

Review

Pat1 proteins: regulating mRNAs from birth to death?

Nancy Standart* and Aline Marnef

Department of Biochemistry, University of Cambridge,
Tennis Court Road, Cambridge, CB2 1QW, UK

* Corresponding author
e-mail: nms@mole.bio.cam.ac.uk

Abstract

The Pat1 protein family has been the subject of several recent extensive investigations of diverse model systems ranging from yeast, flies and worms to man, using a variety of experimental approaches. Although some contradictions remain, the emerging consensus view is that these RNA-binding proteins act in mRNA decay by physically linking deadenylation with decapping and by regulating gene expression as translational repressors. These multiple functions are present in the single invertebrate Pat1 proteins, whereas, in vertebrates, one Pat1 variant represses translation in early development, while a somatic version synthesised in embryogenesis and in adults acts in mRNA decay. At steady state, Pat1 proteins are found enriched in cytoplasmic P(rocessing)-bodies, and related mRNP complexes and granules. Evidence recently obtained from mammalian tissue culture cells shows that Pat1 shuttles in and out of the nucleus, where it localises to nuclear speckles, PML bodies and nucleolar caps, which suggests RNA-related nuclear functions. Less well understood, Pat1 proteins may play additional roles in miRNA silencing and/or biogenesis, as well in the regulation of viral gene expression. Due to the relatively low level of sequence conservation between Pat1 proteins from different species and lacking any discernable motifs, determining their functional domains has proved difficult, as is obtaining a simple unified view of the location of the binding sites of their interacting proteins in all examined species. Questions that remain to be addressed include the following: 1) What are their roles in the nucleus? 2) What is the link, if one exists, between their cytoplasmic and nuclear roles? 3) Do they have specific mRNA targets? 4) Which signalling pathways regulate their P-body localisation in mammalian cells, which may affect quiescent cell survival?

Keywords: 4E-T; HeLa cells; Pat1b; rck/p54/Dhh1; *Xenopus* oocytes.

Introduction: overview of Pat1 proteins

Mainly cytoplasmic, Pat1 proteins have recently attracted considerable attention due to their central roles in several

post-transcriptional processes. In *Saccharomyces cerevisiae*, Δ Pat1p strains show reduced cell growth and viability (1), whereas *patr-1* null mutants are lethal in late embryonic or early larval stages in *Caenorhabditis elegans* (2). Yeast cells and invertebrates possess only one Pat1 protein, whereas there are two differentially expressed Pat1 paralogues in vertebrates, with germline (Pat1a) and somatic (Pat1b) versions. The roles of Pat1 proteins in mRNA metabolism are relatively well-conserved across species and include translation initiation, translational repression as well as mRNA deadenylation and activation of decapping. Accordingly, Pat1 proteins are RNA-binding proteins that localise to P-bodies and are associated with large RNP complexes composed of cytoplasmic proteins with identities in line with Pat1 proteins functions and localisation. In addition, Pat1b was recently shown to be a nucleocytoplasmic protein in human cell lines, and its roles and partners in the nucleus remain to be characterised. Studying the role of Pat1 proteins in the nucleus may unveil a novel control over the life and death of (some?) mRNA, as Pat1 proteins may link transcription and/or processing with their degradation, thereby providing a tighter regulation over these mRNAs.

Pat1 proteins are not highly conserved and the two vertebrate Pat1 proteins have evolved different functions

Pat1 are evolutionarily-conserved proteins found only in eukaryotes. Two Pat1 proteins have evolved in vertebrates, Pat1a and Pat1b, in contrast to the single protein in yeast and invertebrates. Human Pat1a and Pat1b are equidistantly related to yeast Pat1p with ~17% identity (~29% similarity) (3). The level of conservation between the Pat1a and b protein paralogues in vertebrates is, on average, ~25% identity (~40% similarity), as illustrated by the human proteins. Despite being relatively poorly conserved at the sequence level, their roles in gene expression control are common from yeast to man.

One of the striking features of the *Xenopus* Pat1 proteins is their mutually exclusive expression profile in early development. Indeed, xPat1a is confined to oogenesis, whereas xPat1b is predominantly expressed in eggs, embryos and somatic tissues (4, 5). This dramatic switch occurs during meiotic maturation, a process during which the prophase I (meiosis I) arrested *Xenopus* oocytes undergo meiotic progression leading to the formation of an egg arrested at meiosis II and awaiting fertilisation. This process is triggered by a hormonal signal triggering signalling cascades. These cascades ultimately result in dramatic changes in protein expression

patterns, with some proteins being degraded and others newly synthesised at specific times and thereby enabling cell cycle progression (6). The proteolysis of xPat1a during meiosis is accompanied by xPat1b synthesis, and yet the importance of this rapid switch is not understood. We suspect that the expression profile of mammalian Pat1 proteins will resemble that of *Xenopus*, as indirectly evidenced by the lack of human Pat1a in somatic tissues and tissue culture cell lines in which human Pat1b is present, as shown by qPCR and northern blot analysis (4, 7). In other words, vertebrates possess germline and somatic forms of Pat1 proteins, which possibly enable them to perform distinct functions dictated by the cell context.

Indeed, we have shown that xPat1a is a negative regulator of translation in oocytes. This function is likely due to the interaction of xPat1a with the large RNP CPEB (cytoplasmic polyadenylation element-binding protein) repression complex that silences oligo(A)-tailed maternal transcripts containing CPE elements in their 3' UTRs (4, 8, 9). These mRNAs are remarkably stable in oocytes due to a lack of decapping activity until stage 12.5 of embryogenesis, characterised by the midblastula transition (MBT) when zygotic transcription ensues (10). It is at the MBT stage that xPat1b is most abundant (4) and presumably performs significant functions. We speculate that xPat1b may promote mRNA decay then, based on experiments in human cell lines, where Pat1b does not repress translation but instead promotes mRNA deadenylation and mRNA decapping, which ultimately leads to 5'-3' mRNA decay (11–13). Interestingly, translational repression and enhanced mRNA decay are performed by the sole yeast Pat1 protein (14, 15). To obtain stronger evidence that Pat1 proteins act as translational repressors in the germ line and as mRNA decay factors in somatic cells, one should systematically assess both Pat1 functions in oocytes and in somatic cells and check the conservation of function by examining human proteins in the *Xenopus* cell context and vice versa. The first proposition has been studied to some extent, although not yet systematically, and it appears that Pat1b can act as a translational repressor in oocytes in a way similar to Pat1a, whereas Pat1a does not promote mRNA decay (nor translational repression) in the somatic cell context, unlike Pat1b (4, 12). The evidence obtained so far suggests that Pat1b proteins are more flexible in their roles than Pat1a proteins, which seem confined to act in their appropriate cellular context.

Pat1 proteins multiple roles in post-transcriptional processes

Despite being relatively poorly conserved overall and having no readily identifiable motifs or sequences (3, 4, 7, 12), Pat1 proteins have been the subject of numerous investigations over the last few years and have been shown to play key conserved functions in translational regulation, mRNA deadenylation and 5'-3' mRNA decay, with the latter two processes very probably linked by the Pat1 proteins themselves.

A critical step in 5'-3' mRNA decay is the transition from an actively translating mRNA to one targeted for degradation (16). Several studies strongly indicate that Pat1 proteins

play important functions in this transition. First, yeast Pat1p is implicated in translation initiation because of its interaction with eIF4E, eIF4G and PABP1 [poly(A) binding-protein 1] and with the 40S-48S ribosomal subunits, as seen in sucrose gradients, and the observation that it also co-immunoprecipitates (co-IPs) with ribosomal proteins (17–20). This is, however, not a universal feature of Pat1 proteins, as human Pat1b does not interact with eIF4E nor with eIF4G (12). Second, yeast Pat1p and *Xenopus* Pat1 proteins negatively regulate translation. In the case of yeast, overexpressing full-length Pat1p leads to a global decrease in protein synthesis as seen with [³⁵S]-methionine incorporation, without affecting mRNAs levels (15, 21). Translation inhibition was later confirmed by assessing the effect of recombinant Pat1p fragments on a luciferase reporter mRNA in yeast cell extracts (20). Interestingly, although this repression was shown to be cap-independent, it results from reduced interaction between the 43S complex and mRNA (20). Of note, Dhh1, a Pat1 protein partner, plays a redundant function with Pat1 in translational repression and acts in the same manner to silence mRNAs (15, 20). However, Dhh1, unlike Pat1, does not seem stimulate decapping *in vitro*. These findings suggest Dhh1 activates decapping via inhibition of translation initiation, in contrast to Pat1, which both enhances decapping and inhibits translation (16). In *Xenopus* oocytes, xPat1 proteins repress translation of a bound (tethered) luciferase reporter mRNA, but, unlike their yeast counterpart, they do not act as global repressors, as no translational silencing is observed of the control mRNA and there is no reduction in [³⁵S]-methionine incorporation (AM unpublished data, 4, 5). Similarly, hPat1b does not affect [³⁵S]-methionine incorporation in human cell lines in the context of overexpression or knock-down (12). Third, yeast Pat1p, *Drosophila* HPat and human hPat1b all promote 5'-3' mRNA decay. Yeast Pat1p has been reported to act as a decapping activator in deletion strains that accumulated capped but deadenylated mRNAs, and then by the examination of mRNA decapping restoration in complementation assays (14, 17, 21–23). Furthermore, *Drosophila* HPat and human Pat1b promote mRNA deadenylation in tethering assays in tissue culture cells (12, 13, 24).

The role of Pat1 proteins in mRNA decay is also in line with the observation that they interact with the decapping enzymes (Dcp2 and Dcp1a) and their enhancers (Edc3, Ge-1 and rck/p54) and with members of the Ccr4-Caf1-Not1 deadenylation complex, as well as with the exoribonuclease Xrn1 (see Table 1). Many of these protein factors localise in P-bodies where mRNA decay is thought to occur. In the same vein, invertebrate Pat1 proteins and Pat1b localisation to P-bodies is conserved, reinforcing its role in the 5'-3' mRNA decay pathway. Fourth, in line with these functions, Pat1 proteins were demonstrated to bind RNA (4, 11, 21, 24, 25), likely through a novel RNA-binding domain (RBD), as prediction software have failed to identify classical RBDs. Determining the novel RBD of Pat1 proteins and whether they bind mRNAs in a sequence-specific manner is of critical importance to understanding their mode of action.

Table 1 Principal P-body and related factors discussed in this review.

Protein	Features and role(s)	<i>S. cerevisiae</i>	<i>D. melanogaster</i>	<i>C. elegans</i>
Ccr4/Caf1 (Pop2)/Not	MDa complex; deadenylase (also roles in translational repression, transcription regulation, ubiquitylation)	Ccr4/Pop2 (Caf1)/Not	Ccr4/Caf1/Not	CCR4/CAF1 (CCF-1)/Not
CPEB1-4	RNA-binding protein, cytoplasmic polyadenylation factor, translational repressor	<i>absent</i>	Orb 1-2	CPB-1-3, FOG-1
Dcp1(a)	Decapping enzyme catalytic subunit	Dcp1	Dcp1	DCAP-1
Dcp2	Decapping enzyme co-factor	Dcp2	Dcp2	DCAP-2
Edc3/Lsm16	Enhancer of mRNA decapping	Edc3	Edc3	EDC-3
Ge-1/Hedls/ Edc4	Enhancer of mRNA decapping	<i>absent</i>	Ge-1	GE-1
GW182/Tnrc6a-c	miRNP component, binds Ccr4-Not1 complex	<i>absent</i>	Gawky	AIN-1/2
Lsm1-7	Like-Sm protein family, heptameric complex, involved in mRNA decay, binds Pat1 proteins	Lsm1-7	Lsm1-7	Lsm 1-7
Pat1a	RNA-binding protein, translational repressor, in CPEB mRNP, not P-bodies	Pat1	HPat	PATR-1
Pat1b	RNA-binding protein, mRNA decay factor, shuttling protein			
Rap55/Lsm14	RNA-associated protein, translational repressor	Scd6 (Lsm 13)	Trailer hitch (Tral/Lsm15)	CAR-1
Rck/p54/Ddx6	Abundant DEAD-box RNA helicase, enhancer of mRNA decapping, translational repressor	Dhh1	Me31B	CGH-1
Xrn1	5'-3' exoribonuclease	Xrn1	Xrn1/Pacman	XRN-1
eIF4E	Translation initiation factor, cap-binding	eIF4E	eIF4E	eIF4E
4E-T(transporter)	eIF4E-binding protein, nucleocytoplasmic shuttling protein	<i>absent</i>	Cup (related to)	SPN-2/PQN-45

Alphabetical list of P-body components, using human nomenclature. Features and roles, where known, are indicated, as are the corresponding names in yeast, flies and worms. See text for further details.

Where known, and in alphabetical order, are given the protein name origins. CAF1, CCR4-associated factor; CAR-1, cytokinesis, apoptosis and RNA; CCR4, carbon catabolite-repression 4; CGH-1, conserved germline helicase; CPEB, cytoplasmic polyadenylation element-binding; GW182, GW-repeat protein of 182 kDa; Hedls, human enhancer decapping large subunit; HPat, homologous to Pat1; Lsm, like Sm protein; Me31b, maternal expression at 31B; Pat1, protein associated with topoisomerase II; PATR-1, Pat1-related; PQN-45, prion-like-(Q/N-rich)-domain-bearing protein; Rap55, RNA-associated protein of 55 kDa; Rck/p54, a target gene on 11q23 of the t (11;14) (q23;q32) translocation in the B-cell lymphoma cell line RC-K8, of 54 kDa; Scd6, suppressor of clathrin deficiency 6; SPN-2, spindle orientation defective; Tnrc, trinucleotide repeat-containing.

The puzzle over Pat1 proteins domains

To date none of the full-length Pat1 proteins have been purified in recombinant form due to their insolubility (13, 20, A. Marnef, unpublished), and therefore knowledge of their structure is still lacking, as is any information of possible domains. In the light of the near coincident recent publication of seven Pat1 papers in 2010 and the absence of obvious motifs, it is perhaps not surprising that attempts to demarcate potential domain boundaries differ between groups. Nevertheless, one such boundary that separates the proteins into two halves (N- and C-terminal halves) was consistently identified in all Pat1 proteins (4, 5, 11–13, 20, 21, 24). The delineation issue and the wide range of techniques used render the conclusion about each domain function and protein partners fairly complex. This section aims at clarifying these issues, summarised in Figure 1.

Level of conservation

The N-terminal halves of Pat1 proteins are largely poorly conserved apart from a ~50 amino acid long acidic stretch at the very N-termini (see alignments in 7, 12, 24), though mammalian Pat1a proteins appear to have lost part of this region (7, 12). In contrast, the C-terminal half, is for the most part,

well conserved in all Pat1 proteins. The C-terminal portion of hPat1b (the Pat-C region from a.a. 517 to 767), the only predicted extended structured domain, was solved by X-ray crystallography (11). Interestingly, it forms an α - α superhelix, which is typical for the crescent-shaped Pumilio-repeat families of protein, and yet hPat1b's structure arrangement is distinct from Pumilio 1, as hPat1b's α -helix takes a rather irregular L-shaped conformation (11). Commonly, α - α superhelix folds in proteins act as scaffolds for nucleic acid or protein interactions (26). Because of the high sequence conservation in this region across species, it is highly likely that all Pat1 proteins adopt a similar fold.

RNA-binding

The RNA-binding assay used by Pilkington and Parker (21) assessed retention by poly(U)-Sepharose of *in vitro* translated and radiolabelled Pat1p proteins. They found that the full-length protein and two C-terminal regions (III and IV–V) can independently bind RNA, in agreement with Haas et al. (24) who showed that *Drosophila* HPat immunoprecipitates RNA via its C-terminal half, largely mediated by the Mid domain and stimulated by the Pat-C region. The Pat-C region was further shown to directly bind a U₃₀ oligomer in size exclusion chromatography, though other regions were not tested

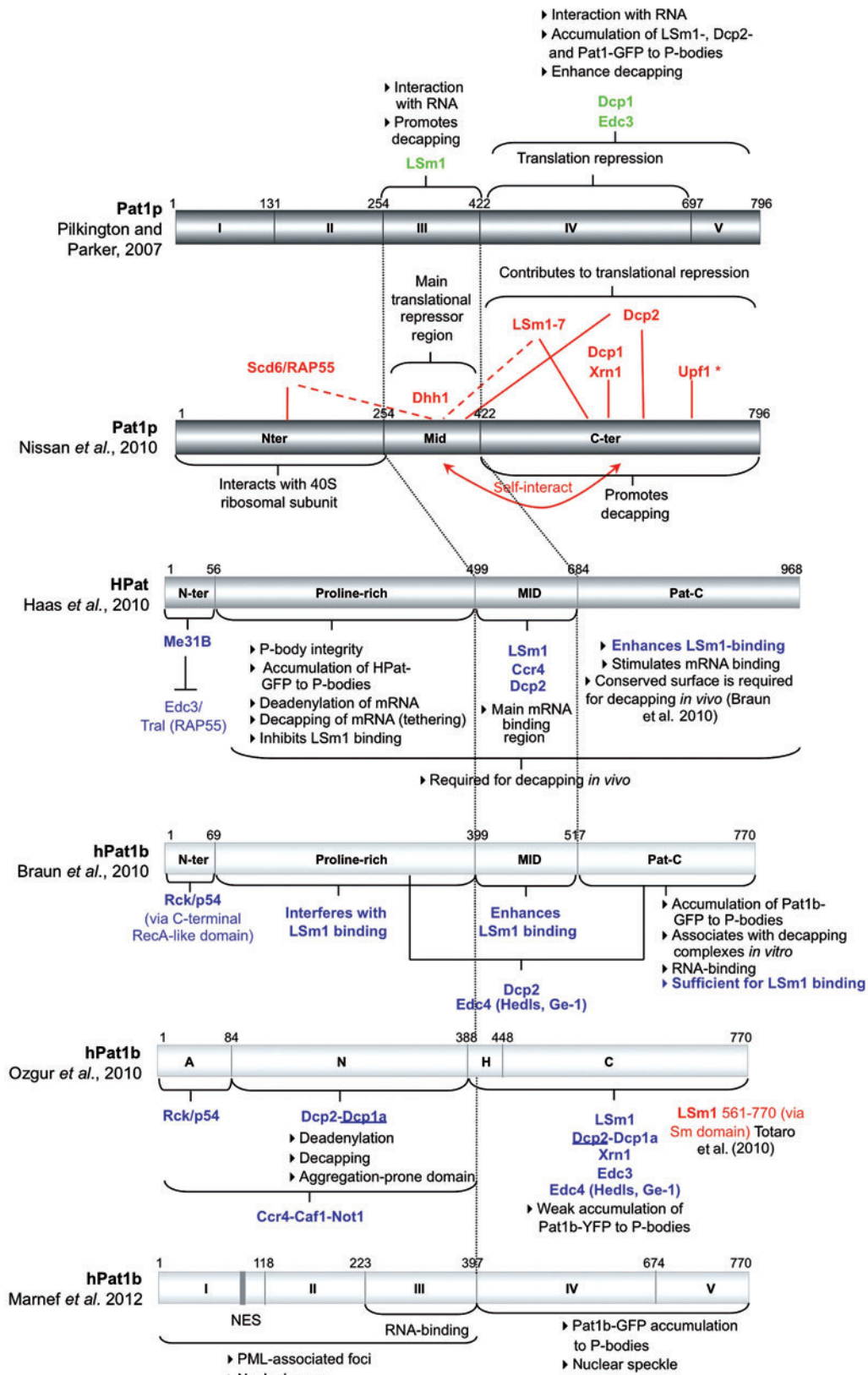


Figure 1 Pat1 protein domains and attributed functions.

Interactions in red were identified using recombinant and purified proteins, with full lines indicating a strong interaction and dashed lines weaker interactions; interactions in green were revealed using yeast-two-hybrid assays, and in blue using co-immunoprecipitation. Underlined proteins indicate their preferred interacting domain. Arrowheads indicate attributed functions.

(11). Our group identified a different RNA-binding region in the case of three vertebrate proteins (xPat1a, xPat1b and hPat1b) using the same technique as Pilkington and Parker (21) and testing all four RNA-homopolymers. These Pat1 proteins preferentially bound poly(G)>poly(U) RNA through a central region (III) in the N-terminal half [with an efficiency similar to that of CPEB, a well-known RNA-binding protein that binds poly(U)], with an α -helix playing an important role in the binding. Similar to Braun et al. (11), we also found that the C-terminal half of hPat1b can bind RNA, though less efficiently than the full-length protein and the central region (4, 25). Altogether, all Pat1 proteins tested bound RNA, though systematic analyses are still lacking largely due to the difficulty of obtaining recombinant proteins.

Translational repression

To date, only yeast Pat1p and *Xenopus* Pat1 proteins have been shown to inhibit protein synthesis. Roy Parker's group assessed the ability of Pat1p domains to repress translation using two methods over the years, with somewhat differing conclusions. In 2008, they tested the effect of overexpressing various truncated forms of Pat1p on [³⁵S]-methionine incorporation *in vivo* and showed the C-terminal half (IV-V) to be the main repressor domain (21). Subsequently, using recombinant regions of Pat1p, they found that it was the Mid-region [III in Pilkington and Parker (21)] that acts as the repressor domain, with the C-terminal domain contributing weakly to the repression of the luciferase reporter mRNA in yeast cell free extracts (20). The manner in which Pat1p repressed translation was further investigated here, and the Mid and C-ter regions were shown to reduce the accumulation of 48S complex formation, probably by preventing 43S association with the mRNA. In *Xenopus* oocytes, using the classical MS2 tethering assay (27), we showed that xPat1a N- and C-terminal halves both repress translation, though they did so less efficiently than the full-length protein (A. Marnef, unpublished). This ability of both domains to repress translation may be due to the fact that the N-terminus co-IPs with Xp54 RNA helicase (rck/p54), whereas the C-terminus interacts with CPEB, the two main constituents of the CPEB RNP repression complex (28).

Deadenylation

Drosophila HPat and human Pat1b proteins trigger deadenylation when tethered to a reporter mRNA, a function that is mediated via the same N-terminal half region (11, 12, 24). However, Pat1 proteins are not involved in poly(A) tail shortening *per se*, as HPat depletion does not prevent deadenylation (24).

Decapping

In yeast, Nissan et al. (20) showed that Pat1p stimulation of decapping is mediated via its C-terminal domain (region

IV–V). Pilkington and Parker (21) had previously reported that this function was performed by region III, corresponding to the Mid-domain (Figure 1), whereas region IV–V enhanced decapping activity. These differences may simply lie in the assays used: examination of the release of a [³²P]-labelled cap (20) or assessing deletion mutants to promote MA2FpG decapping (21). In *Drosophila*, tethered mRNA decapping is promoted by the proline-rich region, whereas, in a complementation assay, PatC is required to restore decapping in depleted cells, with minor contributions by the P-rich and Mid-domains, though not N-ter (11, 24). As proposed by Haas et al. (24), this discrepancy may be reconciled as the RNA-binding abilities of Pat1 Mid and C-ter regions are not required in the tethering assay (although this function may be critical for the way Pat1 induces decapping), in contrast to the complementation assay. In human cells, there is also contrasting evidence regarding the region required for mRNA decapping. Indeed Ozgur et al. (12) identified the N-terminal region N as a decapping domain by analysing the decay and deadenylation status of a reporter mRNA when tethered to hPat1b fragments, whereas Braun et al. (11) reported that Pat-C is required for hPat1b incorporation into active decapping complexes *in vitro*. Altogether, the region responsible for mRNA decapping is not clear-cut, as its definition appears rather sensitive to the experimental assay, as was the case for the repression role of Pat1 proteins. As Pat1 is now considered a scaffold protein, with multiple and complex interactions (Figure 1), these data altogether suggest that more than one domain is involved in decapping and in translational repression.

The function of Pat1 proteins makes sense in terms of their interacting partners

To perform their numerous cytoplasmic functions, Pat1 proteins interact with mRNA and many protein factors involved in translational control, as well as in mRNA decay through separate regions. In this section, protein partners of individual Pat1 proteins will be discussed separately and are shown in Figure 1 (for naming, see Table 1). Individual partners are only discussed in detail if they are of particular interest or there is a case of conflicting evidence.

Yeast Pat1p

Pat1p interacts with Lsm1, Lsm3, Dcp2, Dcp1, Edc3, Dhh1 and Upf1, as shown by yeast-two-hybrid assays (21–23, 29–32) and, in agreement, immunoprecipitates in an RNA-independent manner with Dhh1, Lsm1 and Lsm5 (17, 19, 23, 33, 34). These interactions and more were validated using recombinant proteins in pull-down assays (20) (Figure 1).

Pat1p and Lsm1–7 form a stable complex that can be successfully co-purified from yeast extract (35) and that interacts in an RNA-dependent fashion with the Dcp1–2 complex (19). As Dcp2 binds Pat1p directly (20), *in vivo* Pat1p may interact with Dcp2 independently of the LSm1–7 complex, or the Dcp2-Pat1p interaction is transient.

Surprisingly, Pat1p was also found to self-interact via its Mid- and C-ter domains. This was somewhat unexpected, as one might have predicted a self-interaction to occur through its predicted aggregation-prone region, rich in glutamine/asparagine (Q/N), and proline residues in its N-terminal half (36), which is also a conserved feature in *Drosophila* and human Pat1 proteins (11–13, 24).

***Drosophila* HPat**

In co-IP assays in S2 tissue culture cells, tagged-HPat was shown to bind tagged-Me31B (Dhh1/rck/p54 homologue), -Lsm1, 3 and 7, in the absence of RNA, indicating that it interacts with the entire Lsm1–7 complex, with -Dcp2, and with -Pop2, -Not2, -Not3.5, -Not4 and -CCR4 and, hence, probably with the entire CCR4-NOT complex. No interaction was identified with Edc4 (Hedls/Ge-1 homologue), unlike for hPat1b, nor with Xrn1, in contrast to yeast and human Pat1 proteins (11, 12, 20, 24).

Interestingly, HPat interacts with Me31B through its N-ter region of 56 residues, a binding that is mutually exclusive to that of Edc3, as they both compete for the same Me31B-binding site in the C-terminal Rec-A-like domain. A similar competition was also observed between Edc3 and Tral (Rap55) for Me31B binding (24, 37, 38).

It was proposed that the entire Lsm1–7 complex binds HPat Mid-domain as Lsm1 binds via this region, a binding enhanced by the Pat-C domain and inhibited by the proline-rich region (11, 24). However, these findings must be tempered by the observations made in yeast that revealed dissimilar results when analysing the Lsm1 construct only or when using the entire purified Lsm1–7 complex (20, 21).

Human Pat1b

hPat1b interacts with similar proteins to those that bind the yeast and *Drosophila* Pat1, though the regions involved vary slightly (Figure 1), and there is good agreement between the three groups that studied hPat1b regarding the protein partners associated with each domain. One should also note that Dhh1 (rck/p54/Me31B)-binding in yeast is dissimilar to that in *Drosophila* and humans, even though the first ~56 a.a. involved in the binding are relatively well conserved in yeast (11, 12, 20, 24).

Overall, apart from the deadenylation activity, it is not possible to pinpoint any one conserved Pat1 region as responsible for a specific role or binding specific protein partners. This is somewhat surprising as their functions are relatively well conserved across species. Pat1 may have evolved this flexibility to take advantage of the variation in the non-core 5′-3′ mRNA decay machinery between yeast and vertebrates (illustrated by the absence of an enhancer of decapping Edc4/Ge-1 in yeast), as recently suggested (39), which is in agreement with its proposed role as a scaffold proteins linking deadenylation and decapping.

Pat1 proteins and P-bodies

Consistent with their functions in translational repression and mRNA decay, Pat1 proteins localise to P(rocessing)-bodies in yeast, *C. elegans* embryos, and *Drosophila* S2 and mammalian tissue culture cells (2, 4, 7, 11, 12, 40–42). P-bodies are cytoplasmic foci conserved across eukaryotes that harbour repressed mRNAs, 5′-3′ mRNA decay factors, including Dcp1/2, enhancers of decapping such as Edc3, as well as the Xrn1 exonuclease, translational repressors including rck/p54, CPEB1 and Pat1, miRNP components and only one translation initiation factor, the cap-binding protein translation initiation factor eIF4E and its interacting factor 4E-T, but no ribosomes (reviewed by 43 and 44).

Currently, the molecular details of the P-body pathway in gene expression control remain to be determined, although it is generally thought that silenced mRNAs resident in P-bodies undergo 5′-3′ mRNA decay or return to translation (43–46). Certainly, individual components, for example GW182 and rck/p54, have well-characterised important functions in general and in miRNP-mediated gene expression control. However, the visible presence of P-bodies is apparently not required for mRNA decay mediated by microRNAs, siRNAs, via the NMD pathway or for general mRNA decay/translational repression in yeast (41, 47–49). AU-rich (ARE) mRNAs have been shown to be degraded in mammalian P-bodies (50, 51), and it has also been reported recently that specific mRNA decay and/or translational repression occurs in these cytoplasmic foci (52–56), which, taken together, suggests that they may control a sub-set of mRNAs. To date, however, it has not been possible to biochemically purify these RNP granules to test this directly.

No single component is responsible for P-body assembly; rather, it appears that their formation occurs via multiple redundant protein factors. The core constituents critical for mammalian P-body assembly, for example, include rck/p54, Ge-1, Rap55, Ccr4, GW182, Lsm1, 4E-T and CPEB, and in most cases (where present and tested), their yeast and fly homologues act similarly (40, 57–62). However, Pat1 proteins appear to affect P-body formation differently between species and even cell lines. On the one hand, yeast Pat1p is not required for P-body formation; on the other hand, depletion of *Drosophila* HPat results in P-body disappearance in tissue culture cells. Likewise, loss of *C. elegans* PATR-1 disassembles P-bodies in embryos (2, 15, 40, 41). In HeLa cells, we observed, somewhat surprisingly, that approximately half the cells depleted of Pat1b by siRNA (and shown to lack Pat1b by immunostaining) possess a normal number of P-bodies containing rck/p54, and half do not, which suggests possible cell cycle involvement in P-body formation by Pat1b (4). However, in COS7 cells, Pat1b depletion reduces the number of cells containing large numbers of P-bodies and increases the cells lacking P-bodies, implying a straightforward function in P-body assembly (12).

Fluorescently-tagged Pat1a proteins do not localise to P-bodies in human tissue culture cells, which may simply arise from their absence in somatic cells and, hence, lack of appropriate protein partners. More significantly, Pat1a proteins have

a dominant negative effect on endogenous P-bodies, likely because Pat1a binds a P-body component critical for their formation, namely Lsm1 (4, 12). It would therefore be interesting to further investigate the role of the mammalian Pat1a proteins in mouse oocytes, for example, where the presence of P-body-like foci was revealed by the detection of Dcp1a, Rap55 and rck/p54 (63). Altogether more systematic studies, including careful analysis of binding partners in different cell types and domain swap experiments, may shed further light on the function of the two vertebrate Pat1a/b proteins.

Most studies agree that the localisation of Pat1 proteins to P-bodies is mediated via their C-terminal half, except in *Drosophila*, where it is mediated via the N-terminal half domain (11, 12, 20, 21, 24), (A. Marnef, unpublished) (Figure 1). Yet two common features are shared between human and *Drosophila* Pat1 proteins: 1) the region targeting them to P-bodies is not as efficient as the full-length protein, which suggests that several regions contribute to their accumulation therein; and 2) their N-terminal regions result in a dominant negative effect on endogenous P-bodies, for which the underlying reasons are unclear (4, 12, 24). This is an interesting finding, as these regions promote mRNA deadenylation and mRNA decapping (12, 24), further reinforcing the view that P-bodies are not required for these processes (41).

Link between PKA, Pat1p and P-body formation

In *S. cerevisiae*, P-bodies are not found constitutively, as they are in mammalian cells, but are induced during growth arrest, for example, when cells are starved of glucose and translation is inhibited. Earlier work demonstrated that cAMP-dependent protein kinase (PKA) mutants were resistant to the inhibitory effect of glucose withdrawal on translation (64), which implicates PKA in the translational response to rapid glucose removal. A recent study shows that PKA is a key negative regulator of P-body assembly in yeast; its inactivation is required for P-body formation (65). The effects are due, at least in part, to direct phosphorylation of Pat1p, which disrupts Dhh1 (rck/p54) binding and foci formation. Strikingly, the authors provide evidence that Pat1p phosphorylation and perhaps P-bodies are responsible for the efficient long-term survival of cells in stationary phase and make the intriguing correlation with RNP granules in oocytes that store maternal mRNAs for later use, as we previously suggested (9). Along these lines, we showed that Pat1b undergoes mobility shifts in SDS-PAGE indicative of multiple phosphorylations in *Xenopus* oocytes, eggs and embryos and mapped one major site to Serine 62, which is, however, not conserved in vertebrates (4). The yeast Pat1p PKA phosphorylation site RRRSSY is not obviously shared by vertebrate Pat1 proteins, so it remains to be examined whether such regulation is universal. And last, PKA regulation of foci formation via Pat1p phosphorylation is, at first sight at least, somewhat at odds with previous reports that P-bodies remain present under glucose starvation conditions in strains lacking Pat1p (15, 56, 66, 67). However, as Collier and Parker (15) showed, although a Dhh1-deleted strain also formed P-bodies, a strain lacking

both Pat1 and Dhh1 did not. In other words, it seems that to assemble P-bodies, either Dhh1 or Pat1 is required, and that, when both are present, they need to interact (65).

Possible roles of nuclear human Pat1b

Early studies suggested that yeast Pat1p may have nuclear functions as well as cytoplasmic ones. Indeed, Pat1p was initially characterised and named (Protein associated with topoisomerase II) as a protein that interacts directly and co-immunoprecipitates with topoisomerase II and that can bind ssDNA (1, 68). Later, Pat1p was found in both nuclear and cytoplasmic fractions (18), and though predominantly cytoplasmic in wild-type strains, Pat1p partially relocalised to the nucleus upon Lsm1 deletion (66). Moreover, Pat1p interacted with Crm1p, the nuclear export signal receptor, in a global yeast-two-hybrid screen for proteins with nuclear export activity (69).

Using human cell lines, we recently demonstrated Pat1b to be a nucleocytoplasmic protein for which nuclear export is mediated via a consensus nuclear export sequence (NES) and Crm1, as evidenced with Leptomycin B (LMB) treatment (25). We proposed that all vertebrate Pat1b proteins that shuttle using this NES as the critical hydrophobic residues are conserved. In contrast, its paralogue hPat1a is not a Crm1-dependent nucleocytoplasmic protein, likely reflecting its lack of the N-terminal portion encompassing the Pat1b NES sequence in mammals. These results further underline the differences between the functions of these paralogue sub-families (4).

The shuttling characteristics of Pat1 are not shared by all P-body components, except for 4E-T, as neither Dcp1a, rck/p54, Edc3, Ge-1 nor Xrn1 shuttle into nuclei (4, 70). This is in very good agreement with the genome-wide analysis of the *Schizosaccharomyces pombe* ORFeome, which shows that only Pat1 (SPBC19G7.10c) relocalises from cytoplasmic foci, presumably P-bodies, to the nucleus upon LMB treatment. Dcp1 and Dcp2, Ste13 (rck/p54/Dhh1), Edc3, Sum2 (RAP55/Scd6) and Ccr4 are unaffected by LMB (71), *S. pombe* post-genome database (Ge-1 and 4E-T are absent from yeast).

Strikingly, only 35% of hPat1b is diffuse in the nucleoplasm, whereas the rest display two specific subnuclear localisations. Indeed, most (40%) hPat1b localises to splicing speckles. Splicing speckles are enriched in poly(A)⁺ mRNA and pre-mRNA splicing factors, including SR proteins as well as small nuclear ribonucleoprotein particles (snRNPs) and are often found close to active transcription sites (72). They are thought to provide splicing factors to these transcription sites by acting as assembly/storage/modification compartments (73). In addition, the observation that hPat1b mirrors the localisation of most factors found in speckles upon inhibition of splicing with Spliceostatin A suggests a role for hPat1b in mRNA splicing-related processes (74–76). The potential role in splicing is also in line with the study by Stevens et al. (77), who found yeast Pat1p to be associated with the penta-snRNP. The remaining hPat1b localises to nuclear foci associated with PML bodies (25). PML bodies are dynamic structures implicated in a wide variety of processes and that contain the PML (promyelocytic

leukaemia) protein as well as several seemingly unrelated proteins, including SUMO and CBP. They are thought to play roles in sumoylation, transcriptional regulation and/or nuclear storage (78). Remarkably, hPat1b localisation to these two subnuclear structures depends on active transcription. Therefore, nuclear hPat1b probably requires mRNA to display these two subnuclear localisations, although it is less likely that it exits the nucleus bound to mRNAs, as the bulk of mRNAs are exported via the TAP/Nfx1 (Mex67 in yeast) pathway, rather than via Crm1 (79). Another key feature of nuclear hPat1b upon inhibition of transcription is its relocation to nucleolar caps in ~64% of the cells. When examining the functions of speckle proteins that have been reported to redistribute to nucleolar caps upon ActD treatment, we found that most are implicated in alternative splicing or in miRNA processing or both. Proteins with these properties include EWS (Ewing sarcoma breakpoint region 1 gene), TLS, hnRNPs, p68 RNA helicase and CBP20 (80–82). Indeed, pri-miRNAs have recently been visualised in speckles. However, these are apparently unlikely to represent sites of miRNA transcription or processing but may represent their accumulation/storage sites (83).

Unexpectedly, Pat1b retention in these three nuclear compartments is mediated via distinct regions of the protein. Localisations to PML-associated nuclear foci and nucleolar caps is mediated via its N-terminus and splicing speckles via the C-terminus. These three distinct localisation sites may reflect Pat1b interactions with multiple proteins in the nucleus, likely reflecting its participation in several RNA-related nuclear processes similar to its scaffold roles in the cytoplasm (11–13).

And last, an intriguing coupling between transcription and mRNA decay with a potential role for Pat1 proteins is emerging in yeast. Rpb7 and Rpb4, heterodimer subunits of RNA polymerase II, shuttle between the nucleus and the cytoplasm and mediate transcription, stimulate translation and are also implicated in mRNA decay (84–87). Furthermore, yeast Rpb4 and Rpb7 interact directly with Pat1p in the yeast two hybrid assay (84, 85). Interestingly, the Ccr4-Not1 complex binds Pat1, as well as the core subunits of RNA polymerase II, and plays roles in both transcription regulation and cytoplasmic mRNA deadenylation (88–90). A direct coupling between the regulation of transcription and mRNA degradation was reported to be a common phenomenon employed by approximately 10% of the genes in yeast and to be mediated, in part at least, by Ccr4-Not1 and Rbp4/7 (91). Could Pat1 act similarly and could this be a way of linking transcription and mRNA decay, thereby controlling the level of mRNA from birth to death? The potential transcription-mRNA decay link has been further strengthened recently by two teams that showed that, in yeast, transcription factors and promoters can directly influence the relative stability of mRNAs that they generate (92, 93). Or is the role of Pat1 in the nucleus independent of its cytoplasmic role?

Other roles for Pat1 proteins

In addition to their role in translational repression and mRNA decay, Pat1 proteins may also take part in miRNA silencing.

Drosophila HPat was shown to regulate 15% of Argonaute 1 (AGO1) mRNA targets, as determined by microarray analysis (94), and to interact with GW182, a P-body miRNP component (95). Moreover, nuclear hPat1b may play a role in miRNA biogenesis, as it follows the localisation pattern of many proteins involved in this process. Therefore, future experiments focusing on the potential mRNA function of Pat1 proteins would be useful to shed light on the full range of processes in which Pat1 proteins are involved.

Furthermore, Pat1p was recently reported to be involved in tRNA nuclear-cytoplasmic dynamics together with Dhh1 (96). That study reported that nutrient-deprived yeast cells lacking Dhh1 and Pat1p do not accumulate tRNA within their nuclei, nor do they repress translation or induce P-body formation. Conversely, inhibition of translation initiation and induction of P-body formation by overproduction of Dhh1 or Pat1p causes tRNA nuclear accumulation in nutrient-replete conditions. The coordination between P-body formation, translation repression and tRNA distribution is limited to the early part of this pathway, as loss of mRNA decapping or 5'–3' degradation does not influence tRNA nuclear-cytoplasmic dynamics.

And also, strikingly, as some other P-body components, in particular Dhh1 (rck/p54), Pat1 proteins play as yet unidentified roles in Hepatitis C virus translation and replication, BMV translation and Ty1 retrotransposition (97–101).

Future directions

We envisage three key future studies to refine our knowledge of the roles of Pat1 proteins in RNA-related processes. First, purifying the full-length protein for structural studies, and to assess its direct effect in the cytoplasmic processes in available *in vitro* assays, is of key importance. Second, identification of the mRNA targets of Pat1 proteins and determination of whether they regulate all or a subset of mRNAs will shed light on their principal path of action in the control of gene expression. The localisation of these Pat1-associated mRNAs will also be interesting to examine, as it will reveal whether all Pat1-bound mRNAs go through P-bodies, or only a subset, and whether they are degraded or repressed. On a wider scale, it would also be interesting to know whether Pat1 proteins regulate similar mRNA across species, which might possibly reveal how mRNAs are targeted for deadenylation, decapping and decay. Third, understanding the influence of Pat1 proteins over the birth and death of mRNAs, as is the case for two of its partners Rpb4 and 7, may extend the emerging coupling between transcription and mRNA decay. This could be achieved by studying the role of nuclear Pat1 proteins using Pat1 NES-mutant proteins (to identify associated-protein and mRNAs) and a NLS-mutant construct (once the NLS is defined) to examine how mRNA deadenylation and decay are affected if Pat1 does not shuttle into the nucleus. However, designing or choosing the appropriate functional assay to understand the role of Pat1 proteins in each particular setting (P-bodies, splicing speckles, PML bodies and nucleolar caps) is likely to be less than simple, as highlighted by the

somewhat limited direct evidence obtained so far in the quest to understand the roles of these subcellular structures.

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References

- Wang X, Watt PM, Louis EJ, Borts RH, Hickson ID. Pat1: a topoisomerase II-associated protein required for faithful chromosome transmission in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 1996; 24: 4791–97.
- Boag PR, Atalay A, Robida S, Reinke V, Blackwell TK. Protection of specific maternal messenger RNAs by the P body protein CGH-1 (Dhh1/RCK) during *Caenorhabditis elegans* oogenesis. *J Cell Biol* 2008; 182: 543–57.
- Marnef A, Standart N. Pat1 proteins: a life in translation, translation repression and mRNA decay. *Biochem Soc Trans* 2010; 38: 1602–7.
- Marnef A, Maldonado M, Bugaut A, Balasubramanian S, Kress M, Weil D, Standart N. Distinct functions of maternal and somatic Pat1 protein paralogs. *RNA* 2010; 16: 2094–107.
- Nakamura Y, Tanaka KJ, Miyauchi M, Huang L, Tsujimoto M, Matsumoto K. Translational repression by the oocyte-specific protein P100 in *Xenopus*. *Dev Biol* 2010; 344: 272–83.
- Stanford JS, Lieberman SL, Wong VL, Ruderman JV. Regulation of the G2/M transition in oocytes of *Xenopus tropicalis*. *Dev Biol* 2003; 260: 438–48.
- Scheller N, Resa-Infante P, de la Luna S, Galao RP, Albrecht M, Kaestner L, Lipp P, Lengauer T, Meyerhans A, Díez J. Identification of PatL1, a human homolog to yeast P body component Pat1. *Biochim Biophys Acta* 2007; 1773: 1786–92.
- Radford HE, Meijer HA, de Moor CH. Translational control by cytoplasmic polyadenylation in *Xenopus* oocytes. *Biochim Biophys Acta* 2008; 1779: 217–29.
- Standart N, Minshall N. Translational control in early development: CPEB, P-bodies and germinal granules. *Biochem Soc Trans* 2008; 36: 671–6.
- Gillian-Daniel DL, Gray NK, Astrom J, Barkoff A, Wickens M. Modifications of the 5' cap of mRNAs during *Xenopus* oocyte maturation: independence from changes in poly(A) length and impact on translation. *Mol Cell Biol* 1998; 18: 6152–3.
- Braun JE, Tritschler F, Haas G, Igraja C, Truffault V, Weichenrieder O, Izaurralde E. The C-terminal alpha-alpha superhelix of Pat is required for mRNA decapping in metazoa. *EMBO J* 2010; 29: 2368–80.
- Ozgun S, Chekulaeva M, Stoecklin G. Human Pat1b connects deadenylation with mRNA decapping and controls the assembly of processing bodies. *Mol Cell Biol* 2010; 30: 4308–23.
- Totaro A, Renzi F, La Fata G, Mattioli C, Raabe M, Urlaub H, Achsel T. The human Pat1b protein: a novel mRNA deadenylation factor identified by a new immunoprecipitation technique. *Nucleic Acids Res* 2011; 39: 634–47.
- Hatfield L, Beelman CA, Stevens A, Parker R. Mutations in transacting factors affecting mRNA decapping in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1996; 16: 5830–8.
- Coller J, Parker R. General translational repression by activators of mRNA decapping. *Cell* 2005; 122: 875–86.
- Balagopal V, Fluch L, Nissan T. Ways and means of eukaryotic mRNA decay. *Biochim Biophys Acta* 2012; 1819: 593–603.
- Bonnerot C, Boeck R, Lapeyre B. The two proteins Pat1p (Mrt1p) and Spb8p interact in vivo, are required for mRNA decay, and are functionally linked to Pab1p. *Mol Cell Biol* 2000; 20: 5939–46.
- Wyers F, Minet M, Dufour ME, Vo LT, Lacroute F. Deletion of the PAT1 gene affects translation initiation and suppresses a PAB1 gene deletion in yeast. *Mol Cell Biol* 2000; 20: 3538–49.
- Tharun S, Parker R. Targeting an mRNA for decapping: displacement of translation factors and association of the Lsm1p-7p complex on deadenylated yeast mRNAs. *Mol Cell* 2001; 8: 1075–83.
- Nissan T, Rajyaguru P, She M, Song H, Parker R. Decapping activators in *Saccharomyces cerevisiae* act by multiple mechanisms. *Mol Cell* 2010; 39: 773–83.
- Pilkington GR, Parker R. Pat1 contains distinct functional domains that promote P-body assembly and activation of decapping. *Mol Cell Biol* 2008; 28: 1298–312.
- Bouveret E, Rigaut G, Shevchenko A, Wilm M, Seraphin B. A Sm-like protein complex that participates in mRNA degradation. *EMBO J* 2000; 19: 1661–71.
- Tharun S, He WH, Mayes AE, Lennertz P, Beggs JD, Parker R. Yeast Sm-like proteins function in mRNA decapping and decay. *Nature* 2000; 404: 515–18.
- Haas G, Braun JE, Igraja C, Tritschler F, Nishihara T, Izaurralde E. HPat provides a link between deadenylation and decapping in metazoa. *J Cell Biol* 2010; 189: 289–302.
- Marnef A, Weil D, Standart N. RNA-related nuclear functions of human Pat1b, the P-body mRNA decay factor. *Mol Biol Cell* 2012; 23: 213–24.
- Edwards TA, Pyle SE, Wharton RP, Aggarwal AK. Structure of Pumilio reveals similarity between RNA and peptide binding motifs. *Cell* 2001; 105: 281–9.
- Minshall N, Allison R, Marnef A, Wilczynska A, Standart N. Translational control assessed using the tethered function assay in *Xenopus* oocytes. *Methods* 2010; 51: 165–9.
- Minshall N, Reiter M-H, Weil D, Standart N. CPEB interacts with an ovary-specific eIF4E and 4E-T in early *Xenopus* oocytes. *J Biol Chem* 2007; 282: 37389–401.
- Fromont-Racine M, Mayes AE, Brunet-Simon A, Rain JC, Colley A, Dix I, Decourty L, Joly N, Ricard F, Beggs JD, Legrain P. Genome-wide protein interaction screens reveal functional networks involving Sm-like proteins. *Yeast* 2000; 17: 95–110.
- Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P, Qureshi-Emili A, Li Y, Godwin B, Conover D, Kalbfleisch T, Vijayadamar G, Yang M, Johnston M, Fields S, Rothberg JM. A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* 2000; 403: 623–7.
- Ito T, Chiba T, Ozawa R, Yoshida M, Hattori M, Sakaki Y. A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc Natl Acad Sci USA* 2001; 98: 4569–74.
- Swisher KD, Parker R. Interactions between Upf1 and the decapping factors Edc3 and Pat1 in *Saccharomyces cerevisiae*. *PLoS One* 2011; 6: e26547.
- Coller JM, Tucker M, Sheth U, Parker R. The DEAD box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes. *RNA* 2001; 7: 1717–27.
- Fischer N, Weis K. The DEAD box protein Dhh1 stimulates the decapping enzyme Dcp1. *EMBO J* 2002; 21: 2788–97.

35. Chowdhury A, Mukhopadhyay J, Tharun S. The decapping activator Lsm1p-7p-Pat1p complex has the intrinsic ability to distinguish between oligoadenylated and polyadenylated RNAs. *RNA* 2007; 13: 998–1016.
36. Reijns MA, Alexander RD, Spiller MP, Beggs JD. A role for Q/N-rich aggregation-prone regions in P-body localization. *J Cell Sci* 2008; 121: 2463–72.
37. Tritschler F, Eulalio A, Helms S, Schmidt S, Coles M, Weichenrieder O, Izaurralde E, Truffault V. Similar modes of interaction enable Trailer Hitch and EDC3 to associate with DCP1 and Me31B in distinct protein complexes. *Mol Cell Biol* 2008; 28: 6695–708.
38. Tritschler F, Braun JE, Eulalio A, Truffault V, Izaurralde E, Weichenrieder O. Structural basis for the mutually exclusive anchoring of P body components EDC3 and Tral to the DEAD box protein DDX6/Me31B. *Mol Cell* 2009; 33: 661–8.
39. Fromm SA, Truffault V, Kamenz J, Braun JE, Hoffmann NA, Izaurralde E, Sprangers R. The structural basis of Edc3- and Scd6-mediated activation of the Dcp1:Dcp2 mRNA decapping complex. *EMBO J* 2011; 31: 279–90.
40. Sheth U, Parker R. Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science* 2003; 300: 805–8.
41. Eulalio A, Behm-Ansmant I, Schweizer D, Izaurralde E. P-body formation is a consequence, not the cause of RNA-mediated gene silencing. *Mol Cell Biol* 2007; 27: 3970–81.
42. Gallo CM, Munro E, Rasoloson D, Merritt C, Seydoux G. Processing bodies and germ granules are distinct RNA granules that interact in *C. elegans* embryos. *Dev Biol* 2008; 323: 76–87.
43. Eulalio A, Behm-Ansmant I, Izaurralde E. P bodies: at the crossroads of post-transcriptional pathways. *Nat Rev Mol Cell Biol* 2007; 8: 9–22.
44. Kulkarni M, Ozgur S, Stoecklin G. On track with P-bodies. *Biochem Soc Trans* 2010; 38: 242–51.
45. Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W. Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell* 2006; 125: 1111–24.
46. Parker R, Sheth U. P bodies and the control of mRNA translation and degradation. *Mol Cell* 2007; 25: 635–46.
47. Decker CJ, Teixeira D, Parker R. Edc3p and a glutamine/asparagine-rich domain of Lsm4p function in processing body assembly in *Saccharomyces cerevisiae*. *J Cell Biol* 2007; 179: 437–49.
48. Sweet TJ, Boyer B, Hu W, Baker KE, Collier J. Microtubule disruption stimulates P-body formation. *RNA* 2007; 13: 493–502.
49. Stalder L, Muhlemann O. Processing bodies are not required for mammalian nonsense-mediated mRNA decay. *RNA* 2009; 15: 1265–73.
50. Franks TM, Lykke-Andersen J. TTP and BRF proteins nucleate processing body formation to silence mRNAs with AU-rich elements. *Genes Dev* 2007; 21: 719–35.
51. Takahashi S, Sakurai K, Ebihara A, Kajih H, Saito K, Kontani K, Nishina H, Katada T. RhoA activation participates in rearrangement of processing bodies and release of nucleated AU-rich mRNAs. *Nucleic Acids Res* 2011; 39: 3446–57.
52. Glasmacher E, Hoefig KP, Vogel KU, Rath N, Du L, Wolf C, Kremmer E, Wang X, Heissmeyer V. Roquin binds inducible costimulator mRNA and effectors of mRNA decay to induce microRNA-independent post-transcriptional repression. *Nat Immunol* 2010; 11: 725–33.
53. Lee EK, Kim HH, Kuwano Y, Abdelmohsen K, Srikantan S, Subaran SS, Gleichmann M, Mughal MR, Martindale JL, Yang X, Worley PF, Mattson MP, Gorospe M. hnRNP C promotes APP translation by competing with FMRP for APP mRNA recruitment to P bodies. *Nat Struct Mol Biol* 2010; 17: 732–9.
54. Arribere JA, Doudna JA, Gilbert WV. Reconsidering movement of eukaryotic mRNAs between polysomes and P bodies. *Mol Cell* 2011; 44: 745–58.
55. Cui YH, Xiao L, Rao JN, Zou T, Liu L, Chen Y, Turner DJ, Gorospe M, Wang JY. miR-503 represses CUG-binding protein 1 translation by recruiting CUGBP1 mRNA to processing bodies. *Mol Biol Cell* 2012; 23: 151–62.
56. Lavut A, Raveh, D. Sequestration of highly expressed mRNAs in cytoplasmic granules, P-bodies, and stress granules enhances cell viability. *PLoS Genet* 2012; 8: e1002527.
57. Andrei MA, Ingelfinger D, Heintzmann R, Achsel T, Rivera-Pomar R, Lührmann R. A role for eIF4E and eIF4E-transporter in targeting mRNPs to mammalian processing bodies. *RNA* 2005; 11: 717–27.
58. Ferraiuolo MA, Basak S, Dostie J, Murray EL, Schoenberg DR, Sonenberg N. A role for the eIF4E-binding protein in P-body formation and mRNA decay. *J Cell Biol* 2005; 170: 913–24.
59. Yu JH, Yang WH, Gulick T, Bloch KD, Bloch DB. Ge-1 is a central component of the mammalian cytoplasmic mRNA processing body. *RNA* 2005; 11: 1795–802.
60. Yang WH, Yu JH, Gulick T, Bloch KD, Bloch DB. RNA-associated protein 55 (RAP55) localizes to mRNA processing bodies and stress granules. *RNA* 2006; 12: 547–54.
61. Serman A, Le Roy F, Aigueperse C, Kress M, Dautry F, Weil D. GW body disassembly triggered by siRNAs independently of their silencing activity. *Nucleic Acids Res* 2007; 35: 4715–27.
62. Fan SJ, Marchand V, Ephrussi A. *Drosophila* Ge-1 promotes P body formation and oskar mRNA localization. *PLoS One* 2011; 6:e20612.
63. Swetloff A, Conne B, Huarte J, Pitetti JL, Nef S, Vassalli JD. Dcp1-bodies in mouse oocytes. *Mol Biol Cell* 2009; 20: 4951–61.
64. Ashe MP, De Long SK, Sachs AB. Glucose depletion rapidly inhibits translation initiation in yeast. *Mol Biol Cell* 2000; 11: 833–48.
65. Ramachandran V, Shah KH, Herman PK. The cAMP-dependent protein kinase signaling pathway is a key regulator of P body foci formation. *Mol Cell* 2011; 43: 973–81.
66. Teixeira D, Parker R. Analysis of P-Body Assembly in *Saccharomyces cerevisiae*. *Mol Biol Cell* 2007; 18: 2274–87.
67. Kilchert C, Weidner J, Prescianotto-Baschong C, Spang A. Defects in the secretory pathway and high Ca²⁺ induce multiple P-bodies. *Mol Biol Cell* 2010; 21: 2624–38.
68. Rother RP, Frank MB, Thomas PS. Purification, primary structure, bacterial expression and subcellular distribution of an oocyte-specific protein in *Xenopus*. *Eur J Biochem* 1992; 206: 673–83.
69. Jensen TH, Neville M, Rain JC, McCarthy T, Legrain P, Rosbash M. Identification of novel *Saccharomyces cerevisiae* proteins with nuclear export activity: cell cycle-regulated transcription factor ace2p shows cell cycle-independent nucleocytoplasmic shuttling. *Mol Cell Biol* 2000; 20: 8047–58.
70. Dostie J, Ferraiuolo M, Pause A, Adam SA, Sonenberg N. A novel shuttling protein, 4E-T, mediates the nuclear import of the mRNA 5' cap-binding protein, eIF4E. *EMBO J* 2000; 19: 3142–56.
71. Matsuyama A, Arai R, Yashiroda Y, Shirai A, Kamata A, Sekido S, Kobayashi Y, Hashimoto A, Hamamoto M, Hiraoka Y, Horinouchi S, Yoshida M. ORFeome cloning and global analysis of protein localization in the fission yeast *Schizosaccharomyces pombe*. *Nat Biotechnol* 2006; 24: 841–7.

72. Spector DL, Lamond AI. Nuclear Speckles. *Cold Spring Harb Perspect Biol* 2011; 3 pii: a000646.
73. Lamond AI, Spector DL. Nuclear speckles: a model for nuclear organelles. *Nat Rev Mol Cell Biol* 2003; 4: 605–12.
74. O'Keefe RT, Mayeda A, Sadowski CL, Krainer AR, Spector DL. Disruption of pre-mRNA splicing in vivo results in reorganization of splicing factors. *J Cell Biol* 1994; 124: 249–60.
75. Tanackovic G, Kramer A. Human splicing factor SF3a, but not SF1, is essential for pre-mRNA splicing in vivo. *Mol Biol Cell* 2005; 16: 1366–77.
76. Kaida D, Motoyoshi H, Tashiro E, Nojima T, Hagiwara M, Ishigami K, Watanabe H, Kitahara T, Yoshida T, Nakajima H, Tani T, Horinouchi S, Yoshida M. Spliceostatin A targets SF3b and inhibits both splicing and nuclear retention of pre-mRNA. *Nat Chem Biol* 2007; 3: 576–83.
77. Stevens SW, Ryan DE, Ge HY, Moore RE, Young MK, Lee TD, Abelson J. Composition and functional characterization of the yeast spliceosomal penta-snRNP. *Mol Cell* 2002; 9: 31–44.
78. Lallemand-Breitenbach V, de The H. PML nuclear bodies. *Cold Spring Harb Perspect Biol* 2010; 2: a000661.
79. Rodriguez MS, Dargemont C, Stutz F. Nuclear export of RNA. *Biol Cell* 2004; 96: 639–55.
80. Saitoh S, Chabes A, McDonald WH, Thelander L, Yates JR, Russell P. Cid13 is a cytoplasmic poly(A) polymerase that regulates ribonucleotide reductase mRNA. *Cell* 2002; 109: 563–73.
81. Shav-Tal Y, Blechman J, Darzacq X, Montagna C, Dye BT, Patton JG, Singer RH, Zipori D. Dynamic sorting of nuclear components into distinct nucleolar caps during transcriptional inhibition. *Mol Biol Cell* 2005; 16: 2395–413.
82. Iwamoto F, Stadler M, Chalupníková K, Oakeley E, Nagamine Y. Transcription-dependent nucleolar cap localization and possible nuclear function of DEXH RNA helicase RHAU. *Exp Cell Res* 2008; 314: 1378–91.
83. Pawlicki JM, Steitz JA. Subnuclear compartmentalization of transiently expressed polyadenylated pri-microRNAs: processing at transcription sites or accumulation in SC35 foci. *Cell Cycle* 2009; 8: 345–56.
84. Lotan R, Bar-On VG, Harel-Sharvit L, Duek L, Melamed D, Choder M. The RNA polymerase II subunit Rpb4p mediates decay of a specific class of mRNAs. *Genes Dev* 2005; 19: 3004–16.
85. Lotan R, Goler-Baron V, Duek L, Haimovich G, Choder M. The Rpb7p subunit of yeast RNA polymerase II plays roles in the two major cytoplasmic mRNA decay mechanisms. *J Cell Biol* 2007; 178: 1133–43.
86. Goler-Baron V, Selitrennik M, Barkai O, Haimovich G, Lotan R, Choder M. Transcription in the nucleus and mRNA decay in the cytoplasm are coupled processes. *Genes Dev* 2008; 22: 2022–7.
87. Harel-Sharvit L, Eldad N, Haimovich G, Barkai O, Duek L, Choder M. RNA polymerase II subunits link transcription and mRNA decay to translation. *Cell* 2010; 143: 552–63.
88. Tucker M, Valencia-Sanchez MA, Staples RR, Chen J, Denis CL, Parker R. The transcription factor associated Ccr4 and Caf1 proteins are components of the major cytoplasmic mRNA deadenylase in *Saccharomyces cerevisiae*. *Cell* 2001; 104: 377–86.
89. Chen J, Chiang YC, Denis CL. CCR4, a 3'-5' poly(A) RNA and ssDNA exonuclease, is the catalytic component of the cytoplasmic deadenylase. *EMBO J* 2002; 21: 1414–26.
90. Kruk JA, Dutta A, Fu J, Gilmour DS, Reese JC. The multifunctional Ccr4-Not complex directly promotes transcription elongation. *Genes Dev* 2011; 25: 581–93.
91. Dori-Bachash M, Shema E, Tirosh I. Coupled evolution of transcription and mRNA degradation. *PLoS Biol* 2011; 9: e1001106.
92. Bregman A, Avraham-Kelbert M, Barkai O, Duek L, Guterman A, Choder M. Promoter elements regulate cytoplasmic mRNA decay. *Cell* 2011; 147: 1473–83.
93. Treck T, Larson DR, Moldón A, Query CC, Singer RH. Single-molecule mRNA decay measurements reveal promoter-regulated mRNA stability in yeast. *Cell* 2011; 147: 1484–97.
94. Eulalio A, Rehwinkel J, Stricker M, Huntzinger E, Yang SF, Doerks T, Dorner S, Bork P, Boutros M, Izaurralde E. Target-specific requirements for enhancers of decapping in miRNA-mediated gene silencing. *Genes Dev* 2007c; 21: 2558–70.
95. Jager E, Dorner S. The decapping activator HPat a novel factor co-purifying with GW182 from *Drosophila* cells. *RNA Biol* 2010; 7: 381–5.
96. Hurto RL, Hopper AK. P-body components, Dhh1 and Pat1, are involved in tRNA nuclear-cytoplasmic dynamics. *RNA* 2011; 17: 912–24.
97. Noueiry AO, Diez J, Falk SP, Chen J, Ahlquist P. Yeast Lsm1p-7p/Pat1p deadenylation-dependent mRNA-decapping factors are required for brome mosaic virus genomic RNA translation. *Mol Cell Biol* 2003; 23: 4094–106.
98. Beckham CJ, Light HR, Nissan TA, Ahlquist P, Parker R, Noueiry A. Interactions between brome mosaic virus RNAs and cytoplasmic processing bodies. *J Virol* 2007; 81: 9759–68.
99. Scheller N, Mina LB, Galão RP, Chari A, Giménez-Barcons M, Noueiry A, Fischer U, Meyerhans A, Díez J. Translation and replication of hepatitis C virus genomic RNA depends on ancient cellular proteins that control mRNA fates. *Proc Natl Acad Sci USA* 2009; 106: 13517–22.
100. Checkley MA, Nagashima K, Lockett SJ, Nyswaner KM, Garfinkel DJ. P-body components are required for Ty1 retrotransposition during assembly of retrotransposition-competent virus-like particles. *Mol Cell Biol* 2010; 30: 382–98.
101. Ariumi Y, Kuroki M, Kushima Y, Osugi K, Hijikata M, Maki M, Ikeda M, Kato N. Hepatitis C virus hijacks P-body and stress granule components around lipid droplets. *J Virol* 2011; 85: 6882–92.

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Nancy Standart, an American born in Prague, undertook her undergraduate and graduate studies in the UK, and did post-doctoral work with Klaus Scherrer in Paris, Joan Ruderman in Cambridge, USA and Tim Hunt in Cambridge, UK. Her early research interests focused on control of translation (and mRNA localization) in early development by specific RNA-binding proteins,

first in marine invertebrates and then in *Xenopus*. Currently, the lab studies the role of P-body components in gene expression regulation.



Aline Marnef is from Belgium and did her undergraduate studies in Applied Genetics at U.W.E. in Bristol. During her studies she gained experiences in bacterial genetics, developmental biology, proteomics and mRNP complexes. AM did her PhD degree in Nancy Standart's lab in the Biochemistry Department at the University of Cambridge, where she investigated the

roles of Pat1 proteins and P-bodies in the control of gene expression, in particular at the post-transcriptional level. In 2012, she joined Dr Tamas Kiss' group in Toulouse (France) and now works on nuclear non-coding RNAs.