Review

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The DEAH helicase *DHX36* and its role in G-quadruplex-dependent processes

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Abstract: *DHX36* is a member of the DExD/H box helicase family, which comprises a large number of proteins involved in various cellular functions. Recently, the function of *DHX36* in the regulation of G-quadruplexes (G4s) was demonstrated. G4s are alternative nucleic acid structures, which influence many cellular pathways on a transcriptional and post-transcriptional level. In this review we provide an overview of the current knowledge about *DHX36* structure, substrate specificity, and mechanism of action based on the available models and crystal structures. Moreover, we outline its multiple functions in cellular homeostasis, immunity, and disease. Finally, we discuss the open questions and provide potential directions for future research.

Keywords: immunity; transcription; translation.

Introduction: structure and function of G-quadruplexes

G-quadruplexes (G4s) are secondary structures that can form in nucleic acid sequences with a high guanine content. In the presence of at least four runs of two consecutive guanines, separated by usually 1–12 other nucleotides, the guanines can associate via Hoogsteen base pairing (N1-H-O6 and C2-N-H-N7) to form stacks of planar tetrads (Figure 1A) (Arnott et al. 1974; Gellert et al. 1962). The thermodynamic stability of this fold is positively influenced by the number of possible stacks and negatively by the length of the spacer sequences, which extend as loops from the structure (Tippana et al. 2014). A monovalent cation, preferably K^+, is coordinated in the center between two tetrads and further stabilizes the G4 (Figure 1A, C–F) (Sen and Gilbert 1990; Williamson et al. 1989).

G4 taxonomy is defined by the 5′-3′ directionality of the involved strands, which depends on the torsion angle of the χ-glycosidic bond between ribose and guanine. In the *syn*-conformation, the base is oriented towards the sugar and in the *anti*-conformation it faces in the opposite direction (Figure 1B). In parallel G4s, guanines are coordinated in an *anti*-conformation and all strands are aligned in the same direction, while in antiparallel structures only the strands on opposite sides are in the same orientation (Figures 1C, D). Lastly, the hybrid type constitutes a mixed assembly of parallel and anti-parallel strands (Figure 1E) (Esposito et al. 2007). Of note, most naturally occurring RNA G4s (rG4) are of the parallel type, because the charged 2′-hydroxyl interferes with the *syn*-orientation of the base (Fay et al. 2017), which is not the case for DNA. Apart from unimolecular G4s, a variety of intermolecular structures is also possible (Figure 1F). So far, the relevance of G4 topology *in vivo* has not been determined.

Computational prediction algorithms for potential quadruplex-forming sequences (PQS) and G4-seq revealed more than 360,000 and 700,000 PQS in the human genome, respectively (Chambers et al. 2015; Huppert and Balasubramanian 2005; Marsico et al. 2019). A subset (~10 000) of these was validated by ChIPseq experiments (Hänsel-Hertsch et al. 2016). Moreover, rG4-seq analysis identified ~13,000 PQS in the human transcriptome (Kwok et al. 2016). While individual structural confirmation of many of these sites has not been achieved, their conservation and location at functional regions indicate their importance. PQS are overrepresented at promoters (Eddy et al. 2011; Huppert and Balasubramanian 2006), telomeres (Henderson et al. 1987) and 5′ and 3′ UTRs (Huppert et al. 2008; Maltby et al. 2019; Rouleau et al. 2017). Finally, PQS were found in almost all species including pathogens, such as bacteria and viruses (Saranathan and Vivekanandan 2018), which further strengthens the assumption that G4s function as an ancient regulatory element.

In addition to their evolutionary conservation, genetic and molecular analysis suggests defined cellular functions

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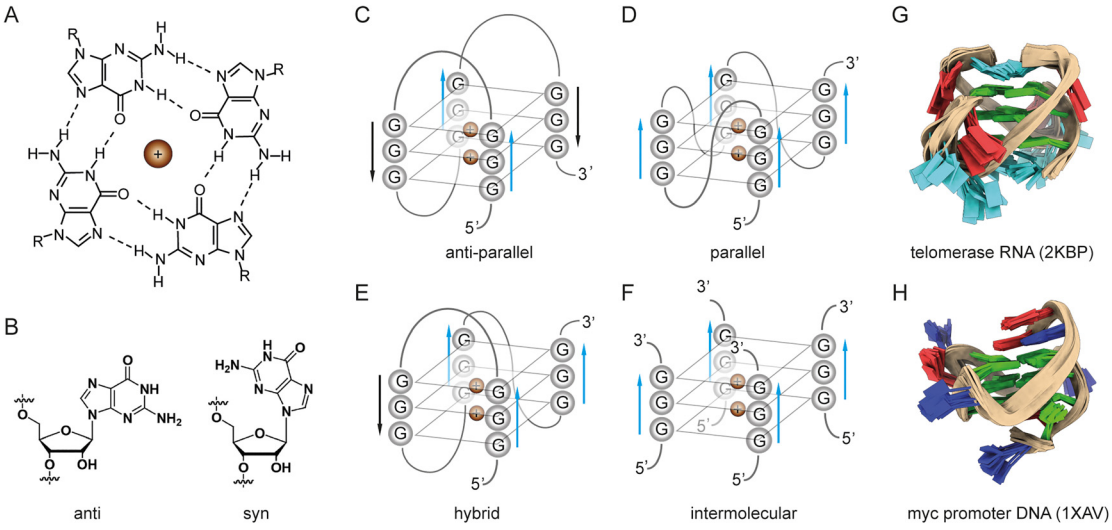

Figure 1: Graphical illustrations of G-quadruplex structures.
(A) Schematic representation of a planar tetrad formed by four guanines with a central monovalent cation (brown circle). (B) Anti and syn conformation of guanosine. (C–F) Examples of possible G-quadruplex topologies. (G, H) NMR solution structures of the G4 in the 5′ region of telomeric RNA and in the promoter region of myc. 3D models were created with ChimeraX. PDB entries are given in parentheses.

for a subset of G4s (Rhodes and Lipps 2015). Due to their stability, and the finding that B-DNA is the preferred conformation within cells, the current model is that G4 formation, function and unfolding is assisted by proteins. Many DNA- and RNA-binding proteins were identified to associate with G4s (Brázda et al. 2014; Sauer and Paeschke 2017; Serikawa et al. 2018). Among these proteins, several helicases have been identified that recognize and unwind G4s *in vitro* and *in vivo* (Sauer and Paeschke 2017).

Many of them belong to the group of DExD/H box helicases (Table 1) (Sauer and Paeschke 2017), a large family of enzymes that share a conserved helicase domain. The C- and N-terminal regions adjacent to this common core are highly divergent and confer specificity for their individual targets. DExD/H helicases perform roles in almost all cellular processes. Apart from their canonical activity of adenosine triphosphate (ATP)-dependent RNA and DNA unwinding they may act as RNA chaperones and in modulating ribonucleoprotein (RNP) complexes (Tanner and Linder 2001). Prominent members include *DHX9* (also RHA, NDHII), which has a wide range of functions from transcription regulation, RNA processing and foreign nucleic acid sensing (Aktaş et al. 2017; Fuller-Pace 2006; Fullam and Schröder 2013; Kim et al. 2010) and *DDX5* which regulates miRNA processing, splicing as well as anti-viral signaling (Cheng et al. 2018; Dardenne et al. 2014). Another important cellular regulator of the G4 landscape is *DHX36* (Creacy et al. 2008; Sauer et al. 2019; Vaughn et al. 2005).

Table 1: Known G4-specific DExD/H box helicases in human cells and their cellular function.

Protein	G4-dependent function	Specificity	Evidence
BLM	Regulation of DNA replication	DNA	Helicase assays (Huber et al. 2002)
DDX5	Expression of myc	RNA	ELISA, CD (Wu et al. 2019)
DHX9	Regulation of transcription	RNA, DNA	Helicase assays (Chakraborty and Grosse 2011)
DDX1	IgG class switch	RNA, DNA	EMSA, pull down (Almeida et al. 2018)
DDX21	Regulation of translation	RNA	Pull down, helicase assays (McRae et al. 2017)
DHX36	Regulation of translation/ transcription	RNA, DNA	PAR-CLIP (Sauer et al. 2019)
FANCJ	Regulation of transcription	DNA	EMSA, helicase assay (Wu and Spies 2016)

DHX36 structure and enzymatic functions

DHX36 was first discovered as a protein binding to AU-rich elements (AREs) and supporting RNA deadenylation and degradation (Tran et al. 2004). Intriguingly, it was demonstrated that it binds to G4s with extraordinarily high affinity for RNA G4s(~39 pM) and DNA G4s (~77 pM) (Creacy et al. 2008). *DHX36* unwinds G4 structures with a much higher efficiency than double-stranded

(ds) DNA (Vaughn et al. 2005; Yangyuoru et al. 2017). The catalytic activity of *DHX36* is dependent on the thermostability of the G4. More stable G4s are unwound much slower by *DHX36*, which may fine-tune the regulatory functions of G4s *in vivo* (Chen et al. 2015). *DHX36* has a specific N-terminal region which harbors a Gly-rich region and a conserved nucleic acid-binding motif (*DHX36*-specific motif [DSM]; Figure 2A, B) (Chalupníková et al. 2008; M.C. Chen et al. 2018; W.-F. Chen et al. 2018). The latter was identified as the region conferring the G4-binding properties of *DHX36* (Lattmann et al. 2010; Meier et al. 2013). In this study it was also shown that the N-terminal domain has two helices (α1 and α2) followed by an unstructured linker region (Figure 2B). The N-terminal helix α1 is positioned on top of the terminal G-plane of a G4 structure creating a hydrophobic interface exposing nonpolar amino acids (Figure 2C). The exposed hydrophobic surface of the G-tetrad is thermodynamically unfavorable in solution. This is mitigated by enzyme binding and provides a possible explanation for the high target affinity of *DHX36*. Moreover, *DHX36* exhibits a strong preference towards binding parallel G4s (Smaldino et al. 2015; Yangyuoru et al. 2017). One reason might be that antiparallel or hybrid types expose polar surfaces via the loops masking the terminal G-planes, which reduces the entropic benefit and may sterically hinder DSM binding. Although important for G4 interaction, the N-terminal domain is not sufficient to disrupt the G4, which is only possible in combination with the conserved DExD/H helicase domain (Figure 2A, B). The N-terminus confers further target specificity and is essential for enzymatic function and subcellular localization (Chalupníková et al. 2008; M.C. Chen et al. 2018; W.-F. Chen et al. 2018; Srinivasan et al. 2020).

Like all members of the DExD/H family, the core helicase portion is comprised of two RecA-like folds (*RecA1* and *2*; Figure 2A, B). These are defined by their ATPase structure of a central beta sheet surrounded by alpha helices. Within the beta strands are the conserved Walker A (GxxxxGKT/S; XXXX: variable quartett) and Walker B (hhhhD; h: hydrophobic amino acid) motifs, which coordinate the γ-phosphate of an ATP and a Mg^{2+} ion, respectively. The hydrolysis of ATP leads to a significant rearrangement of the helicase, from a closed to an open state, in which RecA2 is rotated away from RecA1. This movement relocates RecA2 relative to the template by one nucleotide and flips the 5th nucleotide in the channel so that it stacks against a conserved β-hairpin (M.C. Chen et al. 2018; W.-F. Chen et al. 2018).

The winged-like (WL), ratchet-like (RL), and oligosaccharide-binding-fold-like (OL) subdomains (Figure 2A, B) make additional contacts to the single-stranded (ss) nucleic acid within the binding channel, but are not significantly rearranged during this movement (M.C. Chen et al. 2018; W.-F. Chen et al. 2018).

Solving the structure of *DHX36* from different species with and without bound G4, highlighted the role of exposed polar amino acids of the *RecA2* subdomain in interacting with the phosphate backbone of the G4 (M.C. Chen et al. 2018; W.-F. Chen et al. 2018). A distal loop of the OL region (OI) was also suggested to be involved in G4 positioning by interacting with the first single-stranded nucleotide

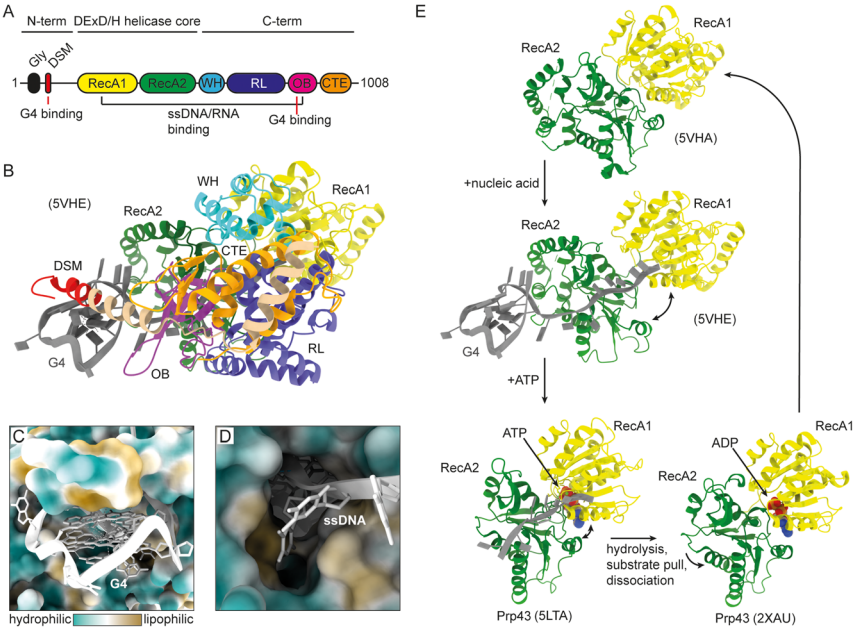

Figure 2: Graphical illustration of *DHX36*. (A) Schematic of the domain organization of human DHX36. (B) Crystal structure of human *DHX36* in complex with myc G4 DNA. (C) Detailed view on the hydrophobic interface between G4 and DSM. (D) View inside the substrate tunnel with bound ssDNA. (E) Simplified model of *DHX36* enzyme activity exemplified by movements of the helicase core. Note that structures of the related helicase Prp43 were chosen as surrogate for the ATP and adenosine diphosphate (ADP) bound states, as these are not yet available for *DHX36*. PDB entries are given in parentheses.

preceding the G4 (M.C. Chen et al. 2018; W.-F. Chen et al. 2018). The nucleic acid: *DHX36* interface in the single-stranded section adjacent to the G4 is mainly formed by hydrogen bonding of the DSM and the OI with the phosphate backbone (M.C. Chen et al. 2018; W.-F. Chen et al. 2018). This may explain the substrate promiscuity of *DHX36* for binding RNA, DNA, or even chimeric targets (Tippana et al. 2019). Together, these parts of *DHX36* form a pocket with charged and hydrophobic surfaces enclosing the G4 structure.

The template channel of *DHX36* can only accommodate single-stranded nucleic acids (Figure 2D) (M.C. Chen et al. 2018; W.-F. Chen et al. 2018), but the binding affinity to unstructured targets is low and sequence-dependent (Creacy et al. 2008; Giri et al. 2011; Yangyuoru et al. 2017). G4s are recognized with similar affinity, whether in conjunction with a single-stranded region or by itself (M.C. Chen et al. 2018; W.-F. Chen et al. 2018). In contrast, the helicase activity of *DHX36* requires a 3′ tail of 9 nt (Tippana et al. 2016). 5′-tailed G4s are not unfolded efficiently by *DHX36* indicating its 3′–5′ helicase activity (Gueddouda et al. 2017). This is corroborated by the fact that positioning the ss DNA/RNA within the enzyme is crucial for the domain rearrangement upon ATP hydrolysis (Gueddouda et al. 2017; Tippana et al. 2019; Yangyuoru et al. 2017). However, the structures for the ATP + nucleic acid to adenosine diphosphate (ADP) state transition have not been determined, yet, but were inferred from structures of related enzymes.

In conclusion, the current model for G4 disruption is as follows: initial binding is achieved by contacts at the G4 structure and single-stranded downstream sequences, which leads to considerable translocations in the RecA-like and OB subdomains. The motion from the ground to nucleic-acid-induced transition-state positions the 5th nucleotide at the RecA2 hairpin and the OI-loop at the first nucleotide downstream of the G4 (M. C. Chen et al. 2018; W.-F. Chen et al. 2018; Srinivasan et al. 2020; Tippana et al. 2016). This was reported to destabilize the G4 structure by exerting a pulling force on the sugar-phosphate backbone of the stack in an ATP-independent fashion (M.C. Chen et al. 2018; W.-F. Chen et al. 2018). However, this interpretation was challenged, because the utilized folding conditions might have caused the observed distortion of the G4 (Guo et al. 2019). Based on model predictions and studies of related enzymes it was suggested that ATP-binding results in the transition to a closed state of RecA1 and A2 reorienting the RecA2 hairpin back to its original position (M.C. Chen et al. 2018; W.-F. Chen et al. 2018; He et al. 2010). ATP hydrolysis enables a RecA domain shift that pulls the template in a 3′–5′ direction through the nucleic acid tunnel, one nucleotide at a time (Figure 2E) (W.-F. Chen et al. 2018). This repetitive motion can be observed in single-molecule FRET experiments (Tippana et al. 2019).

DHX36 also features a C-terminal extension (CTE, Figure 2A). It has been observed that rotation of the CTE is necessary for target binding and enzymatic activity of several DEAH helicases, in contrast to other families (Chen and Ferré-D'Amaré 2017). The CTE is conserved among orthologues of *DHX36* in different species to a higher degree than the N-terminus (Lattmann et al. 2010), but its function has not been studied, yet. This domain and its biological relevance are of particular interest, because one unsolved question in the G4 helicase field is why almost all helicases can unwind G4 structures and how these helicases gain their specificity. Of note, the closest mammalian homolog of the CTE is found in *YTHDC2*, another DExD/H helicase (Bailey et al. 2017; Hazra et al. 2019), which recognizes methylated adenosines (m6A). These data, together with the finding that G4s are proposed to be target structures that facilitate m6A modifications (Fleming et al. 2019), leads to the speculation that *DHX36* might also recognize specific G4s that are m6A modified.

DHX36 is a master regulator of cellular homeostasis

G4s modulate various cellular functions (Bochman et al. 2012; Spiegel et al. 2019). Accordingly, molecular and genetic experiments have been performed to understand the biological function and relevance of *DHX36* binding to G4s. These analyses point towards a model that *DHX36* is a multifunctional helicase that acts on DNA and RNA G4s and supports various biological pathways. *DHX36* is expressed in two isoforms and can be localized in the nucleus and cytoplasm (Iwamoto et al. 2008; Sauer et al. 2019). The predominant localization seems to depend on the cell type and the expressed isoform (Iwamoto et al. 2008; Sauer et al. 2019; Tran et al. 2004). Functionally, the isoforms have also been shown to affect certain target mRNAs differently (Booy et al. 2016; Tran et al. 2004).

DHX36 has been associated with transcriptional activation of several genes involved in cell growth and differentiation by binding and resolving G4s in their respective promoters (Chashchina et al. 2019; Gao et al. 2015; Giri et al. 2011; Huang et al. 2011; Iwamoto et al. 2008; Kim et al. 2011; Schlag et al. 2020). This is of importance, because actively transcribed DNA is temporarily in a single-stranded state and therefore prone to form secondary structures. Once formed within promoters, G4 can either decrease

transcription by blocking DNA polymerase II (PolII) (Eddy et al. 2011), or support transcription by recruiting proteins that effect transcription itself (Cogoi et al. 2010; Raiber et al. 2011). Apart from the interrupted transcription, an internal arrest of PolII has been associated with genomic instability via a topoisomerase 2-dependent mechanism (Magis et al. 2018; Pipier et al. 2020). This suggests a critical role of *DHX36* in maintaining genomic integrity under physiological and stress conditions. One of the major guardians of genome stability is *TP53* (or p53). *TP53* blocks mitotic activity as soon as genomic instability is detected and thereby prevents excessive mutation of the genome upon replication (Williams and Schumacher 2016). It was demonstrated that *DHX36* controls the *TP53*-dependent DNA damage response, which is activated upon ultraviolet (UV) light-induced DNA cross-linking or disruption (Newman et al. 2017). *DHX36* interacts with a G4 in the 3′-UTR polyadenylation site of the *TP53* pre-mRNA and stimulates 3′-end processing, which leads to selective ongoing protein production (Newman et al. 2017).

Another line of evidence connecting *DHX36* function to genome stability is based on its involvement in telomere maintenance. Telomeres are the terminal structures of most eukaryotic chromosomes that protect chromosomes from degradation, end-to-end joining or recognition as double-strand breaks (DSBs) among other functions (Blackburn et al. 2015; Lange 2009). Telomeres are maintained by telomerase, a reverse transcriptase harboring an internal RNA template for the synthesis of telomeric repeats. In the 5′ portion of this RNA, a stem-loop structure (P1) is providing the template boundary for accurate termination of transcription (Chen et al. 2000; Webb and Zakian 2015). Due to its high guanine-content, a G4 can also form within this region, which was shown to disrupt the P1 helix (Gros et al. 2008; Li et al. 2007). Due to the central role of P1, modifications such as structure formation within this region are predicted to alter telomerase function and accuracy. These findings lead to the model that G4 formation within the P1 helix required unwinding to ensure the proper template boundary. Indeed, G4 formation and *DHX36* are both connected to telomere length maintenance, because disruption of the G4 motif as well as down regulation of *DHX36* by siRNA lead to reduced telomerase function and consequently shortened telomeres (Sexton and Collins 2011). DHX36 has been shown to interact with G4s within the telomerase RNA (Booy et al. 2012; Lattmann et al. 2011; Sexton and Collins 2011). Moreover, the current paradigm also suggests G4s as a protective cap to regulate telomerase binding to telomeres (Jurikova et al. 2020). *DHX36* and other helicases can unfold these G4s and by this modulate telomerase function (Smaldino et al. 2015).

A large body of evidence has been gathered for the functions of *DHX36* in post-transcriptional regulation, such as translation and RNA degradation (Murat et al. 2018; Nie et al. 2015; Tran et al. 2004). In a global approach it has been shown that G4-containing mRNAs are located to cytoplasmic stress granules (SGs) upon external stress signals (e.g., arsenite) (Sauer et al. 2019). *DHX36* is recruited to SGs and releases target mRNAs, which then become translational competent (Vester et al. 2018). Moreover, *DHX36* knock-out cells are prone to form SGs, even without external stressors (Sauer et al. 2019). Therefore, it can be assumed that *DHX36* is necessary to reestablish cellular homeostasis after stress. To this end, it is also noteworthy that only a subset of cytoplasmic RNAs are actively targeted to SG upon stress conditions (Sauer et al. 2019).

Most of these RNAs have been found to contain AREs, which may control their recruitment by an interaction with the proteins TIA/TIAR, as well as GC-rich stretches prone to form G4s (Namkoong et al. 2018). This may indeed provide another potential clue for *DHX36* target recognition within SGs, considering that AREs were the first specific sequence motifs associated with the helicase. This is corroborated by a stronger activation of ATPase activity upon binding to single-stranded U-rich oligonucleotides *in vitro*, compared to other sequences (Tran et al. 2004).

Despite higher translation levels, *DHX36* target mRNAs also exhibited decreased stability (Sauer et al. 2019). It has not been clarified, whether this is merely due to the release from SGs, where stored RNAs are protected from decay by association with protein complexes (Decker and Parker 2012), or whether *DHX36* plays an active role in fate determination of these mRNAs.

Considering the enrichment of ARE-containing mRNAs in SGs, a mechanism that may explain the dichotomy of increased translation versus reduced stability (Sauer et al. 2019) is exemplified by the regulation of the Nkx2-5 mRNA. Here, the resolution of a G4 in the 5′ UTR leads to higher translation, while binding of *DHX36* to an ARE in the 3′ UTR targets the mRNA for degradation (Nie et al. 2015). This supports other studies, which found *DHX36*-binding associated with 3′–5′ exosome activation after binding to AREs in the urokinase-type plasminogen activator (uPA) mRNA (Tran et al. 2004). *DHX36* also interacts with the microRNA (miR) machinery on several levels to destabilize target mRNAs (Bicker et al. 2013; Booy et al. 2013). Interestingly, under steady-state conditions, target transcript

stability seems to be less affected by a *DHX36* knock-down, suggesting different regulatory mechanisms involving specific factors (Iwamoto et al. 2008).

Disease potential of *DHX36* mutation, dysregulation or loss

DHX36 is involved in the regulation of many crucial pathways for cellular maintenance. *TP53* is a key protein for the control of genome replication upon DNA damage and stress conditions and is regulated by *DHX36* in a post-transcriptional manner (Newman et al. 2017), as discussed above. Hence, loss of *DHX36* may decrease *TP53* levels in response to genome instability leading to an increased mutation rate during DNA replication.

In addition to MYC (a family of regulatory genes and proto-oncogenes that encode for transcription factors) (Giri et al. 2011), the tyrosine kinase KIT complements the panel of proto-oncogenes that contain G4 motifs in their promoters (Rankin et al. 2005). These genes are critically involved in cell growth and therefore need to be tightly regulated. There is evidence that MYC promoter activity is modulated by a G4. However, while *DHX36* can unwind this structure *in vitro*, it is currently not clear if it is directly involved in MYC expression *in vivo*. In contrast, for KIT a *DHX36*-dependent upregulation of transcription has been reported (Gao et al. 2015).

Previously, it was discovered that *DHX36* is recruited and inhibited by G4 structures of the long non-coding RNA (lncRNA) GSEC (G-quadruplex-forming sequence containing lncRNA), which leads to high motility of colon cancer cells (Matsumura et al. 2016). Moreover, GSEC was found to be upregulated in various other cancer types, which may suggest a common *DHX36*-dependent mechanism in carcinogenesis (Matsumura et al. 2016). Accordingly, *DHX36* has been classified as an oncogene (Davoli et al. 2013) and is upregulated in multiple human cancers (Thul and Lindskog 2017) and cancer cell lines (Nusinow et al. 2020). This may hint towards a so far unexplored roles of *DHX36* in these pathologies.

DHX36 and other helicases may link G4s and antiviral immune responses

G4s are overrepresented in most viral genomes (Lavezzo et al. 2018), but their impact on antiviral immunity remains elusive. Formation of antiviral stress granules (avSG) has been shown to be a common consequence of virus infection (Tsai and Lloyd 2014). In combination with the involvement of *DHX36* in SG regulation discussed above, these findings raise the questions, if G4 formation during viral infection affects *DHX36* expression and function.

Differential regulation of *DHX36* among other conserved DExD/H helicases was shown in zebrafish and carp after viral infection (Mojzesz et al. 2020). This is in agreement with the fact that viral RNA (vRNA) and its surrogate poly(I:C) are effectively sensed by a number of DExD/H helicases (including *DHX36*), which facilitate maximal efficiency of the subsequent immune response (Fullam and Schröder 2013). One of these complexes consists of *RIG-I* and *DHX36* (Yoo et al. 2014). vRNA binding triggers autophosphorylation and activation of the double-stranded RNA-dependent protein kinase PKR. The latter depends on the ATPase activity of *DHX36*, which indicates helicase activity (Yoo et al. 2014). Direct *DHX36* binding to several segments of the Influenza virus genome has been reported to influence *RIG-I*-dependent avSG formation (Yoo et al. 2014). However, a direct involvement of G4s has not been examined, yet.

In murine dendritic cells (mDCs) *DHX36* is found in a multi-helicase complex with *DDX1* and *DDX21*, which acts as a cytoplasmic sensor of viral double-stranded RNA (dsRNA) (Zhang et al. 2011). *DDX1* seems to be mainly responsible for dsRNA binding, whereas *DHX36* and *DDX21* do not directly interact with poly(I:C) but are necessary for the antiviral response (Zhang et al. 2011). Importantly, all these helicases are known for their capability to bind and unwind G4s (Almeida et al. 2018; Creacy et al. 2008; McRae et al. 2017). However, also in this case a clear link to viral G4s remains to be established.

Lastly, *DHX36* together with *DHX9* is involved in the detection of pathogenic DNA in the cytoplasm of human DCs. This was demonstrated by transfections with synthetic mimics of microbial CpG islands (CpG-A and B) and with a DNA virus (Herpes Simplex Virus-1, HSV-1) (Kim et al. 2010). CpG-A is detected by *DHX36*, while *DHX9* recognizes CpG-B (Kim et al. 2010). CpG-A oligonucleotides are characterized by flanking oligo-G stretches (5′-GGGGGACGATCGTCGGG-GGG-3′) in contrast to CpG-B (5′-TCGTCGTTTTGTCGTTTTGT-CGTT-3′). Accordingly, CpG-A oligonucleotides form highly stable intermolecular quadruplexes (Kerkmann et al. 2005), which could link G4s to *DHX36*-specific innate immune responses. Concomitantly, the *DHX36*-dependent activity against HSV-1 (Kim et al. 2010) and the high amount of PQS in the HSV-1 genome (Biswas et al. 2016) may point in the same direction. Additional research is needed to find further clues if and how G4s of pathogens trigger a *DHX36*-dependent immune activation.

Potential direct *DHX36* interactions with viruses

Apart from governing antiviral innate immune responses, *DHX36* may also have a direct influence on the viral life cycle, as many related DExH/D helicases do (Biegel et al. 2017; Cheng et al. 2018; Grünvogel et al. 2015; Matkovic et al. 2018). However, there is only sparse information available in current literature about such interactions and any functional information is lacking (Calderone et al. 2014).

In addition to the interaction with some of the Influenza A virus genome segments, *DHX36* was also shown to bind directly to the Influenza nucleoprotein (NP), non-structural protein 1 (NS1) and the polymerase subdomain (PA) by co-immunoprecipitation (Wang et al. 2017). Another connection was found with the nucleocapsid protein of the porcine reproductive and respiratory syndrome virus (PRRSV) (Jourdan et al. 2012). *DHX36* also binds to the Rev response element (RRE) of HIV, a highly structured RNA region that controls nuclear export of unspliced viral mRNAs (Naji et al. 2011). Although the RRE contains several G-rich sequences, no G4 has been validated, yet. HIV relies on its nucleocapsid protein to destabilize G4s in its genome for efficient reverse transcription (Butovskaya et al. 2019). The viral reverse transcriptase is stalled by G4s, which may lead to a template switch and recombination events associated with immune evasion (Shen et al. 2009). Finally, several conserved G4s were detected in the promoters of lentiviruses, which control gene transcription in a similar fashion as cellular genes (Perrone et al. 2013, 2017). Due to these findings it is tempting to speculate that *DHX36* may also modulate these processes.

In conclusion, a vast amount of knowledge is still to be gained by investigating the host:pathogen interface of *DHX36*.

Perspectives and open questions

DHX36 is a well-characterized protein, but due to its multitude of functions and the fact that G4 biology has only recently gained its current level of interest, the cellular characterization of *DHX36* is far from complete. It is still unclear, which molecular role the N-terminal glycine-rich region or the C-terminal extension play in the enzymatic activity. Notably, the glycine-rich stretch is completely absent in *Drosophila* (Lattmann et al. 2010) and binding of the human enzyme to G4s is not dependent on this segment (M.C. Chen et al. 2018; W.-F. Chen et al. 2018; Lattmann et al. 2010). However, it affects the intracellular localization to SG in human cells (Chalupníková et al. 2008), which may suggest species-dependent protein:protein interactions. Similarly, the function of the CTE is entirely unstudied. The high sequence similarity among species points towards a conserved biological role (Lattmann et al. 2010).

Furthermore, the question of the molecular determinants for target specificity is not finally answered. The structure and charge-based binding model for G4s can explain the preference of *DHX36* for certain types of G4s, but additional sequence specificity for the downstream single stranded region seems likely. As U-rich sequences show enhanced binding and ATPase activation (Tran et al. 2004), it may be interesting to assess whether consensus patterns can guide *DHX36* to specific targets. This will also contribute towards the understanding of the regulation of other G4-binding proteins. Along this line it will be interesting to address how the associated 3′single-stranded regions and loops, as well as the topology of bound G4s (Tippana et al. 2014) may change their recognition by different complexes and thereby determine specific downstream effects.

Apart from these fundamental questions, future research should also investigate the influence of *DHX36* on regulatory networks of oncogenesis. Foremost, global analyses will be required investigating transcription and translation levels of known oncogenes in *DHX36*-depleted cells. In this context it would be interesting to clarify how naturally occurring mutations of *DHX36* are involved in cell transformation. Moreover, large gaps remain in the knowledge about direct interactions of *DHX36* with viral nucleic acids and its role in viral infection. Considering the involvement of *DHX36* in several immune sensor complexes (Fullam and Schröder 2013; Yoo et al. 2014; Zhang et al. 2011), makes *DHX36* a prime candidate for further investigations.

Finally, the discovery of a link between G4 structure and chemical modifications has opened a whole new field of research. It will be interesting to learn how these pathways are functionally linked and if *DHX36* may shape the landscape of the epitranscriptome.

In conclusion, *DHX36* is an important regulator of many cellular pathways and several open questions remain to be answered, which will contribute greatly to our understanding of tissue homeostasis, stress regulation, and immune responses.

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