Review

DEAD/H-Box Helicases in Immunity, Inflammation, Cell Differentiation, and Cell Death and Disease

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Abstract: DEAD/H-box proteins are the largest family of RNA helicases in mammalian genomes, and they are present in all kingdoms of life. Since their discovery in the late 1980s, DEAD/H-box family proteins have been a major focus of study. They have been found to play central roles in RNA metabolism, gene expression, signal transduction, programmed cell death, and the immune response to bacterial and viral infections. Aberrant functions of DEAD/H-box proteins have been implicated in a wide range of human diseases that include cancer, neurodegeneration, and inherited genetic disorders. In this review, we provide a historical context and discuss the molecular functions of DEAD/H-box proteins, highlighting the recent discoveries linking their dysregulation to human diseases. We will also discuss the state of knowledge regarding two specific DEAD/H-box proteins that have critical roles in immune responses and programmed cell death, DDX3X and DDX58, also known as RIG-I. Given their importance in homeostasis and disease, an improved understanding of DEAD/H-box protein biology and protein–protein interactions will be critical for informing strategies to counteract the pathogenesis associated with several human diseases.

Keywords: DEAD/H-box proteins; stress granules; chemotherapy; MAP kinase signaling; PRR; PAMP; DAMP; DDX3X; RIG-I; inflammasome; NLR; NLRP3; caspase; interferon signaling; viral infection; bacterial infection; programmed cell death; pyroptosis; apoptosis; necroptosis; PANoptosis; innate immunity

1. Introduction

RNA helicases play a central role in RNA metabolism, affecting many aspects of cellular homeostasis. DEAD/DEAH (DEAD/H)-box helicases make up the largest family of RNA helicases in humans [1]. The DEAD/H-box helicases are present in every kingdom of life (Figure 1), and their essential role in translation was discovered in the late 1980s [2–6]. Since then, they have been found to be involved in almost every aspect of cell biology, including splicing, RNA metabolism, host responses to bacterial and viral infections, organismal development, stress responses, and programmed cell death (PCD).

DEAD/H-box helicases have a domain architecture containing the conserved amino acid motifs Asp-Glu-Ala-Asp (DEAD) or Asp-Glu-Ala-His (DEAH), and the Helicase_C sequence motif. There are 199 human and 116 mouse protein sequences that contain the DEAD/H-box domain in the InterPro database [7]. Additionally, there are 1913 domain architectures involving the DEAD/H-box domain (PFAM accession number: PF00270), the vast majority of which contain both a Helicase_C domain (PFAM accession number: PF00271) and low-complexity or disordered regions [8]. Furthermore, there are 3268 domain architectures containing the Helicase_C domain, with the most common architectures also containing the DEAD/H-box domain. Phylogenetic analysis shows that most of the DEAD/H-box proteins have orthologs in both humans and mice (Supplementary Data S1). This conservation suggests that the information gleaned from studies...
of these proteins in mouse models may be extrapolated to their roles in humans. Additionally, some of the DEAD/H-box proteins have important paralogs whose relative contributions in biological processes remain poorly understood. An example of one such paralogous relationship can be found between DDX3X, DDX3Y, and DDX3L (also known as D1PAS1) in mice (Supplementary Data S1). DDX3X is one of the most extensively studied DEAD/H-box proteins, though there is still much to learn about DDX3Y and DDX3L. These relationships will be discussed in more detail later in this review. Understanding the functions of the paralogs will be critical for precise therapeutic targeting to avoid unintended adverse events.

In mammals, several DEAD/H-box proteins act as prototypical nucleic acid-sensing pattern recognition receptors (PRRs), as proposed by Janeway [9]. DEAD/H-box proteins have also been associated with a range of human diseases (Supplementary Data S2). These observations bolster the argument for in-depth studies of the DEAD/H-box family of proteins. In this review, we discuss the state of knowledge regarding DEAD/H-box proteins, with a focus on their known roles in the host response to pathogenic challenges, PCD, and disease.

![Figure 1. Distribution of DEAD/H-box domain-containing proteins.](image)

The sunburst plot was downloaded from the Pfam database [8]. In total, 8874 species are represented in the sunburst plot. The Pfam database has 171,615 protein sequences and 2493 domain architectures that contain the DEAD/H-box domain. Arc lengths in the sunburst plot are dependent on the number of protein sequences in each category. A vast majority of DEAD/H-box-containing protein sequences are from eukaryotes (purple), and none are found in viruses. The original interactive sunburst plot can be viewed at the Pfam database using the following link: [https://pfam.xfam.org/family/PF00270#tabview=tab7](https://pfam.xfam.org/family/PF00270#tabview=tab7) (accessed on 20 July 2021).

2. DEAD/H-Box Proteins and the Innate Immune Response to Viral Infections

DEAD/H-box proteins play an important role in many host RNA metabolic processes, and they are often referred to as ribonucleoprotein particle (RNP) modulators [10]. Due to their diverse functions in RNA metabolism, they aid in the host response against viral infection (Figure 2). However, viruses have evolved the ability to use DEAD/H-box proteins for replication. One key example of this ability can be seen in the case of the first DEAD/H-box protein discovered—eukaryotic initiation factor 4A (eIF4A), also known as DDX2 [6]. In one of the earliest reports of a DEAD/H-box protein having a role in the viral life cycle, eIF4A was found to bind Semliki Forest virus mRNA [11]. Subsequent studies have shown that eIF4A binds to several viral mRNAs [12–15], and these studies have made critical contributions to our understanding of eukaryotic translation initiation. eIF4A helps to unwind secondary structures in 5′-untranslated regions (5′-UTR) to facilitate ribosome scanning [16,17]. eIF4A is also important for internal ribosome entry site (IRES)-mediated, cap-independent translation initiation [16,17]. Since several viruses have evolved
to repress host cellular cap-dependent translation and use IRES-mediated translation for viral protein synthesis, eIF4A has emerged as an attractive target for developing antiviral drugs [15,18–23].

Figure 2. Roles of DEAD/H-box proteins in viral replication and antiviral host responses. DEAD/H-box proteins play pleiotropic roles in host responses to viral infections. Some DEAD/H-box proteins promote viral replication through their functions in transcriptional, post-transcriptional, or translational control of gene expression. Some DEAD/H-box proteins act as viral nucleic acid sensors and promote an antiviral host response. Additionally, some have both capabilities. RIG-I (also known as DDX58) is a prototypical example of a DEAD/H-box protein acting as a nucleic acid sensing pattern recognition receptor.

Another well-studied example of viral hijacking of host machinery is the X-linked DEAD/H-box helicase, DDX3X. DDX3X was originally identified for its role in the trans-
lation of host and viral RNA [24–26]. It was initially found to interact with hepatitis C virus (HCV) proteins, but the functional significance of the interaction was unknown at the time [27–29]. Follow-up studies showed that DDX3X promotes HCV replication, and DDX3X has since been implicated in promoting replication for influenza A virus (IAV), West Nile virus (WNV), Japanese encephalitis virus, and human immunodeficiency virus (HIV) [30–35]. DDX3X is required for the nuclear export of HIV, and it also acts as a negative regulator of type I interferons in arenavirus infections [30,31,36]. Though these studies suggest that DDX3X inhibition could be a potential antiviral therapeutic strategy, DDX3X has dual functionality in that it can promote both viral replication and optimal antiviral host response [34,35,37–41]. For example, DDX3X facilitates HIV replication [31], but it also acts as a sensor for aborted HIV transcriptomes [42]. Its importance for both the host and pathogen has made this conserved protein one of the most well-studied DEAD/H-box proteins.

Both the murine Ddx3x and human DDX3X genes are capable of complementing the deficiency of the Ded1 yeast ortholog [25,27]. This conservation may help to explain DDX3X’s dual role in host–pathogen interactions. Viruses have coevolved with organisms expressing a DDX3X ortholog for millions of years. This may have allowed them to develop strategies to blunt DDX3X’s antiviral function and to co-opt its role in gene expression and subcellular transport for viral replication. This is reflected in the observations that several viruses can target DDX3X to inhibit the induction of the type I interferon response [35,41–46]. As viruses have evolved to inactivate the DDX3X-mediated type I interferon response, hosts have similarly found ways to engage other sensors and signaling pathways, or to acquire new functions for existing sensors. For example, DDX3X can promote an NLRP3 inflammasome-mediated pro-inflammatory response to IAV infection [47]. DDX3X can also promote inflammation by enhancing the phosphorylation of the protein phosphatase PP2A to activate NF-kB signaling downstream of TLR3 activation [48].

In addition to DDX3X, there are other DEAD/H-box proteins that mediate host–virus interactions. DDX58 (also known as RIG-I) was implicated in the antiviral host response when it was discovered that RIG-I is induced by porcine reproductive and respiratory syndrome virus (PRRSV) infection [49]. While RIG-I was originally proposed to be a double-stranded RNA (dsRNA) sensor [50–52], later studies showed that RIG-I recognizes uncapped RNA containing a 5′-triphosphate moiety [53,54]. Since endogenous mRNA, with the exception of mitochondrial and chloroplast mRNA, contains a 5′-7-methylguanosine cap structure, the presence of the 5′-triphosphate moiety on cytoplasmic RNA acts as a pathogen-associated molecular pattern (PAMP) for recognition by innate immune sensors [9,55]. RIG-I is the only DEAD/H-box protein that also contains a caspase activation and recruitment domain (CARD). Activation of RIG-I leads to the recruitment of MAVS (mitochondrial antiviral signaling, also known as IPS-1, interferon-β promoter stimulator 1) through CARD–CARD interactions, which in turn leads to the induction of a type I interferon response [56,57]. RIG-I undergoes a conformational change upon ligand binding [58,59] which makes the CARD domain accessible for interaction with MAVS, and which allows for the TRIM25-mediated generation of free K63-linked polyubiquitin chains. These chains associate with RIG-I CARD domains and help to bring MAVS into a complex with RIG-I. RIG-I has two CARD domains, which means that it has a valency of two for CARD–CARD interactions [60]. Multivalency has recently been proposed to be critical for the assembly of biomolecular complexes involved in innate immune signaling and PCD [60–62]. Functionally, the RIG-I bivalency with respect to CARDs could help to bring together different molecules into a single complex, though this remains to be thoroughly investigated.

Though not as well characterized, many other DEAD/H-box proteins have roles in host–virus interactions. Some have a positive impact on viral replication. For instance, DDX1 is a cofactor for the HIV-Rev protein that promotes viral replication [31]. DDX1 also promotes the replication of avian infectious bronchitis virus (IBV), a model coronavirus, by interacting with non-structural protein 14 (NS14) [63]. The role of DDX1 in the replication
of human coronaviruses remains to be determined. If this function of DDX1 is conserved for human coronaviruses, the targeting of DDX1 could be a viable strategy for treating coronavirus infections [64]. DDX1 also forms a complex with two other DEAD/H-box proteins, DDX21 and DHX36, to promote an antiviral type I interferon response through TRIF in myeloid dendritic cells (mDCs) [65]. In this complex, DDX1 binds dsRNA while DDX21 and DHX36 interact with TRIF [65]. The PRK<sup>DDX21</sup> domain of DDX21 interacts with the TIR<sup>TRIF</sup> domain of TRIF through heterotypic domain interactions [65]. The interaction between DHX36 and TRIF is also mediated by heterotypic domain interactions between HA1-DUF<sup>DHX36</sup> and TIR<sup>TRIF</sup> domains [65]. Whether the DDX1-DDX21-DHX36 complex can form in cells other than mDCs remains to be seen.

There are several other examples of DEAD/H-box proteins that are involved in the host–pathogen interaction. DDX5 promotes the viral replication of Japanese encephalitis virus by binding to the viral 3′-UTR [66]. DDX19 can associate with both spliced and unspliced IAV mRNAs, and it binds to IAV polymerase independent of RNA and promotes the nuclear export of viral mRNA [67]. Additionally, DDX56 increases the infectivity of WNV through a mechanism that is independent of viral replication [68]. The helicase activity of DDX56 is required for increased infectivity [69]. It was proposed that DDX56 is required for WNV virion assembly. However, the endogenous function of DDX56 remains poorly understood. A recent study showed that DDX56 is a negative regulator of type I interferon signaling, suggesting that it could have a role in suppressing excess inflammation to maintain organismal homeostasis [70].

DDX9 promotes antiviral signaling by sensing dsRNA and activating the NLRP9b inflammasome in response to rotavirus infection in intestinal epithelial cells [71], although whether DDX9 is sensing a specific secondary structure in the dsRNA that is acting as a PAMP remains to be seen. In plasmacytoid dendritic cells, DDX9 responds to CpG-B to activate NF-κB and cytokine production, including TNF and IL-6 [72]. Additionally, DDX19A and DHX33 promote activation of the NLRP3 inflammasome in response to viral infections [73,74]. DHX15 is another DEAD/H-box protein that is involved in antiviral innate immune responses. It promotes MAVS- and NLRP6-dependent antiviral signaling in enteric encephalomyocarditis virus (EMCV) infection by facilitating NLRP6 binding to viral RNA [75]. Additionally, DHX15 was found to undergo liquid–liquid phase separation with NLRP6 in response to dsRNA [76]. During EMCV infection, DHX15 also promotes interactions with MAVS to induce interferon production and signaling [75]. Furthermore, DHX15 also binds to RIG-I through a heterotypic interaction with CARD<sup>RIG-I</sup> and the viral RNA PAMP to promote type I interferon signaling [77]. Induction of the type I interferon response is dependent on RIG-I, and DHX15 binding promotes ATP hydrolysis upon RNA PAMP binding. Since both DHX15 and RIG-I have ATPase activity, it is not clear which protein is responsible for increased ATP hydrolysis. Additionally, DDX41 plays a role in the host response to viral infections. DDX41 can activate STING-dependent type I interferon and NF-κB and MAP kinase signaling upon dsDNA binding in mDCs [78]. DDX41 binds dsDNA and STING simultaneously to activate this downstream signaling [78].

Together, the data suggest that DEAD/H-box proteins, particularly those involved in positively regulating the viral life cycle, can be targeted to inhibit viral replication. However, there is a need for a comprehensive study on functions of DEAD/H-box proteins during viral infections to clearly identify those which positively regulate viral infection. A deeper understanding of this field will expand the repertoire of host factors that can be therapeutically targeted to treat specific viral infections.

### 3. DEAD/H-Box Proteins and the Innate Immune Response to Bacterial Infections

Although understudied compared to their roles in viral infections, DEAD/H-box proteins also have critical functions in responding to bacterial infections. In response to *Listeria monocytogenes* infection, DDX3X acts as a transcription factor and upregulates the expression of the *Ifnb* gene in macrophages (Figure 3) [79]. DDX3X is phosphorylated by TBK1, which leads to its nuclear translocation and recruitment to the *Ifnb* promoter.
to activate its transcription [79]. In addition, bacterial secondary messengers can act as PAMPs for recognition by innate immune system PRRs such as DDX41. Cyclic di-GMP (c-diGMP) and cyclic di-AMP (c-diAMP) are ubiquitously present bacterial second messengers that are sensed by DDX41 [80]. DDX41 binds to STING and activates type I interferon signaling upon *L. monocytogenes* infection in the mouse splenic dendritic cell line D2SC (Figure 3). DDX41 can sense DNA to activate signaling [78,80], and cytoplasmic delivery of c-diGMP and c-diAMP also activates the type I interferon response in a DDX41-dependent manner [80]. Bacterial DNA is likely also released from lysed cells, and it can activate the host response independently of DDX41, providing another example of the multifaceted nature of PAMP sensing mechanisms used by mammalian hosts to counteract pathogenic infections [47,80–82].

![Diagram showing the role of DEAD/H-box proteins in the host response to bacterial infection.](image)

**Figure 3.** Role of DEAD/H-box proteins in the host response to bacterial infection. DDX3X can activate type I interferon signaling in response to infection with *Listeria monocytogenes* downstream of TBK1. Bacterial secondary messengers can also be sensed by hosts to activate the type I interferon response. DDX41 acts as a sensor for c-diGMP and c-diAMP to activate the STING-dependent type I interferon response. DDX41 can also sense DNA, and may be able to sense bacterial DNA during infection. Solvent-excluded surfaces of DDX3X (PDB ID: 2I4I) [83] and DDX41 (PDB ID: 2P6N) [84] are depicted in the figure. Hydrophobic residues (red) and hydrophilic residues (blue) on the surface of proteins are shown.
TBK1. Bacterial secondary messengers can also be sensed by hosts to activate the type I interferon response. DDX41 acts as a sensor for c-diGMP and c-diAMP to activate the STING-dependent type I interferon response. DDX41 can also sense DNA, and may be able to sense bacterial DNA during infection. Solvent-excluded surfaces of DDX3X (PDB ID: 2I4I) [83] and DDX41 (PDB ID: 2P6N) [84] are depicted in the figure. Hydrophobic residues (red) and hydrophilic residues (blue) on the surface of proteins are shown.

Although DEAD/H-box proteins are canonically classified as RNA-binding proteins, they can bind DNA and have been reported to sense cytosolic DNA [72,78]. While most studies have focused on DEAD/H-box proteins sensing DNA viruses, DNA-sensing DEAD/H-box proteins can also biochemically detect bacterial DNA that is released in the cytoplasm of infected cells. It will be interesting to evaluate whether these DEAD/H-box proteins also have a role in the host response to bacterial infection. Studies to investigate these processes could have important translational impacts and identify new targets for therapeutic interventions in diseases caused by bacterial infections.

4. DEAD/H-Box Proteins in Programmed Cell Death Regulation

The role for programmed cell death (PCD) in organismal development and host responses to pathogenic challenges has been an active area of research for many decades [85,86]. These studies have discovered interlinked PCD pathways that are critical for maintaining organismal homeostasis [81,87–109]. Several DEAD/H-box proteins have been implicated in the regulation of PCD (Figure 4). DEAD/H-box proteins can both promote and inhibit cell death. DDX3X provides a prime example of the contrasting dual roles of DEAD/H-box proteins in PCD (Figure 4). DDX3X can inhibit apoptosis and promote pyroptosis [110,111]. In response to stimulation of the death receptors TRAILR1, TRAILR2, TNFR, and FAS, DDX3X forms an anti-apoptotic complex with GSK3 and CIAP1, which acts as a cap around the cytosolic face of the death receptors and sterically inhibits their activity [111]. This, in turn, inhibits extrinsic apoptosis. Conversely, the inhibition of DDX3X ATPase activity by the small molecule inhibitor RK-33 promotes apoptosis [112]. Additionally, signals that activate apoptosis promote the proteolytic degradation of DDX3X and CIAP1, and DDX3X is a target of CASP8-mediated proteolytic processing [111]. This suggests that a major function of this anti-apoptotic complex guards against the accidental activation of extrinsic apoptosis. However, the molecular events that trigger CASP8 activation to lead to DDX3X cleavage have not been completely elucidated.

In contrast, DDX3X is required for pyroptosis downstream of NLRP3 inflammasome activation [110]. DDX3X was found to be required for NLRP3 inflammasome activation by both potassium efflux-dependent and -independent triggers, but the exact molecular mechanism is not completely understood. It was proposed that the scaffold function of DDX3X promotes prionoid phase transitions, which lead to the formation of complex assemblies that can act as a platform to trigger cell death. Examples of such assemblies include ASC specks, amyloid plaques, Tau fibrils, and PANoptosomes, which are multifaceted macromolecular complexes that regulate PANoptosis, an inflammatory PCD that integrates components from other cell death pathways; the totality of the biological effects in PANoptosis cannot be individually accounted for by pyroptosis, apoptosis, or necroptosis alone [81,92–109]. Since the liquid–liquid phase separation of DDX3X promotes the assembly of stress granules, which are thought to inhibit PCD, it was proposed that, depending on the type of phase transition triggered, DDX3X can promote either cell survival or PCD [110,113]. However, the molecular mechanism by which DDX3X is recruited, either to stress granules or to the NLRP3 inflammasome, requires further study. Taken together, DDX3X has been reported to play a pleiotropic role in PCD, immunologically inhibiting silent apoptosis while promoting inflammatory PCD.
Figure 4. Role of DEAD/H-box proteins in programmed cell death. DDX3X forms an anti-apoptotic complex with cIAP and GSK3 that blocks extrinsic apoptosis induced by ligands, such as TNF, TRAIL, and FASL binding to death receptors. Signals that induce apoptosis target DDX3X for caspase-8 (CASP8)-mediated degradation. Several DEAD/H-box proteins have been reported to drive pyroptosis—a pro-inflammatory programmed cell death pathway. DDX3X is critically important for the activation of the canonical NLRP3 inflammasome downstream of potassium ion efflux, as well as potassium-independent mechanisms. Another DEAD/H-box protein that is involved in triggering NLRP3 inflammasome activation is DHX33. DHX33 acts as a sensor of virus-derived dsRNA, and it activates the NLRP3 inflammasome. DHX9 is a sensor for rotavirus-derived dsRNA for activating the NLRP9b inflammasome. RIG-I (DDX58) itself can also act as an inflammasome sensor. Virus-derived RNA or 5′-triphosphate-containing RNA can trigger assembly of an inflammasome dependent on RIG-I, ASC, and CARD9.

RIG-I is another DEAD/H-box protein that has been extensively studied for its role in PCD (Figure 4). RIG-I induces mitochondrial apoptosis in melanoma and hepatoma cells in response to 5′-triphosphate-containing RNA, as well as the dsRNA analog polyinosinic-polycytidylic acid (poly(I:C)) [114,115]. As discussed above, RIG-I is activated by viral
transcripts, and this leads to the induction of the type I interferon response. While PCD induction by RIG-I was initially thought to be independent of type I interferon signaling, more recent work shows that RIG-I can promote apoptosis downstream of IFNα in melanoma cells [116]. Additionally, combinatorial treatment with a STAT3 inhibitor and IFNα increases apoptosis in melanoma cells [116], suggesting that the activation of RIG-I in conjunction with cancer therapy could potentially improve its therapeutic efficacy. RIG-I activation and the induction of apoptosis could also have a beneficial role in photochemotherapy for treating psoriasis [117]. Furthermore, RIG-I has been proposed to induce IRF3-dependent apoptotic cell death in response to viral infections [118,119]. IRF3-dependent apoptosis in Sendai virus-infected cells is dependent on IRF3 binding to the pro-apoptotic protein BAX, and it is activated by signaling downstream of RIG-I-like receptors [120,121]. In addition to its roles in apoptosis, RIG-I was also reported to assemble an inflammasome in bone marrow-derived dendritic cells [122]. This inflammasome activation requires CARD9 and the inflammasome adaptor ASC. Additionally, in human primary lung epithelial cells, RIG-I can assemble an inflammasome in response to IAV infection in a type I interferon signaling-dependent process [123]. These studies suggest that RIG-I may induce pyroptosis in addition to apoptosis. The CARD domain of RIG-I could be responsible for a RIG-I-containing PCD complex upon ligand binding. Although these studies have shed light on RIG-I-mediated apoptosis and pyroptosis, a complete mechanistic understanding of the process remains elusive.

DDX3X and RIG-I are the most well-studied DEAD/H-box proteins with respect to their role in PCD. However, several other DEAD/H-box proteins have also been implicated in PCD (Figure 4). A screen designed to identify DEAD/H-box proteins involved in the activation of the NLRP3 inflammasome found that DHX33 can form a complex with NLRP3 and ASC that promotes caspase-1 (CASP1) cleavage and inflammasome-dependent cytokine processing [73]. DHX33 essentially acts as an RNA sensor to trigger NLRP3 inflammasome activation. Cytosolic poly(I:C), viral dsRNA, and bacterial RNA promote DHX33-mediated activation of the NLRP3 inflammasome [73]. The interaction between NLRP3 and DHX33 is driven by heterotypic domain interactions between DEADDHX33 and NACHTNLRP3. This interaction might be mediated by intrinsically disordered regions (IDRs) in DEADDHX33 and NACHTNLRP3, in a manner similar to the interaction between DDX3X and NLRP3 [110], but this remains to be tested. DDX19A is another DEAD/H-box protein that has been implicated in NLRP3 inflammasome activation in response to viral infection [74]. PRRSV infection of primary porcine alveolar macrophages activates the NLRP3 inflammasome, and DDX19A is thought to act as an RNA sensor for PRRSV infection [74]. Additionally, DHX9 is a DEAD/H-box protein that is reported to have contrasting roles in PCD. DHX9 acts as a dsRNA sensor in rotavirus-infected intestinal epithelial cells, to facilitate pyroptosis through NLRP9b inflammasome activation [71]. Since DHX9 and NLRP9b do not share a domain for homotypic interactions, their mechanism of interaction is unknown. This may occur through IDRs or with the dsRNA ligand acting as a bridge. In contrast to its role in activating pyroptosis, DHX9 inhibits the lytic replication cycle during infection with Epstein-Barr virus (EBV) [124]. This lytic replication involves PCD, suggesting that DHX9 is a survival factor in EBV-infected cells [125].

Overall, DEAD/H-box proteins play diverse roles in regulating PCD in response to pathogenic infections. However, most of the proteins in the DEAD/H-box family are understudied in the context of PCD, suggesting that there are still discoveries to be made. For example, DDX5 has been most intensely studied for its role in splicing, gene expression, and oncogenesis; however, beyond these roles, the phosphorylation of DDX5 can inhibit TRAIL-induced apoptosis in glioblastoma cells [126]. The double tyrosine phosphorylation of DDX5 downstream of platelet-derived growth factor signaling is associated with the inhibition of CASP8 cleavage and resistance to TRAIL-induced apoptosis in T98G glioblastoma cells [126]. This suggests that further studies to evaluate additional DEAD/H-box proteins in PCD may identify other such functions.
5. DEAD/H-Box Proteins in Cell Differentiation and Organismal Development

Beyond their roles in infection, PCD and host defense, DEAD/H-box proteins are also critically important for cell differentiation and organismal development. One key example is Dhx9, which is one of the earliest DEAD/H-box genes to be discovered. It was originally identified through the observation of a temperature-sensitive mutation in Drosophila melanogaster that led to defective nerve conduction at the restrictive temperature, suggesting a role in neuronal development [127]. Researchers named the gene no action potential (Nap). Two other groups independently identified mutations in the same gene that caused male sterility in D. melanogaster and referred to the gene as maleless (Mle) [128,129]. The human ortholog DHX9 was identified from HeLa cells and was initially named RNA Helicase A [130]; at the time, it was not classified as a DEAD/H-box protein because the characteristic DEAD/H-box sequence motifs had not yet been discovered. Soon after, RNA Helicase A and Mle were found to be orthologs, and they were classified as members of the DEAH family of DEAD/H-box proteins, leading to their subsequent renaming as DHX9 [131]. In D. melanogaster, Dhx9 was found to be important for the proper activation of X-chromosome dosage compensation; its dysfunction led to male sterility. In contrast to D. melanogaster Dhx9, murine Dhx9 is required for normal gastrulation in both male and female embryos [132]. Additionally, there is increased apoptosis in Dhx9−/− mouse embryos compared with the wild type [132], further supporting a role for DHX9 in PCD in addition to organismal development.

DDX17 is another DEAD/H-box protein that has been reported to play a role in cell differentiation and organismal homeostasis. Ddx17 was also discovered in D. melanogaster and was originally named Lighten up (Lip) [133]. DDX17, along with the DEAD/H-box protein DDX5, is involved in regulating alternative splicing during epithelial cell and myotube differentiation in mammals [134]. The sensing of endogenous short interspersed nuclear elements by DDX17 is associated with the assembly of an inflammasome containing NLRP3 and NLRC4 that does not induce pyroptosis but does drive cytokine release, contributing to disease in a murine model of atrophic macular degeneration and IL-18 release in cells from patients with systemic lupus erythematosus [135].

DDX3X and its paralog, DDX3Y, have also been reported to regulate organismal development [136]. In mice, the loss of Ddx3x during hematopoiesis results in an abnormal leukocyte composition in the bone marrow and spleen [137]. Additionally, RIG-I is essential for myelopoiesis through its regulation of the TRIM25-dependent protein ISGylation [138]. Other DEAD/H-box helicases have also been implicated in hematopoiesis in other model systems, including DDX46 and DDX18 in zebrafish [139,140]. Beyond hematopoiesis, the loss of X-linked Ddx3x in mice also leads to female-specific defects in hindbrain development [136]. Males are presumably protected because of the presence of Y-linked Ddx3y. DDX3X is involved in neuronal development in humans as well [141–143]. Mutations in DDX3X lead to RNA metabolism defects that are associated with intellectual disability (discussed in more detail in the next section) [143]. These studies suggest that DEAD/H-box proteins can play critical roles in cell differentiation and organismal development. However, they have been comparatively understudied in this context, due to a lack of in vivo models, likely due to the high chance of embryonic lethality upon complete deletion. In recent years, conditional deletions in specific subsets of cells have allowed scientists to begin to evaluate the role of more DEAD/H-box proteins during development [136]. Recent developments in genome editing technologies, including conditional deletion and activation technologies, should be beneficial for future studies of DEAD/H-box proteins in the context of cell differentiation and organismal development.

6. DEAD/H-Box Proteins in Human Diseases

Owing to their diverse role in RNA metabolism and organismal homeostasis, it is not surprising that aberration in the functions of DEAD/H-box proteins have been implicated in a range of human diseases. For instance, the dysregulation of DEAD/H-box helicases is linked to hematological malignancy, as well as to cancer growth and metastasis. One
relatively well-characterized connection between DEAD/H-box proteins and disease is Bloom syndrome, an autosomal-recessive disorder that leads to stunted growth and genomic instability [144–146]. The gene associated with Bloom syndrome was mapped to human chromosome 15 and later identified as a DEAD/H-box helicase, BLM RecQ-like helicase (BLM) [146,147]. BLM inhibits double-strand breaks during DNA replication that can increase cancer predisposition in cell lines [148]. Consistent with these in vitro findings, Blm−/− mice have an increased predisposition to develop lymphoma, sarcoma, and carcinoma [149]. Since the discovery of the link between BLM and Bloom syndrome, several human gene–disease associations (GDAs) have been discovered involving DEAD/H-box proteins. For example, loss-of-function mutations in Werner syndrome ATP-dependent helicase (WRN) lead to Werner syndrome, a disease that is characterized by premature aging and increased susceptibility to cancer. WRN was discovered through positional cloning and was later found to have DNA helicase activity [150,151]. Subsequent studies showed that WRN is involved in resolving Holiday junctions during DNA recombination, the unwinding of DNA secondary structures, and DNA repair [152–155]. A recent study suggested that WRN is also involved in the nuclear export of mRNA [156].

In addition to BLM and WRN, mutations in DDX3X have been associated with several diseases, specifically several cancers, epilepsy, and female intellectual disability [157–161]. Multiple specific mutations in DDX3X are associated with cancer (Supplementary Data S2). However, there is conflicting evidence regarding the role of DDX3X in tumorigenesis and patient survival. Low levels of DDX3X expression have been associated with poor prognosis in patients with colorectal cancer [160] and in patients who are non-smokers with oral cancer [162], while a high expression of DDX3X is associated with poor prognosis in gliomas [163]. Other studies have reported an oncogenic role for DDX3X in colorectal cancer [159,164]. Additionally, DDX3X depletion has been reported to reduce metastasis in medulloblastoma [165], while this depletion leads to increased malignancy in non-small cell lung cancer [166]. These studies suggest an urgent need to clarify the role for DDX3X in cancers, as it has been proposed as a target for developing anti-cancer therapeutics [112,167–169]. Mechanistically, cancer-associated DDX3X mutants have reduced RNA-dependent ATPase activity, and their expression leads to increased stress granule assembly [170,171]. These findings support the hypothesis that reduced translation can promote tumorigenesis [171]. Since translation is the most energy-intensive process in a cell, a reduced rate of translation caused by DDX3X mutations might allow for the metabolic adaptation of cancer cells for their continued proliferation [172,173]. Additionally, mutations in the DDX3X paralog DDX3Y are also associated with tumors of the reproductive system [174,175]. Loss-of-function mutations in DDX3Y result in male sterility, again suggesting a complex role for DDX3X/Y in cancer [176].

Overall, DEAD/H-box protein GDAs span human diseases and include intellectual disability, cancers, susceptibility to viral infections, and behavioral defects, among others. An analysis of DEAD/H-box protein GDAs from the DisGeNET database highlights the depth and breadth of these associations (Supplementary Data S2) [177]. Overall, the diversity of DEAD/H-box protein GDAs is indicative of the critical role that DEAD/H-box proteins play in maintaining organismal homeostasis. This importance draws attention to the need for a concerted effort to mechanistically characterize DEAD/H-box protein functions to understand their specific roles in each disease, and to identify new therapeutic strategies.

7. Discussion

DEAD/H-box proteins constitute the largest family of RNA helicases in mice and humans. Beyond the many roles for DEAD/H-box proteins discussed above, these proteins are also involved in controlling gene expression and regulating liquid–liquid phase separation-mediated compartmentalization, as well as an ever-growing list of other functions that could not be discussed here due to space limitations [178–182]. They have been implicated in a wide diversity of human diseases. Additionally, the discoveries of un-
expected roles by DEAD/H-box proteins suggest that we are not yet close to having a complete understanding of their biology. Future studies on DEAD/H-box proteins will therefore be mechanistically informative and can lead to the development of novel therapies for a range of human diseases.

**Supplementary Materials:** The following supporting information can be downloaded at: [https://www.mdpi.com/article/10.3390/cells11101608/s1](https://www.mdpi.com/article/10.3390/cells11101608/s1), Data S1: Phylogenetic analysis of human and mouse DEAD/H-box proteins; Data S2: List of human diseases associated with DEAD/H-box protein genes. References [183–185] are cited in the supplementary materials.

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**References**

1. Umate, P.; Tuteja, N.; Tuteja, R. Genome-wide comprehensive analysis of human helicases. *Commun. Integr. Biol.* 2011, 4, 118–137. [CrossRef] [PubMed]
2. Ford, M.J.; Anton, I.A.; Lane, D.P. Nuclear protein with sequence homology to translation initiation factor eIF-4A. *Nature* 1988, 332, 736–738. [CrossRef] [PubMed]
3. Lasko, P.F.; Ashburner, M. The product of the Drosophila gene vasa is very similar to eukaryotic initiation factor-4A. *Nature* 1988, 335, 611–617. [CrossRef] [PubMed]
4. Nishi, K.; Morel-Deville, F.; Hershey, J.W.B.; Leighton, T.; Schnier, J. An eIF-4A-like protein is a suppressor of an Escherichia coli mutant defective in 50S ribosomal subunit assembly. *Nature* 1988, 336, 496–498. [CrossRef] [PubMed]
5. Séraphin, B.; Simon, M.; Boulet, A.; Faye, G. Mitochondrial splicing requires a protein from a novel helicase family. *Nature* 1989, 337, 84–87. [CrossRef]
6. Linder, P.; Lasko, P.F.; Ashburner, M.; Leroy, P.; Nielsen, P.J.; Nishi, K.; Schnier, J.; Slonimski, P.P. Birth of the D-E-A-D box. *Nature* 1989, 337, 121–122. [CrossRef]
7. Blum, M.; Chang, H.-Y.; Chuguransky, S.; Grego, T.; Kandasamy, S.; Mitchell, A.; Nuka, G.; Paysan-Lafosse, T.; Qureshi, M.; Raj, S.; et al. The InterPro protein families and domains database: 20 years on. *Nucleic Acids Res.* 2020, 49, D344–D354. [CrossRef]
8. Mistry, J.; Chuguransky, S.; Williams, L.; Qureshi, M.; Salazar, G.A.; Sonnhammer, E.L.L.; Tosatto, S.C.E.; Paladin, L.; Raj, S.; Richardson, L.J.; et al. Pfam: The protein families database in 2021. *Nucleic Acids Res.* 2021, 49, D412–D419. [CrossRef]
9. Janeway, C.A. Approaching the Asymptote? Evolution and Revolution in Immunology. *Cold Spring Harb. Symp. Quant. Biol.* 1989, 54, 1–13. [CrossRef]
10. Linder, P. Dead-box proteins: A family affair—active and passive players in RNP-remodeling. *Nucleic Acids Res.* 2006, 34, 4168–4180. [CrossRef]
11. Setyono, B.; Van Steeg, H.; Voorma, H.O. Ultraviolet-crosslinking reveals specific affinity of eukaryotic initiation factors for Semliki Forest virus mRNA. *Biochim. Biophys. Acta* 1984, 782, 242–246. [CrossRef]
12. Browning, K.S.; Lax, S.R.; Humphreys, J.; Ravel, J.M.; Jobling, S.A.; Gehrkne, L. Evidence that the 5′-untranslated leader of mRNA affects the requirement for wheat germ initiation factors 4A, 4F, and 4G. *J. Biol. Chem.* 1988, 263, 9630–9634. [CrossRef]
13. Lax, S.R.; Browning, K.S.; Maia, D.M.; Ravel, J.M. ATPase activities of wheat germ initiation factors 4A, 4B, and 4F. *J. Biol. Chem.* 1986, 261, 15632–15636. [CrossRef]
14. Dratewka-Kos, E.; Kiss, I.; Lucas-Lenard, J.; Mehta, H.B.; Woodley, C.L.; Wahba, A.J. Catalytic utilization of eIF-2 and mRNA binding proteins are limiting in lysates from vesicular stomatitis virus infected L cells. *Biochemistry* 1984, 23, 6184–6190. [CrossRef] [PubMed]
15. Pelletier, J.; Sonenberg, N. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* **1988**, *334*, 320–325. [CrossRef] [PubMed]

16. Rogers, G.W., Jr.; Komar, A.A.; Merrick, W.C. elF4A: The godfather of the DEAD box helicases. *Prog. Nucleic Acid Res. Mol. Biol.* **2002**, *72*, 307–331. [CrossRef] [PubMed]

17. Pestova, T.V.; Kolupaeva, V.G.; Lomakin, I.B.; Pilipenko, E.V.; Shatsky, I.N.; Agol, V.I.; Hellen, C.U. Molecular mechanisms of translation initiation in eukaryotes. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 7029–7036. [CrossRef]

18. Su, M.J.; Babbianian, R. Polyadenylated RNA sequences from vaccinia virus-infected cells selectively inhibit translation in a cell-free system: Structural properties and mechanisms of inhibition. *Virology* **1990**, *179*, 679–693. [CrossRef]

19. Belsham, G.J.; Brangwyn, J.K. A region of the 5′ noncoding region of foot-and-mouth disease virus mRNA directs efficient internal initiation of protein synthesis within cells: Involvement with the role of L protease in translational control. *J. Virol.* **1990**, *64*, 5389–5395. [CrossRef]

20. Jang, S.K.; Kräusslich, H.G.; Nicklin, M.J.; Duke, G.M.; Palmenberg, A.C.; Wimmer, E. A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. *J. Virol.* **1988**, *62*, 2636–2643. [CrossRef]

21. Zhou, H.; Xu, M.; Huang, Q.; Gates, A.T.; Zhang, X.D.; Castle, J.C.; Stec, E.; Ferrer, M.; Strulovic, B.; Hazuda, D.J.; et al. Genome-scale RNAi screen for host factors required for HIV replication. *Cell Host Microbe* **2008**, *4*, 495–504. [CrossRef] [PubMed]

22. Biedenkopf, N.; Lange-Grünweiler, K.; Schulte, F.W.; Weißer, A.; Müller, C.; Becker, D.; Becker, S.; Hartmann, R.K.; Grünweiler, A. The natural compound silvestrol is a potent inhibitor of Ebola virus replication. *Antivir. Res.* **2017**, *137*, 76–81. [CrossRef] [PubMed]

23. Elgner, F.; Sabino, C.; Basic, M.; Ploen, D.; Grünweiler, A.; Hildt, E. Inhibition of Zika Virus Replication by Silvestrol. *Viruses* **2018**, *10*, 149. [CrossRef] [PubMed]

24. Park, S.H.; Lee, S.G.; Kim, Y.; Song, K. Assignment of a human putative RNA helicase gene, DDX3, to human X chromosome bands p11.3→p11.23. *Cytogenet. Cell Genet.* **1998**, *81*, 178–179. [CrossRef] [PubMed]

25. Chuang, R.-Y.; Weaver, P.L.; Liu, Z.; Chang, T.-H. Requirement of the DEAD-Box Protein Ded1p for Messenger RNA Translation. *Science* **1997**, 275, 1468–1471. [CrossRef] [PubMed]

26. Nougé, A.O.; Chen, J.; Akhound, P. A mutant allele of essential, general translation initiation factor DED1 selectively inhibits translation of a viral mRNA. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 12985–12990. [CrossRef] [PubMed]

27. Mamiya, N.; Worman, H.J. Hepatitis C Virus Core Protein Binds to a DEAD Box RNA Helicase. *J. Biol. Chem.* **1999**, *274*, 15751–15756. [CrossRef] [PubMed]

28. Owsianka, A.M.; Patel, A.H. Hepatitis C Virus Core Protein Interacts with a Human DEAD Box Protein DDX3. *Virology* **1999**, *257*, 330–340. [CrossRef] [PubMed]

29. You, L.-R.; Chen, C.-M.; Yeh, T.-S.; Tsai, T.-Y.; Mai, R.-T.; Lin, C.-H.; Lee, Y.-H.W. Hepatitis C Virus Core Protein Interacts with Cellular Putative RNA Helicase. *J. Virol.* **1999**, *73*, 2841–2853. [CrossRef]

30. Yedavalli, V.S.R.K.; Neuveut, C.; Chi, Y.-H.; Kleinman, L.; Jeang, K.-T. Requirement of DDX3 DEAD Box RNA Helicase for HIV-1 Rev-RRE Export Function. *Cell* **2004**, *119*, 381–392. [CrossRef] [PubMed]

31. Ariumi, Y.; Kuroki, M.; Abe, K.-i.; Dansako, H.; Ikeda, M.; Wakita, T.; Kato, N. DDX3 DEAD-Box RNA Helicase Is Required for Hepatitis C Virus RNA Replication. *Proc. Natl. Acad. Sci. USA* **2004**, 101, 13922-13926. [CrossRef] [PubMed]

32. Li, C.; Ge, L-L.; Li, P-P.; Wang, Y.; Dai, J-J.; Su, M.; Huang, L.; Shen, Z-Q.; Hu, X-C.; Ishag, H.; et al. Cellular DDX3 regulates Japanese encephalitis virus replication by interacting with viral un-translated regions. *Virology* **2014**, *449*, 70–81. [CrossRef] [PubMed]

33. Chahar, H.S.; Chen, S.; Manjunath, N. P-body components LSM1, GW182, DDX3, DDX6 and XRN1 are recruited to WNV replication sites and positively regulate viral replication. *Virology* **2013**, *436*, 1–7. [CrossRef]

34. Thulasiraman, S.N.; Liu, G.; Pyo, H.M.; Cui, Y.C.; Xu, F.; Ayalew, L.E.; Tikoo, S.K.; Zhou, Y. DDX3 Interacts with Influenza A Virus NS1 and NP Proteins and Exerts Antiviral Function through Regulation of Stress Granule Formation. *PLoS Pathog.* **2015**, *11*, e1007125. [CrossRef] [PubMed]

35. Loureiro, M.E.; Zorzetto-Fernandes, A.L.; Radoshitzky, S.; Chi, X.; Dallari, S.; Marooki, N.; Léger, P.; Foscaldi, S.; Harjono, V.; Sharma, S.; et al. DDX3 suppression type I interferons and favors viral replication during Arenavirus infection. *PLoS Pathog.* **2018**, *14*, e1007125. [CrossRef] [PubMed]

36. Valiente-Echeverría, F.; Hermoso, M.A.; Soto-Rífo, R. RNA helicase DDX3: At the crossroad of viral replication and antiviral immunity. *Rev. Med. Virol.* **2015**, *25*, 286–299. [CrossRef] [PubMed]

37. Schröder, M. Human DEAD-box protein 3 has multiple functions in gene regulation and cell cycle control and is a prime target for viral manipulation. *Biochem. Pharmacol.* **2010**, *79*, 297–306. [CrossRef] [PubMed]

38. Fullam, A.; Schröder, M. DEXD/H-box RNA helicases as mediators of anti-viral innate immunity and essential host factors for viral replication. *Biochem. Biophys. Acta (BBA) Gene Regul. Mech.* **2013**, *1829*, 854–865. [CrossRef] [PubMed]

39. Schröder, M.; Baran, M.; Bowie, A.G. Viral targeting of DEAD box protein 3 reveals its role in TBK1/IKKε-mediated IRF activation. *EMBO J.* **2008**, *27*, 2147–2157. [CrossRef] [PubMed]
Cells 2022, 11, 1608

41. Oshiumi, H.; Ikeda, M.; Matsumoto, M.; Watanabe, A.; Takeuchi, O.; Akira, S.; Kato, N.; Shimotohno, K.; Seya, T. Hepatitis C virus core protein abrogates the DDX3 function that enhances IPS-1-mediated IFN-beta induction. PLoS ONE 2010, 5, e14258. [CrossRef] [PubMed]

42. Gringhuis, S.I.; Hertoghs, N.; Kaptein, T.M.; Zijlstra-Willems, E.M.; Sarrami-Forooshani, R.; Sprokholt, J.K.; Teijlingen, N.H.V.; Koostra, N.A.; Boonman, T.; Dort, K.A.V.; et al. HIV-1 blocks the signaling adaptor MAVS to evade antiviral host defense after sensing of abortive HIV-1 RNA by the host helicase DDX3. Nat. Immunol. 2017, 18, 225–235. [CrossRef] [PubMed]

43. Kalverda, A.P.; Thompson, G.S.; Vogel, A.; Schröder, M.; Bowie, A.G.; Khan, A.R.; Homans, S.W. Poxvirus K7 Protein Adopts a Bcl-2 Fold: Biochemical Mapping of Its Interactions with Human DEAD Box RNA Helicase DDX3. J. Mol. Biol. 2009, 385, 843–853. [CrossRef] [PubMed]

44. Oda, S-I.; Schröder, M.; Khan, A.R. Structural Basis for Targeting of Human RNA Helicase DDX3 by Poxvirus Protein K7. Structure 2009, 17, 1528–1537. [CrossRef] [PubMed]

45. Wang, H.; Ryu, W.-S. Hepatitis B Virus Polymerase Blocks Pattern Recognition Receptor Signaling via Interaction with DDX3: Implications for Immune Evasion. PLoS Pathog. 2010, 6, e1000986. [CrossRef] [PubMed]

46. Yu, S.; Chen, J.; Wu, M.; Chen, H.; Kato, N.; Yuan, Z. Hepatitis B virus polymerase inhibits RIG-I- and Toll-like receptor 3-mediated beta interferon induction in human hepatocytes through interference with interferon regulatory factor 3 activation and dampening of the interaction between TBK1/IKKe and DDX3. J. Gen. Virol. 2010, 91, 2080–2090. [CrossRef]

47. Kesavardhana, S.; Samir, P.; Zheng, M.; Malireddi, R.K.S.; Karki, R.; Sharma, B.R.; Place, D.E.; Briard, B.; Vogel, P.; Kanneganti, T.-D. DDX3X coordinates host defense against influenza virus by activating the NLRP3 inflammasome and type 1 interferon response. J. Biol. Chem. 2021, 296, 100579. [CrossRef]

48. Wang, X.; Wang, R.; Luo, M.; Li, C.; Wang, H.X.; Huan, C.C.; Qu, Y.R.; Liao, Y.; Mao, X. (DEAD)-box RNA helicase 3 modulates NF-κB signal pathway by controlling the phosphorylation of PP2A-C subunit. Oncotarget 2017, 8, 33197–33213. [CrossRef]

49. Zhang, X.; Wang, C.; Schook, L.B.; Hawken, R.J.; Rutherford, M.S. An RNA helicase, RHHV-1, induced by porcine reproductive and respiratory syndrome virus (PRRSV) is mapped on porcine chromosome 10q13. Microb. Pathog. 2000, 28, 267–278. [CrossRef]

50. Kato, H.; Sato, S.; Yoneyama, M.; Yamamoto, M.; Uematsu, S.; Matsui, K.; Tsujimura, T.; Takeda, K.; Fujita, T.; Takeuchi, O.; et al. Cell Type-Specific Involvement of RIG-I in Antiviral Response. Immunity 2005, 23, 19–28. [CrossRef]

51. Sumpter, R.; Loo, Y.-M.; Foy, E.; Li, K.; Yoneyama, M.; Fujita, T.; Lemon, S.M.; Gale, M. Regulating Intracellular Antiviral Defense and Permissiveness to Hepatitis C Virus RNA Replication through a Cellular RNA Helicase, RIG-I. J. Virol. 2005, 79, 2689–2699. [CrossRef] [PubMed]

52. Yoneyama, M.; Kikuchi, M.; Natsukawa, T.; Shinobu, N.; Imaiizumi, T.; Miyagishi, M.; Taira, K.; Akira, S.; Fujita, T. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat. Immunol. 2004, 5, 730–737. [CrossRef] [PubMed]

53. Hornung, V.; Ellegast, J.; Kim, S.; Brzózka, K.; Jung, A.; Kato, H.; Poeck, H.; Akira, S.; Conzelmann, K.-K.; Schlee, M.; et al. 5′-Triphosphate RNA Is the Ligand for RIG-I. Science 2006, 314, 994–997. [CrossRef]

54. Pichlmair, A.; Schulz, O.; Tan, C.P.; Näslund, T.I.; Liljeström, P.; Weber, F.; Reis e Sousa, C. RIG-I-Mediated Antiviral Responses to Single-Stranded RNA Bearing 5′-Triphosphate RNA Is the Ligand for RIG-I. Cell 2006, 129, 997–1001. [CrossRef] [PubMed]

55. Shatkin, A.J. Capping of eucaryotic mRNAs. Cell 1976, 9, 645–653. [CrossRef]

56. Kawai, T.; Takahashi, K.; Sato, S.; Coban, C.; Kumar, H.; Kato, H.; Ishii, K.J.; Takeuchi, O.; Akira, S. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat. Immunol. 2005, 6, 981–988. [CrossRef] [PubMed]

57. Seth, R.B.; Sun, L.; Ea, C.-K.; Chen, Z.J. Identification and Characterization of MAVS, a Mitochondrial Antiviral Signaling Protein that Activates NF-κB and IRF3. J. Gen. Virol. 2005, 86, 2080–2090. [CrossRef] [PubMed]

58. Kowalinski, E.; Lunardi, T.; McCarthy, A.A.; Louber, J.; Brunel, J.; Grigorov, B.; Gerlier, D.; Cusack, S. Structural Basis for the Sensing of Abortive HIV-1 RNA by the Host Helicase DDX3. Cell 2010, 142, 669–682. [CrossRef]

59. Luo, D.; Ding, S.C.; Vela, A.; Kohlway, L.; Lindenbach, B.D.; Pyle, A.M. Structural Insights into RNA Recognition by RIG-I. Cell 2011, 147, 409–422. [CrossRef]

60. Samir, P.; Kanneganti, T.-D. Hidden Aspects of Valency in Immune System Regulation. Trends Immunol. 2019, 40, 1082–1094. [CrossRef]

61. Li, P.; Banjade, S.; Cheng, H.-C.; Kim, S.; Chen, B.; Guo, L.; Llaguno, M.; Hollingsworth, J.V.; King, D.S.; Banani, S.F.; et al. Phase transitions in the assembly of multivalent signalling proteins. Nature 2012, 483, 336–340. [CrossRef] [PubMed]

62. Samir, P.; Malireddi, R.K.S.; Kanneganti, T.-D. The PANoptosome: A Deadl y Protein Complex Driving Pyroptosis, Apoptosis, and Necroptosis (PANoptosis). Front. Cell. Infect. Microbiol. 2020, 10, 238. [CrossRef] [PubMed]

63. Xu, L.; Khadjiah, S.; Fang, S.; Wang, L.; Tay, F.P.L.; Liu, D.X. The Cellular RNA Helicase DDX1 Interacts with Coronavirus Nonstructural Protein 14 and Enhances Viral Replication. J. Virol. 2010, 84, 8571–8583. [CrossRef] [PubMed]

64. Wu, C.-H.; Chen, P.-J.; Yeh, S.-H. Nucleocapsid Phosphorylation and RNA Helicase DDX1 Recruitment Enables Coronavirus Transition from Discontinuous to Continuous Transcription. Cell Host Microbe 2014, 16, 462–472. [CrossRef]

65. Zhang, Z.; Kim, T.; Bao, M.; Facchinetti, V.; Jung, S.Y.; Ghaffari, A.A.; Qin, J.; Cheng, G.; Liu, Y.-J. DDX1, DDX21, and DHX36 Helicases Form a Complex with the Adaptor Molecule TRIF to Sense dsRNA in Dendritic Cells. Immunity 2011, 34, 866–878. [CrossRef] [PubMed]
66. Li, C.; Ge, L.-L.; Li, P.-P.; Wang, Y.; Sun, M.-X.; Huang, L.; Ishag, H.; Di, D.-D.; Shen, Z.-Q.; Fan, W.-X.; et al. The DEAD-box RNA helicase DDX5 acts as a positive regulator of Japanese encephalitis virus replication by binding to viral 3′ UTR. *Antivir. Res.* **2013**, *100*, 487–499. [CrossRef]

67. Diot, C.; Fournier, G.; Dos Santos, M.; Magnus, J.; Komarova, A.; van der Werf, S.; Munier, S.; Naffakh, N. Influenza A Virus Polymerase Recruits the RNA Helicase DDX19 to Promote the Nuclear Export of Viral mRNAs. *Sci. Rep.* **2016**, *6*, 33763. [CrossRef]

68. Xu, Z.; Anderson, R.; Hobman, T.C. The Capsid-Binding Nucleolar Helicase DDX56 Is Important for Infectivity of West Nile Virus. *J. Virol.* **2011**, *85*, 5571–5580. [CrossRef]

69. Xu, Z.; Hobman, T.C. The helicase activity of DDX56 is required for its role in assembly of infectious West Nile virus particles. *Virology* **2012**, *433*, 226–235. [CrossRef]

70. Li, D.; Fu, S.; Wu, Z.; Yang, W.; Yu, Y.; Shu, H.; Liu, X.; Zheng, H. DDX56 inhibits type I interferon by disrupting assembly of IRF3–IP05 to inhibit IRF3 nucleus import. *J. Cell Sci.* **2020**, *133*, 290409. [CrossRef]

71. Zhu, S.; Ding, S.; Wang, P.; Wei, Z.; Pan, W.; Palm, N.W.; Yang, Y.; Yu, H.; Li, H.-B.; Wang, G.; et al. Nlrp9b inflammasomes restrict rotavirus infection in intestinal epithelial cells. *Nature* **2017**, *546*, 667–670. [CrossRef] [PubMed]

72. Kim, T.; Pazhoor, S.; Bao, M.; Zhang, Z.; Hanabuchi, S.; Facchinetti, V.; Bover, L.; Plumas, J.; Chaperot, L.; Qin, J.; et al. Aspartate-glutamate-alanine-histidine box motif (DEAH)/RNA helicase A helicases sense microbial RNA in human plasmacytoid dendritic cells. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 15181–15186. [CrossRef] [PubMed]

73. Mitoma, H.; Hanabuchi, S.; Kim, T.; Bao, M.; Zhang, Z.; Sugimoto, N.; Liu, Y.-J. The DDX33 RNA Helicase Senses Cytosolic RNA and Activates the NLRP3 Inflammasome. *Immunity* **2013**, *39*, 123–135. [CrossRef]

74. Li, J.; Hu, L.; Liu, Y.; Huang, L.; Mu, Y.; Cai, X.; Weng, C. DDX19A Senses Viral RNA and Mediates NLRP3-Dependent Inflammasome Activation. *J. Immunol.* **2015**, *195*, 5732–5747. [CrossRef] [PubMed]

75. Wang, P.; Zhu, S.; Yang, L.; Cui, S.; Pan, W.; Jackson, R.; Zheng, Y.; Rongvaux, A.; Sun, Q.; Yang, G.; et al. Nlrp6 regulates intestinal antiviral innate immunity. *Science* **2015**, *350*, 826–830. [CrossRef] [PubMed]

76. Shen, C.; Li, R.; Negro, R.; Cheng, J.; Vora, S.M.; Fu, T.M.; Wang, A.; He, K.; Andreeva, L.; Gao, P.; et al. Phase separation drives RNA virus-induced activation of the NLRP6 inflammasome. *Cell* **2021**, *184*, 5759–5774. [CrossRef]

77. Pattabhi, S.; Knoll, M.L.; Gale, M.; Loo, Y.-M. DHX15 Is a Coreceptor for RLR Signaling That Promotes Antiviral Defense Against RNA Virus Infection. *J. Interferon Cytokine Res.* **2019**, *39*, 331–346. [CrossRef]

78. Zhang, Z.; Yuan, B.; Bao, M.; Lu, N.; Kim, T.; Liu, Y.-J. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat. Immunol.* **2011**, *12*, 959–965. [CrossRef]

79. Soulat, D.; Burckstummer, T.; Westermayer, S.; Goncalves, A.; Bauch, A.; Stefanovic, A.; Hantschel, O.; Bennett, K.L.; Decker, T.; Superti-Furga, G. The DEAD-box helicase DDX3X is a critical component of the TANK-binding kinase 1-dependent innate immune response. *EMBO J.* **2008**, *27*, 2135–2146. [CrossRef]

80. Parvatiyar, K.; Zhang, Z.; Teles, R.M.; Ouyang, S.; Jiang, Y.; Iyer, S.S.; Zaver, S.A.; Schenk, M.; Zeng, S.; Zhong, W.; et al. The helicase DDX41 recognizes the bacterial secondary messengers cyclic di-GMP and cyclic di-AMP to activate a type I interferon immune response. *J. Interferon Cytokine Res.* **2015**, *39*, 2135–2146. [CrossRef] [PubMed]

81. Lee, S.; Karki, R.; Wang, Y.; Nguyen, L.N.; Kalathur, R.C.; Kanneganti, T.D. AIM2 forms a complex with pyrin and ZBP1 to drive RNA virus-induced activation of the NLRP6 inflammasome. *Cell* **2021**, *184*, 5759–5774. [CrossRef] [PubMed]

82. Li, J.; Hu, L.; Liu, Y.; Huang, L.; Mu, Y.; Cai, X.; Weng, C. DDX19A Senses Viral RNA and Mediates NLRP3-Dependent Inflammasome Activation. *J. Immunol.* **2015**, *195*, 5732–5747. [CrossRef] [PubMed]

83. Hogbom, M.; Collins, R.; van den Berg, S.; Jenvert, R.M.; Karlberg, T.; Kotenyova, T.; Flores, A.; Karlsson Hedestam, G.B.; Decker, T.; Superti-Furga, G. The DEAD-box helicase DDX3X is a critical component of the TANK-binding kinase 1-dependent innate immune response. *EMBO J.* **2008**, *27*, 2135–2146. [CrossRef]

84. Schutz, P.; Karlberg, T.; van den Berg, S.; Collins, R.; Lettio, H.; Hogbom, M.; Holmborg-Schiavone, L.; Tempel, W.; Park, H.W.; Hammarstrom, M.; et al. Comparative structural analysis of human DEAD-box RNA helicases. *PLoS ONE* **2010**, *5*, e12791. [CrossRef]

85. Nagata, S.; Tanaka, M. Programmed cell death and the immune system. *Nat. Rev. Immunol.* **2017**, *17*, 333–340. [CrossRef]

86. Cohen, J.J.; Duke, R.C.; Fadok, V.A.; Sellins, K.S. Apoptosis and Programmed Cell Death in Immunology. *Annu. Rev. Immunol.* **1992**, *10*, 267–293. [CrossRef]

87. Riera Romo, M. Cell death as part of innate immunity: Cause or consequence? *Immunology* **2021**, *163*, 399–415. [CrossRef]

88. Malireddi, R.K.S.; Kesavardhana, S.; Kanneganti, T.D. ZBP1 and TAK1: Master Regulators of NLRP3 Inflammasome/Pyroptosis, Apoptosis, and Necroptosis (PNAP). *Front. Cell. Infect. Microbiol.* **2019**, *9*, 406. [CrossRef]

89. Ketelut-Carneiro, N.; Fitzgerald, K.A. Apoptosis, Pyroptosis, and Necroptosis—Oh My! The Many Ways a Cell Can Die. *J. Mol. Biol.* **2021**, *434*, 167378. [CrossRef]

90. Newton, K.; Dixit Vishva, M.; Kayagaki, N. Dying cells fan the flames of inflammation. *Science* **2021**, *374*, 1076–1080. [CrossRef]

91. Wang, Y.; Kanneganti, T.-D. From pyroptosis, apoptosis and necroptosis to PANoptosis: A mechanistic compendium of programmed cell death pathways. *Comput. Struct. Biotechnol. J.* **2021**, *19*, 4641–4657. [CrossRef] [PubMed]

92. Kuriaiko, T.; Man, S.M.; Malireddi, R.K.; Karki, R.; Kesavardhana, S.; Place, D.E.; Neale, G.; Vogel, P.; Kanneganti, T.D. ZBP1/DAI is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways. *Sci. Immunol.* **2016**, *1*, aag2045. [CrossRef] [PubMed]
93. Malireddi, R.K.S.; Karki, R.; Sundaram, B.; Kancharana, B.; Lee, S.; Samir, P.; Kanneganti, T.D. Inflammatory Cell Death, PANoptosis, Mediated by Cytokines in Diverse Cancer Lineages Inhibits Tumor Growth. *Immunohorizons* 2021, 5, 568–580. [CrossRef] [PubMed]

94. Kesavardhana, S.; Malireddi, R.K.S.; Burton, A.R.; Porter, S.N.; Vogel, P.; Pruett-Miller, S.M.; Kanneganti, T.-D. The Zα2 domain of ZBP1 is a molecular switch regulating influenza-induced PANoptosis and perinatal lethality during development. *J. Biol. Chem.* 2020, 295, 8325–8330. [CrossRef]

95. Banoth, B.; Tuladhar, S.; Karki, R.; Sharma, B.R.; Briard, B.; Kesavardhana, S.; Burton, A.; Kanneganti, T.-D. ZBP1 promotes fungi-induced inflammasome activation and pyroptosis, apoptosis, and necroptosis (PA(N)optosis). *J. Biol. Chem.* 2020, 295, 18276–18283. [CrossRef]

96. Christgen, S.; Zheng, M.; Kesavardhana, S.; Karki, R.; Malireddi, R.K.S.; Banoth, B.; Place, D.E.; Briard, B.; Sharma, B.R.; Tuladhar, S.; et al. Identification of the PANoptosome: A molecular platform triggering pyroptosis, apoptosis, and necroptosis (PANoptosis). *Front. Cell. Infect. Microbiol.* 2020, 10, 237. [CrossRef]

97. Karki, R.; Sharma, B.R.; Lee, E.; Banoth, B.; Malireddi, R.K.S.; Samir, P.; Tuladhar, S.; Mummareddy, H.; Burton, A.R.; Vogel, P.; et al. Interferon regulatory factor 1 regulates PANoptosis to prevent colorectal cancer. *ICI Insight* 2020, 5, 136720. [CrossRef]

98. Zheng, M.; Williams, E.P.; Malireddi, R.K.S.; Karki, R.; Banoth, B.; Burton, A.; Webby, R.; Channappanavar, R.; Jonsson, C.B.; Kanneganti, T.-D. Impaired NLRP3 inflammasome activation/pyroptosis leads to robust inflammatory cell death via caspase-8/RIPK3 during coronavirus infection. *J. Biol. Chem.* 2020, 295, 14040–14052. [CrossRef]

99. Gurung, P.; Burton, A.; Kanneganti, T.-D. NLRP3 inflammasome plays a redundant role with caspase 8 to promote IL-1β–mediated osteomyelitis. *Proc. Natl. Acad. Sci. USA* 2016, 113, 4452–4457. [CrossRef]

100. Lukens, J.R.; Gurung, P.; Vogel, P.; Johnson, G.R.; Carter, R.A.; McGoldrick, D.J.; Bandi, S.R.; Calabrese, C.R.; Walle Vande, L.; Lamkanfi, M.; et al. Dietary modulation of the microbiome affects autoinflammatory disease. *Nature* 2014, 516, 246–249. [CrossRef]

101. Malireddi, R.K.; Ippagunta, S.; Lamkanfi, M.; Kanneganti, T.D. Cutting edge: Proteolytic inactivation of poly(ADP-ribose) polymerase 1 by the Nlrp3 and Nlrc4 inflammasomes. *J. Immunol.* 2010, 185, 3127–3130. [CrossRef] [PubMed]

102. Malireddi, R.K.S.; Gurung, P.; Kesavardhana, S.; Samir, P.; Burton, A.; Mummareddy, H.; Vogel, P.; Pelletier, S.; Burgula, S.; Kanneganti, T.-D. Innate immune priming in the absence of TAK1 drives RIPK1 kinase activity–independent pyroptosis, apoptosis, and necroptosis, and inflammatory disease. *J. Exp. Med.* 2020, 217, e20191644. [CrossRef] [PubMed]

103. Malireddi, R.K.S.; Kesavardhana, S.; Karki, R.; Kancharana, B.; Burton, A.R.; Kanneganti, T.D. RIPK1 Distinctly Regulates PANoptosis by Independently Mediating the TLR3 and TLR7 Pathways to Enhance PANoptosis and IL-1β Production. *Cell Death Differ.* 2020, 27, 573–587. [CrossRef] [PubMed]

104. Zheng, M.; Karki, R.; Samir, P.; Burton, A.; Kanneganti, T.D. Caspase-6 Is a Key Regulator of Innate Immunity, Inflammasome Activation, and Cell Death. *J. Immunol.* 2020, 205, 149–168.e117. [CrossRef] [PubMed]

105. Karki, R.; Sharma, B.R.; Tuladhar, S.; Williams, E.P.; Zalduondo, L.; Samir, P.; Zheng, M.; Sundaram, B.; Banoth, B.; Malireddi, R.K.S.; et al. Synergism of TNF-α and IFN-γ Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Shock Syndromes. *Cell* 2021, 184, 149–168.e117. [CrossRef] [PubMed]

106. Malireddi, R.K.S.; Gurung, P.; Mavuluri, J.; Dasari, T.K.; Klco, J.M.; Chi, H.; Kanneganti, T.D. TAK1 restricts spontaneous NLRP3 activation and cell death to control myeloid proliferation. *J. Exp. Med.* 2018, 215, 1023–1034. [CrossRef] [PubMed]

107. Lamkanfi, M.; Kanneganti, T.D.; Van Damme, P.; Vanden Berghe, T.; Vanoverberghe, I.; Vandekerckhove, J.; Vandenaebbelee, P.; Gevaert, K.; Nunez, G. Targeted peptidecentric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes. *J. Exp. Med.* 2014, 217, 2379–2391. [CrossRef] [PubMed]

108. Gurung, P.; Burton, A.; Kanneganti, T.-D. NLRP3 inflammasome plays a redundant role with caspase 8 to promote IL-1β–mediated osteomyelitis. *Proc. Natl. Acad. Sci. USA* 2016, 113, 4452–4457. [CrossRef]

109. Lukens, J.R.; Gurung, P.; Vogel, P.; Johnson, G.R.; Carter, R.A.; McGoldrick, D.J.; Bandi, S.R.; Calabrese, C.R.; Vande Walle, L.; Lamkanfi, M.; et al. Dietary modulation of the microbiome affects autoinflammatory disease. *Nature* 2014, 516, 246–249. [CrossRef]

110. Malireddi, R.K.; Ippagunta, S.; Lamkanfi, M.; Kanneganti, T.D. Cutting edge: Proteolytic inactivation of poly(ADP-ribose) polymerase 1 by the Nlrp3 and Nlrc4 inflammasomes. *J. Immunol.* 2010, 185, 3127–3130. [CrossRef] [PubMed]

111. Malireddi, R.K.S.; Gurung, P.; Kesavardhana, S.; Samir, P.; Burton, A.; Mummareddy, H.; Vogel, P.; Pelletier, S.; Burgula, S.; Kanneganti, T.-D. Innate immune priming in the absence of TAK1 drives RIPK1 kinase activity–independent pyroptosis, apoptosis, and necroptosis, and inflammatory disease. *J. Exp. Med.* 2020, 217, e20191644. [CrossRef] [PubMed]

112. Malireddi, R.K.S.; Kesavardhana, S.; Karki, R.; Kancharana, B.; Burton, A.R.; Kanneganti, T.D. RIPK1 Distinctly Regulates PANoptosis by Independently Mediating the TLR3 and TLR7 Pathways to Enhance PANoptosis and IL-1β Production. *Cell Death Differ.* 2020, 27, 573–587. [CrossRef] [PubMed]

113. Samir, P.; Kanneganti, T.-D. DDX3X Sits at the Crossroads of Liquid–Liquid and Prionoid Phase Transitions Arbitrating Life and Death. *DNA Cell Biol.* 2020, 39, 1091–1095. [CrossRef] [PubMed]

114. Besch, R.; Poeck, H.; Hohenauer, T.; Senft, D.; Häcker, G.; Berking, C.; Hornung, V.; Endres, S.; Ruzicka, T.; Rothenfusser, S.; et al. Proapoptotic signaling induced by RIG-I and MDA-5 results in type I interferon–independent apoptosis in human melanoma cells. *J. Clin. Investig.* 2009, 119, 2399–2411. [CrossRef]

115. Peng, S.; Geng, J.; Sun, R.; Tian, Z.; Wei, H. Polysinosinic-polyctydidylic acid liposome induces human hepatoma cells apoptosis which correlates to the up-regulation of RIG-I like receptors. *Cancer Sci.* 2009, 100, 529–536. [CrossRef]
116. Li, Y.; Song, Y.; Li, P.; Li, M.; Wang, H.; Xu, T.; Yu, X.; Yu, Y.; Tai, Y.; Chen, P.; et al. Downregulation of RIG-I mediated by ITGB3/c-SRC/STAT3 signaling confers resistance to interferon-α-induced apoptosis in tumor-repopulating cells of melanoma. *J. Immunothe. Cancer* 2020, 8, e001111. [CrossRef]

117. Chowdhari, S.; Saini, N. Gene expression profiling reveals the role of RIG1 like receptor signaling in p53 dependent apoptosis induced by PUVA in keratinocytes. *Cell. Signal.* 2016, 28, 25–33. [CrossRef]

118. Chattopadhyay, S.; Sen, G.C. RIG-I-like receptor-induced IRF3 mediated pathway of apoptosis (RIPA): A new antiviral pathway. *Protein Cell* 2017, 8, 165–168. [CrossRef]

119. Peters, K.; Chattopadhyay, S.; Sen, G.C. IRF-3 Activation by Sendai Virus Infection Is Required for Cellular Apoptosis and Avoidance of Persistence. *J. Virol.* 2008, 82, 3500–3508. [CrossRef]

120. Chattopadhyay, S.; Yamashita, M.; Zhang, Y.; Sen, G.C. The IRF-3/Bax-Mediated Apoptotic Pathway, Activated by Viral Cytoplasmic RNA and DNA, Inhibits Virus Replication. *J. Virol.* 2011, 85, 3708–3716. [CrossRef]

121. White, C.L.; Chattopadhyay, S.; Sen, G.C. Phosphatidylinositol 3-Kinase Signaling Delays Sendai Virus-Induced Apoptosis by Preventing XIAP Degradation. *J. Virol.* 2011, 85, 5224–5227. [CrossRef] [PubMed]

122. Poeck, H.; Bseieder, M.; Cross, O.; Finger, K.; Roth, S.; Rebsamen, M.; Hannenschläger, N.; Schlee, M.; Rothenfusser, S.; Barchet, W.; et al. Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1β production. *Nat. Immunol.* 2010, 11, 63–71. [CrossRef] [PubMed]

123. Pothlichet, J.; Meunier, I.; Davis, B.K.; Ting, J.Y.; Skamene, E.; von Messling, V.; Vidal, S.M. Type I IFN Triggers RIG-I/TLR3/NLRP3-dependent Inflammasome Activation in Influenza A Virus Infected Cells. *PLoS Pathog.* 2013, 9, e1003256. [CrossRef] [PubMed]

124. Fu, W.; Verma, D.; Burton, A.; Swaminathan, S. Cellular RNA Helicase DHX9 Interacts with the Essential Epstein-Barr Virus (EBV) Protein SM and Restricts EBV Lytic Replication. *J. Virol.* 2019, 93, e0244-18. [CrossRef] [PubMed]

125. Styles, C.T.; Paschos, K.; White, R.E.; Farrell, P.J. The Cooperative Functions of the EBNA3 Proteins Are Central to EBV Persistence and Latency. *Pathogens* 2018, 7, 31. [CrossRef]

126. Yang, L.; Lin, C.; Sun, S.Y.; Zhao, S.; Liu, Z.R. A double tyrosine phosphorylation of P68 RNA helicase confers resistance to TRAIL-induced apoptosis. *Oncogene* 2007, 26, 6082–6092. [CrossRef]

127. Wu, C.F.; Ganetzky, B.; Jan, L.Y.; Jan, Y.N.; Benzer, S. A Drosophila mutant with a temperature-sensitive block in nerve conduction. *Proc. Natl. Acad. Sci. USA* 1978, 75, 4047–4051. [CrossRef]

128. Belote, J.M.; Lucchesi, J.C. Control of X chromosome transcription by the maleless gene in Drosophila. *Nature* 1980, 285, 573–575. [CrossRef]

129. Fukunaga, A. Sterility in D. melanogaster due to nucleocytoplasmic interactions. *J. Hered.* 1980, 71, 349–352. [CrossRef]

130. Lee, C.G.; Hurwitz, J. A new RNA helicase isolated from HeLa cells that catalytically translocates in the 3′ to 5′ direction. *J. Biol. Chem.* 1992, 267, 4398–4407. [CrossRef]

131. Lee, C.G.; Hurwitz, J. Human RNA helicase A is homologous to the maleless protein of Drosophila. *J. Biol. Chem.* 1993, 268, 16822–16830. [CrossRef]

132. Lee, C.-G.; Soares, V.d.C.; Newberger, C.; Manova, K.; Lacy, E.; Hurwitz, J. RNA helicase A is homologous to the maleless protein of Drosophila. *Proc. Natl. Acad. Sci. USA* 1995, 92, 13709–13713. [CrossRef] [PubMed]

133. Csink, A.K.; Linsk, R.; Birchler, J.A. The Lighten up (Lip) gene of Drosophila melanogaster, a modifier of retroelement expression, position effect variegation and white locus insertion alleles. *Genetics* 1994, 138, 153–163. [CrossRef] [PubMed]

134. Dardenne, E.; Polay Espinoza, M.; Fattet, L.; Germann, S.; Lambert, M.P.; Neil, H.; Zonta, E.; Mortada, H.; Gratadou, L.; Deygas, M.; et al. RNA helicases DDX5 and DDX17 dynamically orchestrate transcription, miRNA, and splicing programs in cell differentiation. *Cell Rep.* 2014, 7, 1900–1913. [CrossRef]

135. Wang, S.A.-O.; Narendran, S.A.-O.; Hirahara, S.; Varshney, A.; Pereira, F.A.-O.; Apicella, I.; Ambati, M.; Ambati, V.L.; Yerramothu, P.; Ambati, K.; et al. DDX17 is an essential mediator of sterile NLRC4 inflammasome activation by retrotransposon RNAs. *Sci. Immunol.* 2021, 6, abi4493. [CrossRef]

136. Patmore, D.M.; Jassim, A.; Nathan, E.; Gilbertson, R.J.; Tahan, D.; Hoffmann, N.; Tong, Y.; Smith, K.S.; Kanneganti, T.D.; Suzuki, H.; et al. DDX3X Suppresses the Susceptibility of Hindbrain Lineages to Medulloblastoma. *Dev. Cell* 2013, 22, 2532–2542. [CrossRef]

137. Szappanos, D.A.-O.; Tschismarov, R.; Perlot, T.; Westermayer, S.; Fischer, K.; Platanitis, E.; Kallinger, F.; Novatchkova, M.; Lassnig, C.; Müller, M.A.-O.; et al. The RNA helicase DDX3X is an essential mediator of innate antimicrobial immunity. *PLoS Pathog.* 2018, 14, e1007397. [CrossRef]

138. Wu, S.F.; Xia, L.; Shi, X.D.; Dai, Y.J.; Zhang, W.N.; Zhao, J.M.; Zhang, W.; Weng, X.A.-O.; Lu, J.; Le, H.Y.; et al. RIG-I regulates myeloid differentiation by promoting TRIM25-mediated ISGylation. *Proc. Natl. Acad. Sci. USA* 2020, 117, 14395–14404. [CrossRef]

139. Hirabayashi, R.; Hozumi, S.; Higashijima, S.-I.; Kikuchi, Y. Ddx46 is required for multi-lineage differentiation of hematopoietic stem cells in zebras. *Stem Cells Dev.* 2013, 22, 2532–2542. [CrossRef]

140. Payne, E.M.; Bolli, N.; Rhodes, J.; Abdel-Wahab, O.I.; Levine, R.; Hedvat, C.V.; Stone, R.; Khanna-Gupta, A.; Sun, H.; Kanki, J.P.; et al. Ddx18 is essential for cell-cycle progression in zebrafish hematopoietic progenitors and is mutated in human AML. *Blood* 2011, 118, 903–915. [CrossRef]
141. Kellaris, G.; Khan, K.; Baig, S.M.; Tsai, I.C.; Zamora, F.M.; Ruggieri, P.; Natowitz, M.R.; Katsanis, N. A hypomorphic inherited pathogenic variant in DXD3X causes male intellectual disability with additional neurodevelopmental and neurodegenerative features. *Hum. Genom.* 2018, 12, 11. [CrossRef] [PubMed]

142. Scala, M.; Torella, A.; Severino, M.; Morana, G.; Castello, R.; Accogli, A.; Verrico, A.; Vari, M.S.; Cappuccio, G.; Pinelli, M.; et al. Three de novo DXD3X variants associated with distinctive brain developmental abnormalities and brain tumor in intellectually disabled females. *Eur. J. Hum. Genet.* 2019, 27, 1254–1259. [CrossRef] [PubMed]

143. Lennox, A.L.; Hoye, M.L.; Jiang, R.; Johnson-Kerner, B.L.; Suit, L.A.; Venkataramanan, S.; Sheehan, C.J.; Alsina, F.C.; Fregeau, B.; Aldinger, K.A.; et al. Pathogenic DXD3X Mutations Impair RNA Metabolism and Neurogenesis during Fetal Cortical Development. *Neuron* 2020, 106, 404–420.e408. [CrossRef] [PubMed]

144. Bloom, D. The syndrome of congenital telangiectatic erythema resembling lupus erythematosus in dwarfs; probably a syndrome entity. *AMA Am. J. Dis. Child.* 1954, 88, 754–758. [CrossRef]

145. Mohaghegh, P.; Karow, J.K.; Brosh, R.M., Jr.; Bohr, V.A.; Hickson, I.D. The Bloom’s and Werner’s syndrome proteins are DNA helicases. *EMBO J.* 2000, 19, 3428–3435. [CrossRef]

146. Fry, M.; Loeb, L.A. Human Werner syndrome DNA helicase unwinds tetrahelical structures of the fragile X syndrome repeat sequence d(CGG)n. *Nucleic Acids Res.* 2001, 29, 2843–2849. [CrossRef] [PubMed]

147. Gray, M.D.; Shen, J.C.; Kamath-Loeb, A.S.; Blank, A.; Sopher, B.L.; Martin, G.M.; Oshima, J.; Loeb, L.A. The Werner syndrome protein is a DNA helicase. *Nat. Genet.* 1997, 17, 100–103. [CrossRef] [PubMed]

148. Wang, W.; Seki, M.; Narita, Y.; Sonoda, E.; Takeda, S.; Yamada, K.; Masuko, T.; Katada, T.; Enomoto, T. Possible association of BLM in decreasing DNA double strand breaks during DNA replication. *EMBO J.* 2000, 19, 3428–3435. [CrossRef]

149. Iglesias-Pedraz, J.M.; Fossatti-Jara, D.M.; Valle-Riestra-Felice, V.; Cruz-Visalaya, S.R.; Ayala Felix, J.A.; Comai, L. WRN modulates BLM in decreasing DNA double strand breaks during DNA replication. *EMBO J.* 2000, 19, 3428–3435. [CrossRef]

150. Su, C.Y.; Lin, T.C.; Lin, Y.F.; Chen, M.H.; Lee, C.H.; Wang, H.Y.; Lee, Y.C.; Liu, Y.P.; Chen, C.L.; Hsiao, M. DDX3 as a strongest DDX3X biomarker correlates with poor survival in Human Gliomas. *Int. J. Mol. Sci.* 2015, 16, 15578–15591. [CrossRef] [PubMed]

151. Wu, D.W.; Lee, M.C.; Wang, J.; Chen, C.Y.; Cheng, Y.W.; Lee, H. DXD3 loss by p53 inactivation promotes tumor malignancy via the MDM2/Slug/E-cadherin pathway and poor patient outcome in non-small-cell lung cancer. *Oncogene* 2014, 33, 1515–1526. [CrossRef]

152. Karow, J.K.; Constantinou, A.; Li, J.L.; West, S.C.; Hickson, I.D. The Bloom’s syndrome gene product promotes branch migration of Holliday junctions. *Proc. Natl. Acad. Sci. USA* 2000, 97, 6004–6008. [CrossRef] [PubMed]

153. Imamura, O.; Fujita, K.; Itoh, C.; Takeda, S.; Furuichi, Y.; Matsumoto, T. Werner and Bloom helicases are involved in DNA repair in a complementary fashion. *Oncogene* 2000, 21, 954–963. [CrossRef] [PubMed]

154. McDaniel, L.D.; Schultz, R.A. Elevated sister chromatid exchange phenotype of Bloom syndrome cells is complemented by BRCA2. *Science* 1994, 262, 424–429. [CrossRef]

155. Iglesias-Pedraz, J.M.; Fossatti-Jara, D.M.; Valle-Riestra-Felice, V.; Cruz-Visalaya, S.R.; Ayala Felix, J.A.; Comai, L. WRN modulates BRCA2 in decreasing DNA double strand breaks during DNA replication. *EMBO J.* 2000, 19, 3428–3435. [CrossRef] [PubMed]

156. Snijders Blok, L.; Madsen, E.; Juusola, J.; Gilissen, C.; Baralle, D.; Reijnders, M.R.; Venselaar, H.; Helsmoortel, C.; Cho, M.T.; van der Wall, E.; et al. Identification of the DEAD box RNA helicase DDX3 as a therapeutic target in colorectal cancer. *Oncotarget* 2015, 6, 28312–28326. [CrossRef]

157. Heyne, H.O.; Hoischen, A.; et al. Mutations in DDX3X Are a Common Cause of Unexplained Intellectual Disability with Gender-Specific Features. *Nat. Genet.* 2018, 50, 1048–1053. [CrossRef] [PubMed]

158. Karow, J.K.; Constantinou, A.; Li, J.L.; West, S.C.; Hickson, I.D. The Bloom’s syndrome gene product promotes branch migration of Holliday junctions. *Proc. Natl. Acad. Sci. USA* 2000, 97, 6004–6008. [CrossRef] [PubMed]

159. Koeleman, B.P.; et al. De novo variants in neurodevelopmental disorders with epilepsy. *Nat. Genet.* 2018, 50, 1048–1053. [CrossRef] [PubMed]

160. Huang, D.Y.; Tsai, W.C.; Chiou, H.Y.; Feng, S.W.; Lin, C.; Li, Y.F.; Huang, L.C.; Lin, M.H. DDX3X biomarker correlates with poor survival in Human Gliomas. *Int. J. Mol. Sci.* 2015, 16, 15578–15591. [CrossRef] [PubMed]

161. Lennox, A.L.; Hoye, M.L.; Jiang, R.; Johnson-Kerner, B.L.; Suit, L.A.; Venkataramanan, S.; Sheehan, C.J.; Alsina, F.C.; Fregeau, B.; Aldinger, K.A.; et al. Pathogenic DXD3X Mutations Impair RNA Metabolism and Neurogenesis during Fetal Cortical Development. *Neuron* 2020, 106, 404–420.e408. [CrossRef] [PubMed]

162. Lee, M.C.; Wang, J.; Cheng, Y.W.; Lee, H. DXD3 loss by p53 inactivation promotes tumor malignancy via the MDM2/Slug/E-cadherin pathway and poor patient outcome in non-small-cell lung cancer. *Oncogene* 2014, 33, 1515–1526. [CrossRef]
167. Tantravedi, S.; Vesuna, F.; Winnard, P.T., Jr.; Martin, A.; Lim, M.; Eberhart, C.G.; Berlinicke, C.; Raabe, E.; van Diest, P.J.; Raman, V. Targeting DDX3 in Medulloblastoma Using the Small Molecule Inhibitor RK-33. *Transl. Oncol.* 2019, 12, 96–105. [CrossRef]

168. Xie, M.; Vesuna, F.; Tantravedi, S.; Bol, G.M.; Heerma van Voss, M.R.; Nugent, K.; Malek, R.; Gabrielson, K.; van Diest, P.J.; Tran, P.T.; et al. RK-33 Radiosensitizes Prostate Cancer Cells by Blocking the RNA Helicase DDX3. *Cancer Res.* 2016, 76, 6340–6350. [CrossRef]

169. Samal, S.K.; Routray, S.; Veeramachaneni, G.K.; Dash, R.; Botlagunta, M. Ketorolac salt is a newly discovered DDX3 inhibitor to treat oral cancer. *Sci. Rep.* 2015, 5, 9882. [CrossRef]

170. Epling, L.B.; Grace, C.R.; Lowe, B.R.; Partridge, J.F.; Enemark, E.J. Cancer-associated mutants of RNA helicase DDX3X are defective in RNA-stimulated ATP hydrolysis. *J. Mol. Biol.* 2015, 427, 1779–1796. [CrossRef]

171. Valentin-Vega, Y.A.; Wang, Y.D.; Parker, M.; Patmore, D.M.; Kanagaraj, A.; Moore, J.; Rusch, M.; Finkelstein, D.; Ellison, D.W.; Gilbertson, R.J.; et al. Cancer-associated DDX3X mutations drive stress granule assembly and impair global translation. *Sci. Rep.* 2016, 6, 25996. [CrossRef] [PubMed]

172. Roux, P.P.; Topisirovic, I. Regulation of mRNA translation by signaling pathways. *Cold Spring Harb. Perspect. Biol.* 2012, 4, a012252. [CrossRef] [PubMed]

173. Buttgereit, F.; Brand, M.D. A hierarchy of ATP-consuming processes in mammalian cells. *Biochem. J.* 1995, 312 Pt 1, 163–167. [CrossRef] [PubMed]

174. Vogt, P.H.; Besikoglu, B.; Bettendorf, M.; Frank-Herrmann, P.; Zimmer, J.; Bender, U.; Knauer-Fischer, S.; Choukair, D.; Sinn, P.; Lau, Y.C.; et al. Gonadoblastoma Y locus genes expressed in germ cells of individuals with dysgenetic gonads and a Y chromosome in their karyotypes include DDX3Y and TSPY. *Hum. Reprod.* 2019, 34, 770–779. [CrossRef]

175. Gueler, B.; Sonne, S.B.; Zimmer, J.; Hilscher, B.; Hilscher, W.; Graem, N.; Rajpert-De Meyts, E.; Vogt, P.H. AZFa protein DDX3Y is differentially expressed in human male germ cells during development and in testicular tumours: New evidence for phenotypic plasticity of germ cells. *Hum. Reprod.* 2012, 27, 1547–1555. [CrossRef]

176. Foresta, C.; Ferlin, A.; Moro, E. Deletion and expression analysis of AZFa genes on the human Y chromosome revealed a major role for DBY in male infertility. *Hum. Mol. Genet.* 2000, 9, 1161–1169. [CrossRef]

177. Pinero, J.; Ramirez-Anguita, J.M.; Sauch-Pitarch, J.; Ronzano, F.; Centeno, E.; Sanz, F.; Furlong, L.I. The DisGeNET knowledge platform for disease genomics: 2019 update. *Nucleic Acids Res.* 2020, 48, D845–D855. [CrossRef]

178. Hondele, M.; Sachdev, R.; Heinrich, S.; Wang, J.; Vallotton, P.; Fontoura, B.M.A.; Weis, K. DEAD-box ATPases are global regulators of phase-separated organelles. *Nature* 2019, 573, 144–148. [CrossRef]

179. Sarkar, M.; Ghosh, M.K. DEAD box RNA helicases: Crucial regulators of gene expression and oncogenesis. *Front. Biosci.* 2016, 21, 225–250. [CrossRef]

180. Arul Nambi Rajan, A.; Montpetit, B. Emerging molecular functions and novel roles for the DEAD-box protein Dbp5/DDX19 in gene expression. *Cell. Mol. Life Sci.* 2021, 78, 2019–2030. [CrossRef]

181. Zhang, L.; Li, X. DEAD-Box RNA Helicases in Cell Cycle Control and Clinical Therapy. *Cells* 2021, 10, 1540. [CrossRef] [PubMed]

182. Cargill, M.; Venkataraman, R.; Lee, S. DEAD-Box RNA Helicases and Genome Stability. *Genes* 2021, 12, 1471. [CrossRef] [PubMed]

183. Felsenstein, J. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution* 1985, 39, 783–791. [CrossRef]

184. Nei, M.; Kumar, S. *Molecular Evolution and Phylogenetics*; Oxford University Press: New York, NY, USA, 2000.

185. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* 2018, 35, 1547–1549. [CrossRef]