SURVEY AND SUMMARY

The DBHS proteins SFPQ, NONO and PSPC1: a multipurpose molecular scaffold

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ABSTRACT

Nuclear proteins are often given a concise title that captures their function, such as 'transcription factor,' 'polymerase' or 'nuclear-receptor.' However, for members of the *Drosophila behavior/human splicing* (DBHS) protein family, no such clean-cut title exists. DBHS proteins are frequently identified engaging in almost every step of gene regulation, including but not limited to, transcriptional regulation, RNA processing and transport, and DNA repair. Herein, we present a coherent picture of DBHS proteins, integrating recent structural insights on dimerization, nucleic acid binding modalities and oligomerization propensity with biological function. The emerging paradigm describes a family of dynamic proteins mediating a wide range of protein–protein and protein–nucleic acid interactions, on the whole acting as a multipurpose molecular scaffold. Overall, significant steps toward appreciating the role of DBHS proteins have been made, but we are only beginning to understand the complexity and broader importance of this family in cellular biology.

INTRODUCTION

The control of gene expression involves the dynamic interplay between proteins and nucleic acids. To regulate and integrate numerous components and pathways throughout gene regulation, the cell needs factors that can bridge DNA, RNA and protein. One such example of bridging proteins is the 'multifunctional' *Drosophila behavior/human splicing* (DBHS) family.

The DBHS proteins are defined by highly conserved tandem N-terminal RNA recognition motifs (RRMs),

a NonA/paraspeckle domain (NOPS) and a C-terminal coiled-coil [1] (Figure 1A). Outside this conserved region, members of the family differ significantly, both in length and sequence complexity. Found exclusively within vertebrates and invertebrates; the family has expanded and diversified to produce multiple paralogs [2]. In humans, there are three members of the family: splicing factor proline/glutamine rich (SFPQ, a.k.a. PSF), Non-POU domain-containing octamer-binding protein (NONO, a.k.a. p54nrb) and paraspeckle protein component 1 (PSPC1 a.k.a. PSP1). In contrast, invertebrates have one or two members (e.g. protein no-on-transient A (NonA) and NonA-like in *Drosophila melanogaster*, and NONO-1 in *Caenorhabditis elegans*).

DBHS proteins have a nuclear localization signal at their C-terminus and are largely regarded as nuclear factors. DBHS proteins are found in the nucleoplasm, and under various conditions can be found within subnuclear bodies termed paraspeckles, localized to chromatin, or DNA damage foci [3–5]. In addition there is emerging evidence that DBHS proteins function cytoplasmically and on the cell surface in defined cell types [6,7]. The function of SFPQ has been reviewed elsewhere [8]; however, structural and biological data suggest that DBHS proteins rarely function alone. Here, we present a unified picture of DBHS protein function by recognizing the family as dynamic factors mediating protein–protein and protein–nucleic acid interactions. These interactions are facilitated by novel DBHS protein structures and largely regulated by post-translational modifications and availability of interaction partners. The cellular pool of DBHS protein is thus constantly updated, regulated and relocalized to facilitate dynamic and context-dependent function.

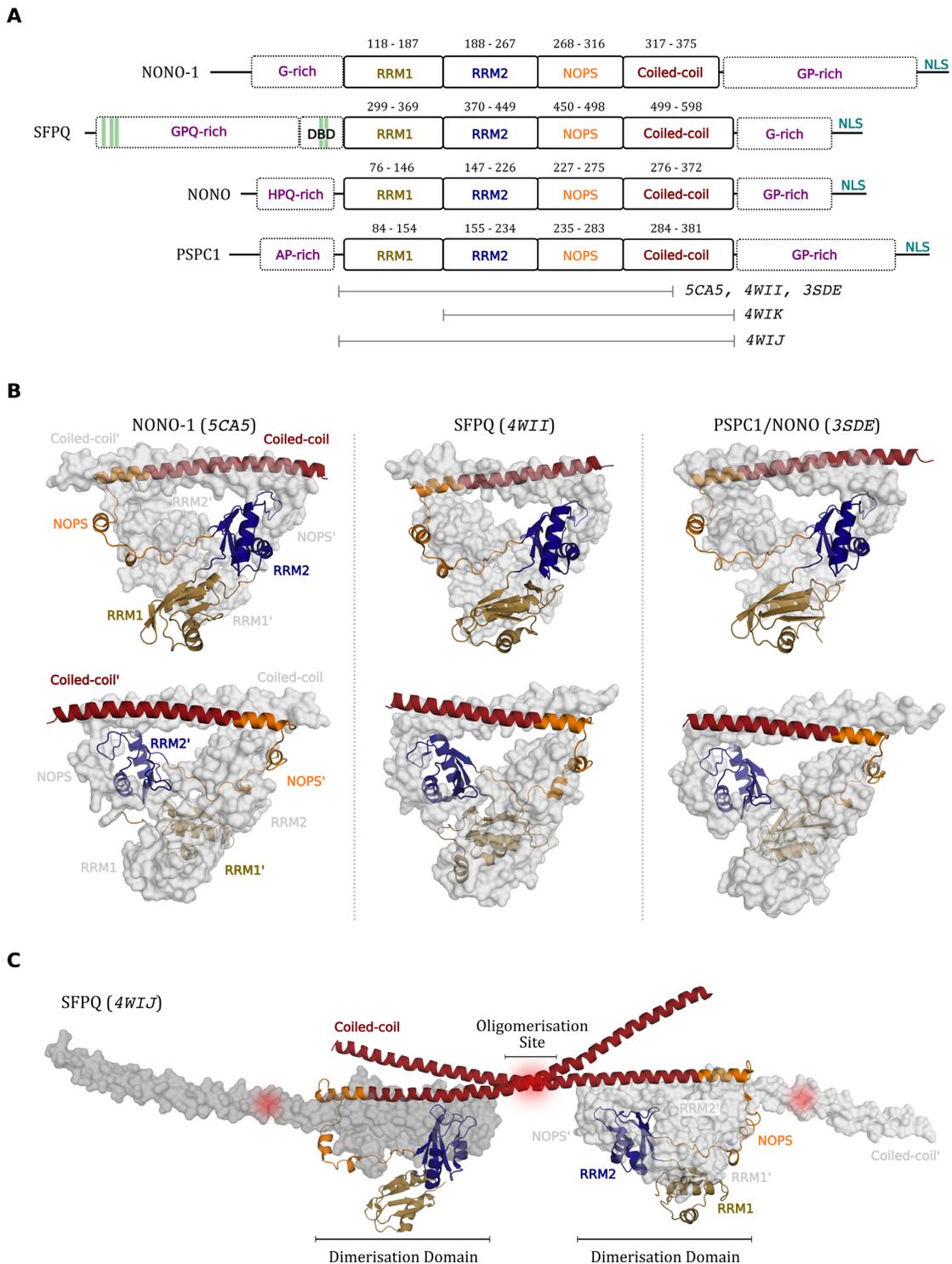*To whom correspondence should be addressed. Tel: +61 08 6488 3297; Email: archa.fox@uwa.edu.au

Figure 1. DBHS protein domain architecture and structure. (**A**) Schematic representation of DBHS protein domain architecture with the structurally characterized RNA recognition motifs (RRM) RRM1 (brown) and RRM2 (blue), NonA/ paraspeckle (NOPS) (orange) and coiled-coil (red) domains indicated. The uncharacterized DNA-binding domain (DBD) of SFPQ and other low complexity regions of each paralog are indicated in dashed boxes. The RGG motifs are represented in green within the SFPQ schematic. The corresponding amino acid boundaries for each protein are indicated above the schematic for NONO-1 (*C. elegans*), SFPQ, NONO and PSPC1 (*H. sapiens*). (**B**) X-ray crystal structures of NONO-1 (5CA5) (2), SFPQ (4WII) (12) and PSPC1/NONO (3SDE) (11). The first subunit of each dimer is illustrated as a domain colored cartoon and the second subunit as a molecular surface (gray). Directly below is an additional representation of each dimer from an identical perspective where the converse is shown; the first subunit of the dimer is illustrated as a surface (gray) and the second subunit of the dimer illustrated as a domain colored cartoon. (**C**) X-ray crystal structure of the SFPQ homodimer (4WIJ) (12) illustrating two SFPQ homodimers interacting via their coiled-coil oligomerization motif (highlighted by a red mark). The additional coiled-coil interaction sites within partnered chains are highlighted by a red mark. The dimerization domain and coiled-coil mediated oligomerization site are indicated. Prime (′) denotes the partner chain. Domains are colored consistently throughout.

DBHS PROTEIN STRUCTURE AND FUNCTION

DBHS proteins are nucleic acid- and protein-binding dimers capable of forming higher order oligomeric complexes (Figure 1). In their structured core DBHS proteins are remarkably modular, possessing both protein–protein and protein–nucleic acid binding sites that enable them to behave as a 'molecular scaffold (Figure 2). Beyond the structured regions, the N- and C- terminal low-complexity regions contribute significantly to the functional diversity observed for DBHS paralogs.

DBHS domain architecture

All members of the DBHS protein family possess a conserved core of ∼300 amino acids defined as the 'DBHS region' (Figure 1A). The DBHS region encompasses the tandem dissimilar RRMs, the protein–protein interaction NOPS domain and the coiled-coil domain. In DBHS proteins, the tandem RRMs are distinct from one another and are separated by a flexible seven-amino acid linker (2). The RRM is one of the most abundant and well-characterized nucleic acid binding domains, present in 0.5–1.0% of human genes (reviewed in (9,10). A canonical RRM has a $\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$ topology where aromatic residues on the β-sheets π-stack nucleotides with additional contacts from charged and sequence-specificity determining side chains (9). DBHS RRM1 is described as canonical, with conserved aromatic and charged residues exposed to the solvent (2,11,12). In contrast, the DBHS RRM2 lacks the conserved aromatic residues and has additional extended β-turns within loop 3 and loop 5, one of which shows high conservation (2) and resembles a double-stranded DNA/RNA recognition motif (13). The NOPS domain definition, derived from a Pfam alignment, stretches from the end of RRM2 to the coiled-coil domain where it functions almost exclusively in mediating DBHS dimerization. However, some surface-exposed basic residues within the NOPS domain may be involved in nucleic acid binding (2). The C-terminal end of the DBHS region features the highly charged coiled-coil domain known to facilitate dimerization and oligomerization. The coiled-coil dimerization domain forms an unusual right-handed antiparallel coiled-coil (12). While modular in their core domain architecture, DBHS proteins possess sequences that are likely to be intrinsically disordered. These intrinsically disordered regions contain low-complexity sequences (or low-complexity domains, LCDs) that flank the DBHS region and are sites for post-translational modification and potentially drive dynamic phase separation (14).

Obligatory dimerization

The first indication that DBHS proteins function as dimers came in 1993 with the purification of SFPQ/NONO heterodimers from *HeLa* cells (1). Subsequently, yeast two hybrid experiments, immuno-precipitation and other experiments on endogenous proteins, confirmed that DBHS proteins interact reciprocally (15–19) and others have consistently reported copurification and *in vitro* interaction to confirm DBHS dimerization. Atomic resolution structures for DBHS protein dimers have been determined from both vertebrates and invertebrates (2,11,12). The structures show DBHS monomers forming a globular core with emerging antiparallel-coiled coils (Figure 1B). As a result of this, the putatively RNA-binding β-sheet surface of RRM2 faces a 20-Å 'void' in the core of the dimer (11). The obligatory dimerization is mediated by reciprocal interactions between RRM2 of one monomer, the partnered NOPS and the distal coiled-coil domain (2,11,12). The interface involves contacts from across the entire DBHS region but is dominated by a highly conserved cluster of hydrophobic interactions between RRM2 and the NOPS domain. Consistent with the role of the RRM2, NOPS and coiled-coil domains in dimerization, removal of RRM1 does not hinder the ability of SFPQ to form an obligate homodimer (12). The dimerization interface is highly conserved (2) such that *Chironomus tentans* Hrp65 can form stable heterodimers with human SFPQ and *D. melanogaster* NonA (20). Consistent with obligatory dimerization, mutation of residues within the NOPS-RRM2 dimerization interface results in localization and functional defects (11). Thus, the DBHS region forms a compact and intimately intertwined core dependent on a complex series of contacts between RRM2, NOPS and coiled-coil domains. Unsurprisingly, deletion of either RRM2 and/or the NOPS domain results in a loss of function, presumably due to a loss of dimer integrity. Similarly, over expression of individual parts of the proteins not capable of dimerizing, such as an RRM, or coiled-coil region in isolation, should be considered dimerization incompetent and therefore functionally limited.

Recognizing SFPQ, NONO and PSPC1 as fundamentally dimeric means that some past literature, where they are annotated as individual functional units, may need to be reinterpreted. Nevertheless, we have included many such studies in this review as their functional insights are important. DBHS dimerization is a dynamic process whereby a given dimer (homo or hetero) can readily exchange interaction partner to form an alternative dimerization state and in turn regulate function. For example, alternative dimerization between differing Hrp65 isoforms dictates their subcellular localization in *C. tentans* (20,21). Dimerization state may also be dependent on the relative abundance of each paralog. For example, mouse Sertoli cells have higher expression of SFPQ and PSPC1 compared to NONO (18), in contrast to *HeLa* cells where NONO and SFPQ are more abundant than PSPC1 (17). Sertoli cells contain all heterodimer combinations (PSPC1/SFPQ, SFPQ/NONO and PSPC1/NONO), whereas *HeLa* cells predominantly contain SFPQ/NONO and PSPC1/NONO. Different dimers may have different cell-type specific functions, as DBHS proteins can functionally compensate for each other in some biological scenarios, but not others. For example, SFPQ overexpression causes increased exon inclusion in a splicing minigene reporter, but NONO overexpression had no effect (22). In contrast, knockout of NONO can be compensated by an upregulation of PSPC1 to form a functional heterodimer with SFPQ in DNA repair (23). However, there are examples where SFPQ and NONO do not compensate for the loss of PSPC1 (24) and PSPC1 and SFPQ cannot compensate for the loss of NONO in intellectual disability in humans (25). Future studies cannot ignore the dynamic
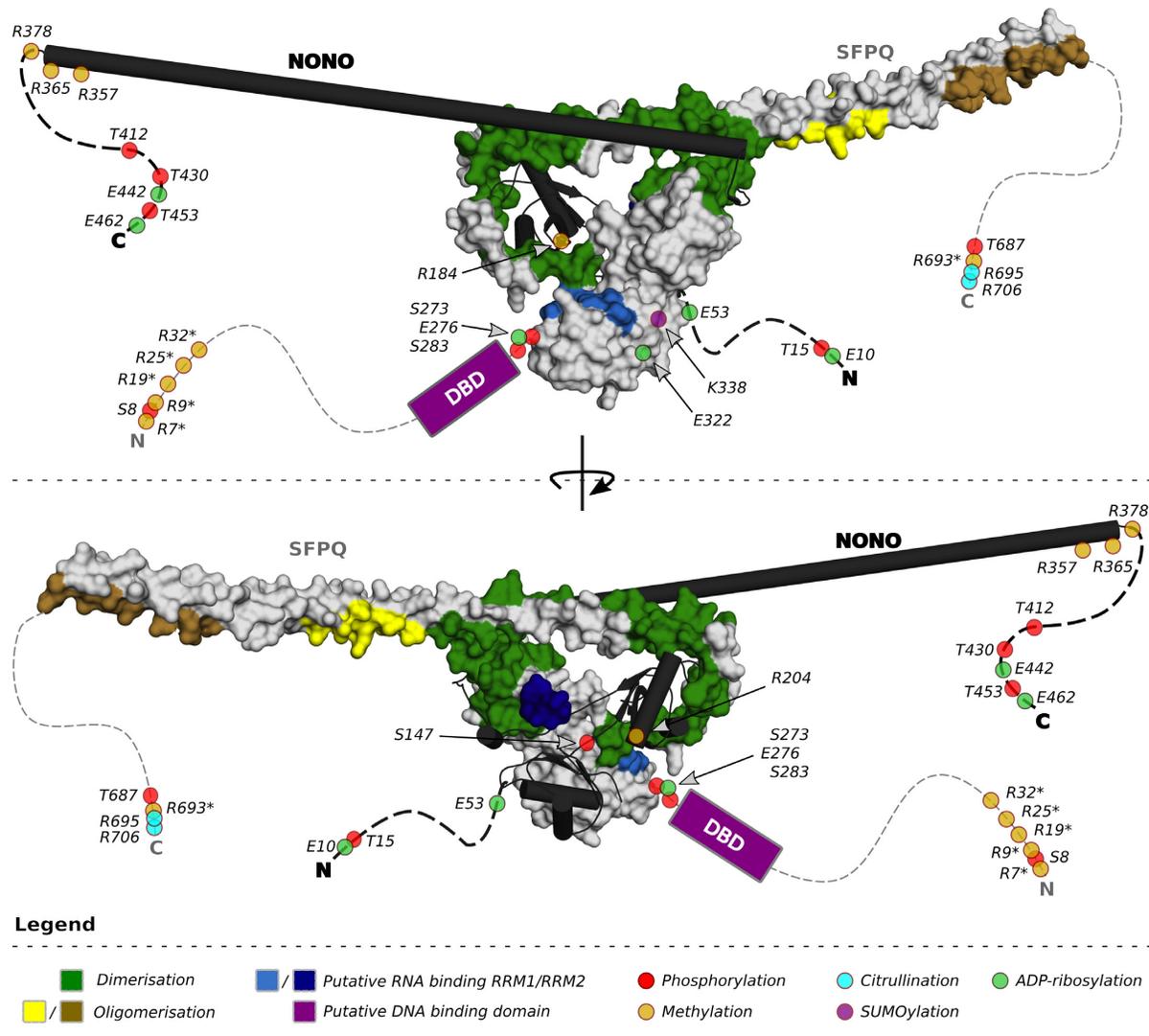
Figure 2. DBHS protein-binding sites and post-translation modifications mapped to the X-ray crystal structure of SFPQ (4WIJ). The structure illustrates a putative SFPQ/NONO heterodimer (colored surface/black cartoon, respectively) with the remaining N- and C-terminal uncharacterized and low-complexity domains modeled as flexible chains at the corresponding termini of the structure (dashed lines). Interaction sites within the X-ray crystal structure are colored; dimerization interface (green), coiled-coil oligomerization motif (yellow), secondary oligomerization site (brown), putative RNA-binding surface of RRM1 (light blue), putative RNA-binding loop of RRM2 (dark blue). The structurally uncharacterized DNA-binding domain (12) of SFPQ is also illustrated (purple box). Mapped as colored circles to the SFPQ and NONO chains are reported sites of post-translational modification and corresponding amino acid number; phosphorylation (red), methylation (orange), citrullination (teal), SUMOylation (purple) and ADP-ribosylation (pale green). Methylation sites that are also subject to citrullination are indicated with an asterisk.

expression and interplay between DBHS protein paralogs, especially given functional overlap and redundancy.

Coiled-coil mediated oligomerization

Oligomerization and functional aggregation are emerging as important to DBHS function. DBHS structures show an extended α-helical coiled-coil projecting out from the core dimer interface (2,11,12). Truncation and mutagenesis of this coiled-coil region resulted in aberrant subnuclear localization and physiological defects in several DBHS proteins and it was postulated that these defects resulted in perturbed coiled-coil mediated oligomerization (11,17,26,27). Recently, the SFPQ homodimer structure confirmed that the α-helical 'arms' project out from the

dimerization core and provide an interface for oligomerization via a highly conserved motif present within the extended coiled-coil domain (2,12) (Figure 1C). This interface takes part, in a concentration-dependent manner, in a classical heptad-repeat coiled-coil interaction with another DBHS protein dimer (28). The formation of higher order oligomers by SFPQ is not only essential for the structure of the mammalian paraspeckle, but also for the cooperative enhancement of nucleic acid binding (11,12). C-terminal to the coiled-coil oligomerization motif, there are regions of highly conserved charged residues that provide an interface for further coiled-coil type interactions (12), consistent with the coiled-coil acting as a molecular ruler for DBHS protein interactions (29). The molecular scaffolding brought about

by combined nucleic acid binding and coiled-coil mediated oligomerization is not uncommon, for example, coiled-coil mediated interactions feature heavily in centrosome assembly (30). Other aggregation-prone paraspeckle proteins also form higher-order oligomers to stabilize paraspeckles, although not through coiled-coils, instead through reversible prion-like protein aggregation (14). It may well be that oligomerization by coiled-coil domains and prion-like domain interactions, are both examples of reversible and dynamic 'functional aggregation,' an emerging concept in cell biology that is driven by local concentrations of molecules such as DBHS proteins that readily oligomerize, or aggregate. This functional aggregation property, as well as their abundance, may explain why DBHS proteins are often identified in mass spectrometry/proteomic studies (31), even in negative control samples.

Sequence and structure specific RNA interaction

In spite of structural data and the presence of canonical nucleic acid recognition motifs, precisely how DBHS proteins bind nucleic acids is still unknown. What is known is that DBHS proteins recognize a broad spectrum of nucleic acids. *In vitro*, SFPQ and NONO can bind to any single-stranded polynucleotide (32–35) with preference for single-stranded RNA (ssRNA) over single-stranded DNA (ssDNA) (33,34). While both NONO and SFPQ are reported to have a preference for short G-rich RNA (35–40), SFPQ exhibits the highest affinity for poly-U (32) and NONO poly-G (35). NONO has also been reported to bind long stretches of poly(ADP-ribose) (PAR) using RRM1 (41) (similar to the serine/arginine-rich splicing factor 1 (ASF/SF2) (42) (reviewed in (43)), an interaction potentially heightened by coiled-coil mediated oligomerization given the preference for longer stretches of PAR. In addition, DBHS proteins also bind structured nucleic acids. For example, NONO/SFPQ homo- or heterodimers interact with the U5 small-nuclear RNA (snRNA) stem *in vitro*, an interaction dependent on both the sequence and structure of the target RNA (15). SFPQ and NONO also interact with the stem loop in the 5′-splice site of pre-mRNA (35,44,45), the terminal stem-loop of the hepatitis delta virus RNA (46) and inverted repeat Alu elements (IR*Alus*) that form long dsRNA regions and can be subject to extensive RNA editing (38,47).

Despite this broad range of target RNAs, some degree of sequence and/or secondary structure driven specificity is observed. It is tempting to suggest that the canonical RRM1 facilitates interactions with unstructured nucleic acids, whilst additional complex mechanisms, likely involving DBHS oligomerization and RRM2, mediate structured RNA binding. Supporting the notion of distinct binding modes, DBHS binding to double-stranded nucleic acid is independent of binding single stranded nucleic acids (33,34). The interaction of DBHS proteins with nucleic acid may also be consolidated by amino acids proximal to the highly conserved surfaces of RRM1 and RRM2. For example, the N-terminal region preceding NONO RRM1 is implicated in binding to the 5′-splice site of pre-mRNA (44). Similarly, Arginine residues in the coiled-coil region of SFPQ undergo post-translation modifications influencing RNA

binding (48), albeit with an unknown mechanism. It is clear that while we understand nucleic acid binding to some extent, more data are required to deconvolute the observed promiscuity of DBHS protein RNA interaction.

DNA interaction

SFPQ, NonA and Hrp65 all possess RGG motifs N-terminal to their first RRM, some of which constitute the putative 'DNA-binding domain' (Figure 1A). While the precise role of the RGG/RG motif (reviewed in (49)) is unclear, in the context of the N-terminal LCD of SFPQ; they are required for the interaction with dsDNA (12,50) and any of the five RGG motifs could also serve as a module for sensing PAR at sites of DNA damage (41,43). Indeed, the role of the RGG motifs are regulated by a swathe of post-translational modifications, including methylation, citrullination and ADP-ribosylation, with potential regulatory roles discussed further below (48,51,52).

ROLES IN TRANSCRIPTIONAL REGULATION

DBHS proteins mediate transcriptional repression, activation, initiation, elongation and termination (Figure 3 [1–4]). Utilizing their behavior as a molecular scaffold, DBHS proteins associate synergistically with a broad spectrum of transcription factors, DNA and RNA; acting bifunctionally as positive and negative transcriptional regulators. Thus, as with many transcription factors, their precise role is context dependent (53).

Transcriptional repression

Transcriptional repression by DBHS proteins appears largely driven by and dependent on SFPQ, either in a homodimer, or heterodimer context. Several studies have described how SFPQ binds directly to target gene promoters, subsequently recruiting epigenetic silencers such as Sin3A and HDAC (54–56). Through recruitment of epigenetic regulators, SFPQ/NONO can act on hormone receptors such as the thyroid and retinoid X receptors (57), or in complex with steroidogenic factor 1 (SF-1), repress the human *CYP17* gene or genes involved in circadian rhythms (58–61). There is a dearth of characterized SFPQ DNA recognition elements, but one example is the palindromic sequence CTGAGTC, an insulin-like growth factor response element in specific gene promoters (62–65). SFPQ/NONO can also negatively regulate transcription by sequestering activators away from target promoters. For example, direct binding of SFPQ/NONO to the progesterone receptor can prevent its binding to DNA (54). In other transcriptional contexts, NONO represses genes responsive to the cAMP pathway (66). SFPQ and NONO can associate with silencer motifs in the promoter of the *phosphate carrier* (*PiC*) gene (67); and more recently, as transcriptional repressors of *Interleukin-8* (*IL8*) (68). Reports suggesting that NONO can directly interact with promoter elements repressing transcription may have over looked SFPQ heterodimerization, although it cannot be ruled out that NONO may possess some hitherto uncharacterized DNA-binding ability (69,70).
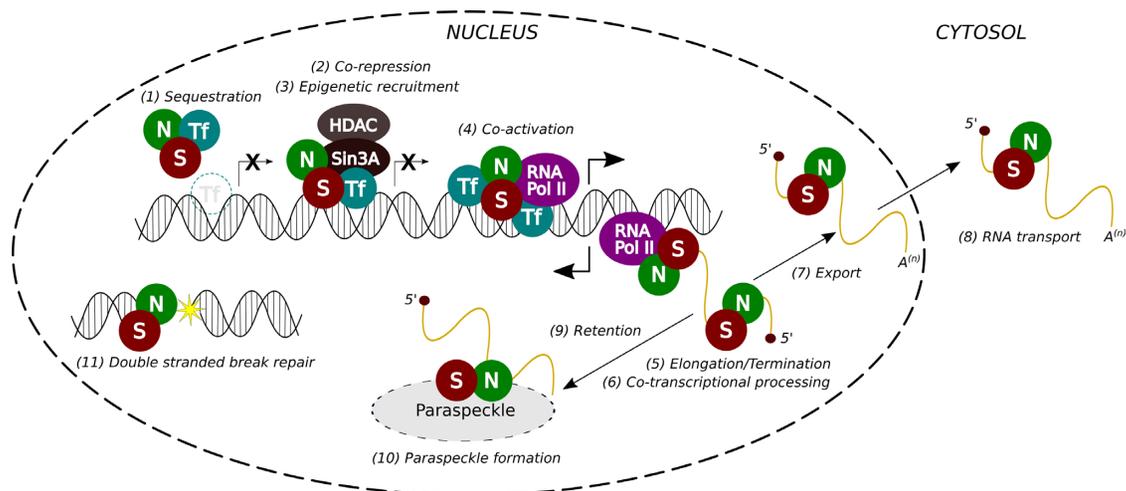
Figure 3. Simplified schematic representation of DBHS protein function. The DBHS proteins SFPQ (S) and NONO (N) are represented as simple red and green spheres respectively. (1) SFPQ and NONO can sequester transcription factors away from target promoters, (2) act as co-repressors at target promoters and (3) in complex with repressors stimulate epigenetic silencing. (4) Both SFPQ and NONO are associated with co-activation of transcription through (5) elongation up to termination. (6) SFPQ and NONO also remain associated with nascent mRNA to facilitate co-transcriptional processing, (7) mRNP export and (8) cytosolic trafficking. (9,10) By virtue of their involvement in paraspeckle formation and integrity, SFPQ and NONO can facilitate nuclear RNA retention. SFPQ, NONO and PSPC1 are also involved in double stranded break repair (11).

Transcriptional activation

Transcriptional activation by DBHS proteins appears to be driven by NONO, and in many cases involves binding and processing of the nascent RNA transcript as well as DBHS interaction with transcriptional machinery. In contrast to DBHS transcriptional silencing, the landscape of transcriptional activation appears to be influenced by the presence of the nascent RNA, which likely presents as a scaffold for DBHS binding. NONO associates with promoters of many transcriptionally active genes, as revealed by ChIP-seq experiments, suggesting a pervasive role in transcription (71). There are examples of target genes upregulated by DBHS proteins, such as *oct4* in embryonic stem cells (72). Transcriptional activation by NONO is often associated with a synergistic interaction with other activators. For example, NONO interacts with photoreceptor transcription factors enhancing rhodopsin expression and regulates the co-transcriptional splicing of rod-specific genes (71). NONO is recruited by transducers of regulated cAMP response element binding proteins facilitating the association of RNA polymerase II (RNAPII) with cAMP-dependent promoters (73). In some cases, NONO is prevented from activating transcription by direct binding to an inhibitor protein, for example binding by a suppressor of cytokine signaling (SOCS3), in this case the NONO-SOCS3 complex can be disrupted by IL-1β leading to increased transcriptional activity of the *Mucin8* gene (74). The recruitment of NONO into transcriptionally active contexts is also closely linked to RNA. ILF3 up regulates *survivin* in complex with NONO, E2F1 and E2F2 in an RNA-dependent manner (75).

It is difficult to say whether the above transcriptional activation functions can be attributed to NONO exclusively, or a functional DBHS heterodimer. For example, a complex of SFPQ/NONO and SF-1 maintains basal and cAMP-dependent transcription of *Cyp17* and *Rbp4* (39,58–60). Less widely expressed, PSPC1 as a putative heterodimer

with SFPQ forms a complex with LMX1b and nuclear receptor related 1 protein (NURR1), activating genes in neuron development (76,77). Likewise, SFPQ acts as an essential co-activator for the transcription of adenosine deaminase B2 (ADARB2), a role now used as a reporter for functional transcriptional activity of SFPQ (12,78).

The apparent ability of DBHS proteins to elicit both transcriptional co-repressor and co-activator activity is exemplified by SFPQ, NONO and PSPC1 all being found as either transcriptional co-activators or co-repressors of Androgen Receptor (AR)-mediated transcription in different studies and contexts (18,79,80). Clearly further work is required to understand this duality of function, however potentially DBHS dimer composition, modification status, cell-type specific expression and localization could all be involved (18,55).

Transcriptional elongation and termination

DBHS proteins can remain associated with the carboxyl-terminal domain (CTD) of RNAPII throughout initiation, elongation and termination. In several cases, SFPQ and NONO interact with both the phosphorylated and unphosphorylated CTD of RNAPII and nascent RNA simultaneously (81). For example, SFPQ and NONO simultaneously interact with the conserved mRNA 5′ splice site, RNAPII CTD and snRNPs (15,44). The role of DBHS proteins in elongation appears to be preserved from invertebrates to humans, where Hrp65 maintains an active transcriptional elongation complex via actin recruitment (82,83). At the end of mRNA production, SFPQ and NONO are also required for 5′-3′ exoribonuclease 2 (XRN2) recruitment and efficient transcriptional termination (84).

POST-TRANSCRIPTIONAL PROCESSING AND EXPORT

It has been suggested that DBHS proteins may couple transcription to post-transcriptional processing (85), namely through a persistent association with nascent RNA (Figure 3 [5-8]). While potential binding to the majority of transcripts suggests nonspecific binding (80,86), it is nevertheless clear that some substrate specificity and activity is apparent.

Transcript splicing, polyadenylation and stabilization

SFPQ was first identified in a stable complex with polypyrimidine tract-binding protein (PTB), required for pre-mRNA splicing (32). Other studies have identified SFPQ and/or NONO as spliceosome-associated proteins (36,87,88) and shown that NONO/SFPQ associate with U5 snRNA early in formation of the spliceosome (15). NONO directly interacts with the 5′ splice site (44) and SFPQ is found in large pre-assembled spliceosomal complexes (89). Despite these associations, DBHS proteins are not essential components of the spliceosomal machinery *per se*, but are rather involved in co-transcriptional and alternative splicing. Specifically, SFPQ has been identified as a regulator of splicing for *CD45* (90), neuronal cell-specific genes (91), the preprotachykini (*PPT*) minigene (22), the microtubule-binding protein Tau (45) and spinal muscular atrophy genes *SMN1*/*SMN2* (92). Similarly, NONO is cited in rod-specific gene expression (71), phosphodiesterase splicing (93) and together SFPQ and NONO bind to specific A-U rich elements in pre-mRNA such as *TNF-α* (94). SFPQ/NONO also facilitates pre-mRNA 3′-end processing by promoting polyadenylation and pre-mRNA cleavage (84,95,96).

Beyond post-transcriptional processing, DBHS proteins are thought to contribute to maintaining transcript stability. For example, the stability of some histone coding mRNA is thought to involve SFPQ through either a direct or indirect interaction with the transcript (97). Similarly, SFPQ and NONO are known to regulate the stability of noncoding RNA, such as the long non-coding RNA *NEAT1* (98). Given their diffuse localization and broad nucleic acid specificity; it is highly likely that DBHS proteins function akin to a histone in degenerately coating nascent transcripts for stabilization.

Regulation of RNA localization and translation

DBHS proteins can also remain associated with the processed mRNP once formed. In neuronal cells SFPQ and NONO are components of large RNA transport granules in the neurites (99), a phenomenon important for local translation at the synapse. SFPQ and NONO are also snRNA export stimulatory factors, accelerating the recruitment of the phosphorylated adapter for RNA export (PHAX) for efficient nuclear export of snRNA (100). In invertebrates, NonA has been show to facilitate intranuclear mobility of mRNP particles, where it forms a complex with nuclear export factor 1 (NXF1) (101). Similarly, Hrp65 has been implicated in regulating mRNA localization and transport

(21). There is also evidence for DBHS protein function in internal ribosome entry site (IRES) regulation (102,103). The IRES can initiate translation independent of a 5′-cap by recruitment of specific RNA-binding proteins (104). For example, SFPQ, in complex with Annexin A2, binds directly to the IRES of the p53 mRNA and regulates its activity (102). Similarly, NONO and hnRNPM associate with the fibroblast growth factor 1 (FGF1) IRES in differentiating myoblasts and this 'loading' may be initiated when the RNA is transcribed in the nucleus (103).

SUBNUCLEAR STRUCTURES AND COMPLEXES

DBHS proteins are highly mobile inside the cell nucleus, but they can be triggered by binding to local high concentrations of various nucleic acids to form microscopically visible nuclear bodies, paraspeckles or large complexes such as DNA repair foci (Figure 3 [9–11]).

Formation and function of paraspeckles

Paraspeckles are ribonucleoprotein bodies located within the interchromatin space of mammalian cell nuclei (98) (reviewed in (4,5,105)). Paraspeckle proteins are defined by the colocalization of SFPQ, NONO or PSPC1 with the long noncoding RNA *NEAT1* (4,17,106–108). Both SFPQ and NONO are essential for paraspeckle formation and integrity, as siRNA knockdown of either protein prevents paraspeckle formation (107). The DBHS proteins directly bind *NEAT1* and likely stabilize the RNA, as loss of these proteins results in reduced *NEAT1* levels (107). Furthermore, DBHS proteins are also integral to one paraspeckle regulatory mechanism where they bind structured edited RNAs derived from transcribed inverted repeat elements, resulting in nuclear retention of these RNAs in the paraspeckle (38,47,109,110). DBHS oligomerization (12) and contacts with other paraspeckle proteins such as RBM14 (14) are also important for the paraspeckle structure. While the precise functional role of the paraspeckle is unclear, a general consensus that paraspeckles fine-tune gene expression under stress conditions is emerging (reviewed in (4,5,105,111,112)). One mechanism for gene regulation is that paraspeckles sequester a subset of nuclear proteins, including DBHS proteins, effectively depleting the available nuclear pool of these factors with flow-on effects on the target genes of these proteins (68,78,113–115).

Localization to DNA damage sites

DBHS proteins are implicated in double-stranded break (DSB) repair where they assist in homology directed repair or nonhomologous end joining (NHEJ). SFPQ promotes homologous DNA-pairing, strand invasion, D-loop formation and topoisomerase activity in a variety of cell types (116–119). SFPQ/NONO is found within the DSB preligation complex with the Ku protein and substrate DNA (120) and directly interacts with RAD51 (50,121), TopBP1 (122) and Matrin3 (123), recruiting proteins to sites of DNA damage (114) and stimulating both homologous and nonhomologous repair (41,50,121,123,124). Collectively, the DBHS proteins promote end joining of homologous DNA by direct interaction with DNA ends and

recruitment/stabilization of a preligation complex (125). In the context of DNA repair, DBHS proteins have redundant roles. For example, knockout of NONO in embryonic fibroblasts is compensated by PSPC1 up regulation, with a subsequent involvement of PSPC1 in the DSB repair pathway (23). PSPC1 is also involved in repair of cisplatin-induced DNA damage in certain cell-types with knockdown of PSPC1 causing cell death and bypassing of the G1/S checkpoint in *HeLa* cells (24). Interestingly, there is an emerging theme of RNA-binding proteins playing distinct roles in DNA damage responses and DBHS proteins add to this repertoire (reviewed in (126)).

Emerging evidence places localized poly(ADP-ribose) polymerase (PARP) activity early in the cellular response to DNA damage, where protein LCDs containing RGG motifs directly associate with PAR, forming phase separated compartments at sites of DNA damage (127). Mechanistically analogous to paraspeckle nucleation through combined nucleic acid recognition and prion-like interactions (14); DBHS interaction with PAR may serve as a scaffold to nucleate other subnuclear or cytosolic structures (128,129).

CIRCADIAN RHYTHM AND CELL CYCLE

Circadian rhythm is the change in abundance of proteins in response to an ∼ 24 h cycle. DBHS proteins are involved in coordinating cell cycle and circadian rhythm by regulating different nodes of the circadian network (130,131). In mammalian cells, the *Period* (*Per1* and *Per2*) proteins control a negative transcriptional feedback loop that generates oscillations in transcript abundance (132). DBHS proteins modulate this by interacting with PER proteins and antagonizing their function (133). Beyond PER binding, NONO co-activates circadian genes in a cAMP-dependent manner, by recruiting RNAPII to cAMP-dependent promoters (73). While NONO is not rhythmic in its abundance (130), SFPQ protein levels appear to oscillate with the circadian cycle (61). Akin to NONO; SFPQ can directly interact with the nuclear PER complex, moreover it can also recruit Sin3A-HDAC to drive deacetylation and repression of the *Per1* promoter (61). Loss of NONO does not significantly affect circadian rhythms in mammals, suggesting perhaps compensation by SFPQ or PSPC1, but loss of NonA, one of two DBHS fly proteins, results in arrhythmic flies (133). The NONO: PER complex, formed as a function of oscillating PER levels, directly co-activates the promoter of the G1-S checkpoint protein p16-INK4A (130). NONO null tissues show increased cell proliferation, reduced expression of INK4A, but an unaffected circadian clock (130). This cell cycle defect can be rescued with over expression of NONO, but not PSPC1 or SFPQ, suggesting that this role is exclusive to NONO (130). Thus, the nucleoplasmic pool of different DBHS protein has both redundant and independent functions as transcriptional co-activators and co-repressors for circadian clock-regulated genes, which combined, contribute to both the cell and circadian cycles (130,134).

DBHS PROTEIN CLINICAL SIGNIFICANCE

With roles in almost every step of gene regulation, it is not surprising that perturbation of DBHS protein function has consequences for the cell and organism. Broadly, DBHS proteins are rapidly emerging as clinically relevant in the contexts of development, innate immunity and cancer (Figure 4). Furthermore, *SFPQ*, *NONO* and *PSPC1* all belong to a class of human genes with the lowest tolerance for missense and loss of function mutations, suggesting strong involvement in selectable phenotypes in humans (Exome Aggregation Consortium ExAC, Cambridge, MA, URL: http://exac.broadinstitute.org, accessed March 2016).

Neurobiology and Development

Most recently, mutations in NONO have been identified that lead to patients with intellectual disability, defects that neither PSPC1 nor SFPQ can compensate for (25). Consistent with this, the NONO knockout mouse exhibits a similar neurological defect (23). Loss of the zebrafish SFPQ ortholog leads to a subset of neuronal cells failing to differentiate and arrested development in the zebrafish embryo due to improper brain formation (27,135,136). At the molecular level, PSPC1 and SFPQ are components of transcriptional and post-transcriptional complexes implicit in the regulation of genes required for neuronal differentiation and development (76,77,91,99,137). SFPQ and NONO directly interact with c-Jun N-terminal Kinase (JNK1) in an RNA-dependent manner where they are necessary for neuronal growth (138). Similarly, in neuronal cells, SFPQ and NONO directly interact with Protein degylcase-1 (DJ-1) to carry out a neuroprotective role (139). In photoreceptor development, NONO acts as an enhancer and post-transcriptional splicing regulator for rod-specific genes such as *rhodopsin* (71). Finally, via its role in progesterone signaling, SFPQ derepression of the PR may trigger labor (140), a function also attributed to NONO (56). Given the pervasive role of DBHS proteins in both transcriptional and post-transcriptional events in many cell types, not just neuronal cells, it is interesting to speculate that additional clinical roles may be masked by functional redundancy of the three mammalian DBHS proteins.

Innate immunity

Host cells respond to viral infection by inducing innate immunity pathways. In turn, viral systems hijack host cell components for the purpose of driving viral replication, often utilizing the host defense factors. DBHS proteins are heavily involved in the innate immune response to viruses and can bind directly to viral RNAs, bind to 'decoy' host ncRNAs, or interact with proteins to alter the transcriptional status of immune related genes. For example, SFPQ binds to the hepatitis delta-virus RNA and is used for the viral replication-cycle (46). SFPQ is also used for influenza A virus transcription and post-transcriptional processing (141,142). Interestingly, knockdown of NONO had no effect on influenza A viral replication (142); however, it is possible that PSPC1 expression may compensate for the loss of NONO. SFPQ is also implicated in both the transcription and maturation of HIV pre-mRNA, facilitating viral pre-mRNA nuclear export (143). NONO is also a regulator of early and late stages of HIV-1 infection in T-cells (144). As mentioned above, SFPQ can be sequestered
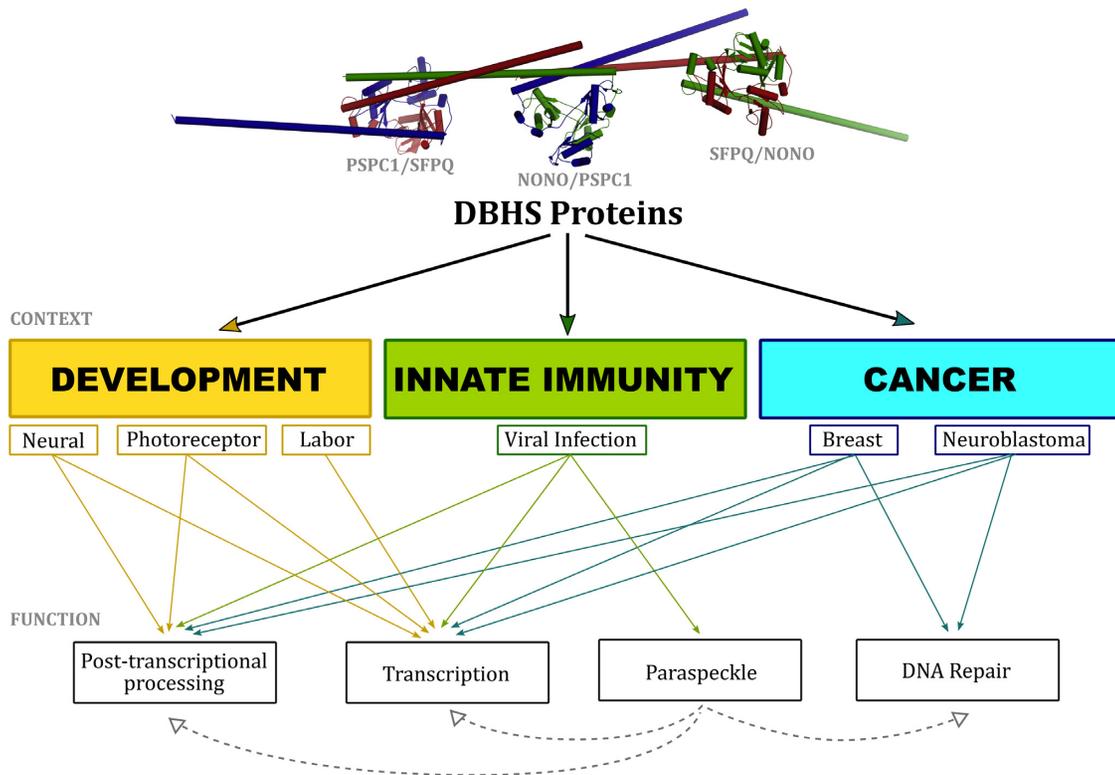
Figure 4. Context-dependent functions of mixed dimers and oligomers of DBHS protein with clinical significance. Broadly, DBHS proteins function in the clinical contexts of development, innate immunity and cancer. Corresponding examples are shown in colored boxes. The functional or mechanistic manifestation of that clinical context is indicated with a line. Note that paraspeckles may be involved in regulating DBHS protein partitioning for the other functions shown (indicated by dashed lines).

into enlarged paraspeckles by NEAT1 binding to relieve repression of the IL-8 promoter. The cell uses this mechanism in an innate immune response to infection by Herpes Simplex virus-1 (68). Epstein–Barr Virus (EBV) infection also triggers an innate immune response mediated by a paraspeckle/DBHS protein pathway (145) and SFPQ binds directly to EBV-genome encoded ncRNAs (146). It is highly likely that the broad recognition of nucleic acids coupled with DBHS oligomerization and potential recruitment of additional proteins plays key roles in DBHS binding to viral RNAs.

DBHS proteins in cancer

As with many gene regulators, DBHS proteins can be either tumor suppressors or oncogenes in a variety of transformed contexts. As tumor suppressors, breast tumors with loss of NONO are associated with significantly increased tumor size, presumably due to enhanced proliferation (147), perhaps consistent with NONO as a regulator of cell cycle (130). Similarly, release of SFPQ from DNA elements, or disruption of the SFPQ/PTBP2 tumor suppression complex allows for rapid cell proliferation and migration (64,65,148). In contrast, NONO is reported as highly expressed in examples of malignant melanoma (149), malignant pleural mesothelioma (150), malignant breast cancers (151,152) and neuroblastoma (153). Increased NONO abundance correlates with enhanced melanoma progression (149), malignant progression of breast tumors (151) and

dysregulation of lipid metabolism (152). It is likely that the association of NONO with pervasive active transcription is being utilized to alter gene expression in these contexts. For example, driving advanced neuroblastoma and poor patient outcomes; NONO facilitates simultaneous interaction with the long noncoding RNA lncUSMycN and the N-*Myc* mRNA leading to post-transcriptional up regulation of the potent neuroblastoma oncoprotein N-Myc (153). SFPQ and NONO have also been linked to promoting invasion and growth in colorectal cancers (154–156), and prostate cancer progression through AR- mediated activity (80,157,158). SFPQ is reported as an oncogene through fusion with the Transcription Factor E3 (TFE3) in papillary renal cell carcinoma (159) and neuroblastoma (160), or fused with Abelson murine leukemia 1 (ALB1) in acute lymphoblastic leukemia (161). In this latter case it is possible that the dimerization and oligomerization properties of SFPQ are harnessed by the kinase fusion to promote constitutive oncogenic kinase activity. Interestingly, SFPQ was recently reported as redistributing to the cell membrane in malignant cell lines of leukemia patients, however the functional implications of this are not known (6).

REGULATING DBHS PROTEIN FUNCTION

The precise functional context of a given DBHS protein appears to be dependent on combinations of cell-type, exogenous stimuli, dimerization state, protein and/or nucleic acid interaction partner, subnuclear localization, post-

translational modification and time of day. Thus, all of these elements have regulatory potentially in the DBHS context.

Protein interaction partner

While the dimeric and oligomeric state of a DBHS protein certainly influences its role, protein interactions outside of these also regulate their function. One such example is NONO activity in response to cAMP: at some cAMP responsive promoters, NONO drives transcriptional activation through interaction with the CREB/TORC complex to recruit RNAPII (73), whereas at other cAMP responsive promoters, NONO interacts with RASD1 to instead selectively repress transcription (66). A similar scenario is apparent for AR mediated transcription (18,54). A more recent study showed SFPQ displays inhibited RNA binding when in a complex with TRAP150, resulting in altered post-transcriptional processing (162). From a structural perspective, it is not yet clear how multiple, sometimes simultaneous, protein–protein interactions are mediated by DBHS proteins. It is likely that protein and/or nucleic acid binding may induce dynamic changes in DBHS structure, thus 'revealing' specific interaction sites that may be coupled to higher-order associations as a result of oligomerization. Furthermore, the highly variant low-complexity domains flanking the DBHS region likely play a significant role in contributing additional protein–protein interactions.

Nucleic acid partner

Nucleic acids regulate DBHS protein function through competition at mutually exclusive binding sites, allosteric modification or via delocalizing a subset of DBHS protein. The repressor activity of SFPQ can be alleviated via an allosteric mechanism whereby binding of the murine noncoding RNA *VL30* to SFPQ competes with its promoter binding, releasing it and resulting in transcriptional activation (64,65). In a different context, SFPQ is responsible for transcriptional activation at the promoter of *ADARB2*, and this is attenuated by *NEAT1* lncRNA mediated sequestration of SFPQ into the paraspeckle (78). Similarly, induction of *NEAT1* facilitates expression of *IL-8* by relocating SFPQ to paraspeckles, relieving repression of *IL-8* (68). Consistent with lncRNA regulation of SFPQ activity, the binding of the lncRNA *MALAT1* to SFPQ has been shown to disrupt the PTBP2: SFPQ tumor suppressor complex (148). Further, NONO interacts with the lncRNA *lncUSMycN* and the N-*Myc* mRNA to post-transcriptionally upregulate N-Myc expression, acting as an oncogene driving neuroblastoma progression (153). Recently, a study illustrated that synthetic oligonucleotides can drive degradation of nuclear DBHS protein, potentially by interrupting native dimerization (163), perhaps hinting at a regulatory role of noncoding RNA in DBHS protein degradation.

Post-translational modification

DBHS proteins are substrates for a number of post-translation modifications (Figure 2). The phosphorylation of SFPQ by Protein Kinase C inhibits its binding to RNA, but stimulates its association with ss and dsDNA promoting

D-loop formation (118). In contrast, Mnk1 and Mnk2 selectively phosphorylate SFPQ at Ser8 and Ser283, proximal to RRM1, enhancing RNA binding to the 3′UTR of TNF-α (94). In T-cells, GSK3 phosphorylates SFPQ at T687 promoting interaction with TRAP150, preventing SFPQ from binding to CD45 pre-mRNA (90). NONO is also phosphorylated in the region proximal to the coiled-coil domain (T412, T430 and T453) during mitosis (164). The phosphorylation of these T–P motifs provides binding sites for the peptidyprolyl isomerase (Pin1) that may lead to subsequent conformational changes of this region (164). Thr15 in the NONO N-terminus is also phosphorylated by CDK1 during mitosis, with consequences for RNA binding to simple substrates *in vitro* (35). Interestingly, the *in vitro* phosphorylation of Thr15 disrupts binding of NONO to all homo-ribopolymers excluding poly-G (35), suggesting that the N-terminal 53 residues of NONO may allosterically regulate RNA-binding ability. Finally, NONO is also a substrate for Protein phosphatase 1 that associates with NONO RRM1 and influences NONO post-transcriptional splicing (165). Phosphorylation of DBHS proteins also drives altered subnuclear or cellular localization. In murine neuroblastoma cells, SFPQ and NONO associate with the nuclear envelope in response to tyrosine phosphorylation (166). While mechanistically unclear, the phosphorylation of SFPQ at N- and C-terminal Tyr residues proximal to the DBHS region drives cytosolic localization inhibiting cell proliferation (167,168). Similarly, hyperphosphorylation of the N-terminal half of SFPQ drastically alters its subnuclear localization pattern in apoptosis (169).

Methylation, SUMOylation, citrullination and ADP-ribosylation of DBHS proteins also regulate their nucleic acid binding. The methylation of conserved Arg residues C-terminal to the highly charged coiled-coil oligomerization motif negates the binding of NONO to structured RNAs such as mRNA containing IR*Alu*s and dsRNA (170). Furthermore, the highly conserved β2-β3 Arg184 and Arg204 of NONO are also reported to be methylated, however, the function of these sites are unknown (170). The N-terminal 'DNA binding' RGG motifs of SFPQ can also be mono- and di-methylated (51,171). This methylation of RGG does not perturb SFPQ dimerization, but promotes mRNA binding via an unknown mechanism (48). In contrast, the *in vitro* citrullination of SFPQ prevented RGG methylation and decreased mRNA association, potentially highlighting a dynamic control of SFPQ functionality regulated by methylation and citrullination switches (48). DBHS proteins can also be post-translationally modified with the addition of small ubiquitin-like modifiers (SUMO). SUMOylation of SFPQ on the surface of RRM1 (residues 337–340) is required for interaction with HDAC1, promoting deacetylase activity and inhibiting activity at the human tyrosine hydroxylase promoter (172). Finally, DBHS proteins are likely regulated by ADP-ribosylation both within the DBHS region and adjacent LCDs. NONO and SFPQ were identified as direct substrates of PARP-1 where they are modified on a series of glutamate residues (52). While these modifications are not functionally characterized, there is an emergent field describing ADP-ribose polymers as important modulators of transcriptional regulators (128,173–176). Furthermore, in the context of DNA

damage responses, ADP-ribosylation of SFPQ and NONO might promote delocalization from DNA damage sites by outcompeting DNA and PAR.

Subcellular localization

Apart from the subnuclear partitioning of DBHS protein into paraspeckles, other foci, or the nucleoplasmic pool, DBHS proteins can also reside outside the nucleus. SFPQ was identified early on as a cell surface antigen in myoblast cells ([177]). Confocal microscopy experiments have shown SFPQ localization on the surface of brain microvascular endothelial cells where it is thought to be involved in invasive meningitis ([178]). Furthermore, SFPQ can be relocated to the cell surface membrane in multidrug-resistant cancer ([6]). As mentioned above, both NONO and SFPQ are observed within the cytoplasm of hippocampal neurons associated with RNA transport granules ([99]). Consistent with cytoplasmic localization, by virtue of an interaction with the HERMES protein, SFPQ and NONO can be found as components of cytoplasmic RNP granules in retinal cells ([7]). Additionally, as described earlier, post-translational phosphorylation of SFPQ at C-terminal Tyr residues drives cytoplasmic localization ([168]). Given their abundance and dynamic nature, the cytoplasmic or extracellular role of DBHS proteins may have been underestimated to date.

CONCLUSION

Since the review published by Shav-Tal and Zipori in 2002 ([3]), the body of literature on DBHS proteins has increased dramatically, with novel contributions helping us to understand their biological roles. The emerging paradigm for DBHS protein function describes a family of nuclear mediators essential for seeding and bridging multiple nuclear processes, as well as several cytoplasmic roles. By virtue of their modular design, paralogs, swathe of modifications, resulting broad nucleic acid specificity and varied protein interaction partners; DBHS proteins are able to act as dynamic nuclear elements mediating protein–protein and protein–nucleic acid interactions in a variety of contexts. In this manner, DBHS proteins can effectively couple gene transcription to post-transcriptional processing and recruit factors to DNA damage foci. However, we are still lacking in our understanding of the precise mechanistic detail of the DBHS interactome, particularly beyond the core structured region. Nevertheless, moving forward, we now have a sound framework to reliably investigate this remarkably adaptable and versatile protein family. Further research is required to appreciate what mediates the dynamic and sometimes simultaneous DBHS protein association with RNA, DNA and protein. Understanding the mechanistic 'decisions' that are made to dictate DBHS protein partitioning will moreover be therapeutically invaluable.

FUNDING

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