are targets for SARS-CoV-2. As well as associating with DDR proteins, it has been confirmed that SARS-CoV-2 proteins affect DDR pathways. For example, SARS-CoV-2 infection results in phosphorylation of ATM and DNA-PKcs, an increase in DNA repair foci containing phosphorylated ATM substrates and degradation of Chk1 [136]. It seems that there is a requirement for certain DDR pathways for optimal viral replication as addition of the ATR inhibitor, berzosertib, has potent anti-viral activity, inhibiting Chk1 phosphorylation [190].

The effects of SARS-CoV-2 on the host genome and on the cellular DDR are comparable to those seen with other RNA and DNA viruses. Infection with the RNA viruses HIV-1, HTLV-1 and HCV generates ROS, which can cause DNA damage (reviewed in [43]). In the case of HCV (and HBV), this has been linked to hepatocellular carcinoma. Similarly, ROS production is also a consequence of DNA virus infection, such as is observed with, among others, HBV, EBV and HPV [42, 252]. Although our knowledge of the effects of SARS-CoV-2 on the DDR is not extensive, again they appear to be similar to those reported for other viruses. Thus, there is an activation of ATM, and DNA-PK signalling, as is generally observed, and a generation of γH2AX repair foci [42, 46, 136]. A point of some concern may be whether SARS-CoV-2 virus or viral proteins and/or RNA persist in patients suffering from long COVID and whether these could cause genome instability over the longer term. If this were the case, a rise in cancer cases might be seen in future.

Funding information
This work received no specific grant from any funding agency.

Conflicts of interest
The author declares that there are no conflicts of interest.

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